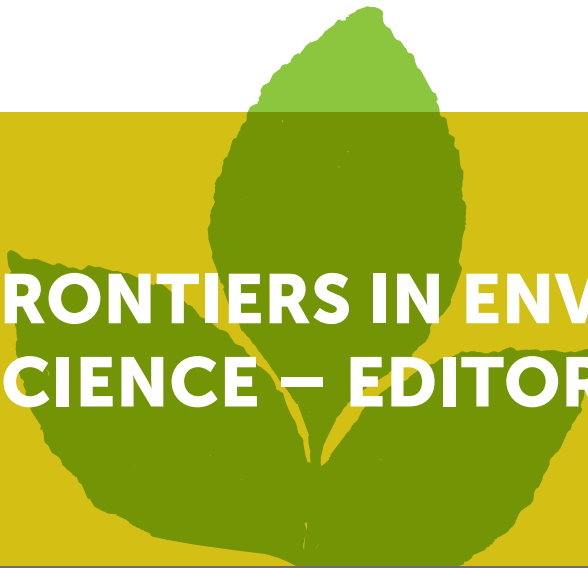




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Field-Scale Co-fermentation of Solid Waste From Urine-Diverting Dry Toilets (UDDT-SW) and Banana Waste to Produce Undissociated Carboxylic Acids to Inactivate *Ascaris* Eggs

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Innovative, low-cost methods for inactivating pathogens in human fecal material (HFM) are needed, particularly in expanding urban areas where conventional sewer systems and centralized wastewater treatment plants are not feasible. To address this challenge, we have developed a bioprocess that utilizes open cultures of anaerobic bacteria (i.e., microbiomes) to produce carboxylic acids using HFM as substrate. When the pH is sufficiently low, the carboxylic acids exist in the undissociated form and inactivate pathogens. Here, we used real solid waste (SW) collected from container-based, urine-diverting dry toilets (UDDT) in Nairobi, Kenya to conduct lab-scale and field-scale trials. Through these trials, we investigated operating conditions required to use carboxylic acid fermentation in sanitation waste treatment processes. We tested three different inoculum treatments and determined that the microbiome in UDDT-SW is well-suited to produce carboxylic acids without the need for an external inoculum. We also tested co-fermentation of UDDT-SW with carbohydrate-rich food waste as a means of reducing the pH. We found that when food waste was incorporated in a way that maintained the pH between 4.8 and 5.2, then the food waste was quickly converted to carboxylic acids, and the low pH created high concentrations of undissociated carboxylic acids. The resulting concentrations of undissociated carboxylic acids resulted in *Ascaris* inactivation within 15 days. However, we found that a temperature $\geq 30^{\circ}\text{C}$ is required for carboxylic acid production to occur.

Keywords: carboxylic acids, volatile fatty acids, *Ascaris*, sanitation, pathogen inactivation

INTRODUCTION

As a global community, we struggle to manage our waste in a safe and sustainable manner. Management of human excreta is particularly concerning due to the implications it has for human health and dignity, and yet 4.5 billion people still lack access to safely managed sanitation services (WHO and UNICEF, 2017). The provision of a sanitation

facility alone does not address the challenge because excreta must also be either safely contained on-site or transported, and treated off-site. Connection to a sewer system also does not guarantee access to safely managed sanitation. Of the 2.8 billion people using sewer connections, only 1.9 billion people are expected to have safely managed sanitation due to sewer lines that empty directly into the environment or wastewater treatment plants with insufficient treatment (WHO and UNICEF, 2017). Sewer systems are also frequently unfeasible due to high costs, uncertain land tenure, lack of appropriate space, and challenging topography among others (World Bank, 2019). Instead, a variety of treatment approaches and technologies are required to address the diverse challenges faced in achieving universal safe sanitation, including innovative methods for inactivating pathogens in excreta.

One method under investigation uses anaerobic fermentation of human fecal material (HFM) to produce carboxylic acids (Harroff et al., 2017). Complex organic molecules are first broken down to short-chain carboxylic acids (e.g., acetic acid, propionic acid, and *n*-butyric acid) through hydrolysis and primary fermentation. Oxidation of an electron donor, such as ethanol, sugars, amino acids, or lactate, is then coupled to chain elongation of the short-chain carboxylic acids to produce *n*-butyric acid (C4), *n*-caproic acid (C6), and even *n*-caprylic acid (C8) (Agler et al., 2011; Spirito et al., 2014). Acetic acid (C2) is often the dominant primary fermentation product, and hydrocarbon chains are elongated with two carbon molecules at a time. Therefore, molecules with even-numbered hydrocarbon chains are more common than those with odd-numbered chains, but both are produced. Previous work has shown that HFM can be used as substrate to produce up to 257 mM *n*-butyric acid, 11.3 mM *n*-valeric acid, and 27.1 mM *n*-caproic acid (Harroff et al., 2017).

Carboxylic acids have been used as food preservatives for decades due to their antimicrobial properties (Freese et al., 1973), and past work has demonstrated that for short-chain (one to five carbons) and medium-chain (six to eight carbons) carboxylic acids, antimicrobial activity increases as the length of the hydrocarbon chain increases (Abdul and Lloyd, 1985; Salsali et al., 2006; Royce et al., 2013). More recent work has demonstrated that carboxylic acids are also toxic to *Ascaris* eggs, which are commonly used as indicators for pathogen inactivation in waste treatment processes due to their high resistance to inactivation (Butkus et al., 2011; Harroff et al., 2017; Riungu et al., 2018a). The importance of hydrocarbon chain length has also been demonstrated for *Ascaris* inactivation. When exposure time, pH, and temperature were constant, *n*-caproic acid (C6) concentrations required for a 3-log reduction in *Ascaris* viability were more than an order of magnitude smaller than concentrations required for *n*-butyric acid (C4) (Harroff et al., 2017).

Carboxylic acids exist in both a dissociated (carboxylate) and undissociated (carboxylic acid) form, depending on the pH in the solution. When the pH is below the pKa (~4.8 for the carboxylic acids of interest here), a majority of the molecules are in the undissociated form with a hydrogen atom attached to the carboxyl group. At a pH above the pKa, a majority of the

molecules exist as the dissociated carboxylate with the hydrogen detached. The specific concentrations can be calculated using the Henderson-Hasselbalch equation, $pH = pK_a + \log \left(\frac{[A^-]}{[HA]} \right)$, where $[A^-]$ represents the concentration of the dissociated form, and $[HA]$ represents the concentration of the undissociated form. Throughout this paper, we will explicitly specify if we are referring to the undissociated form. If the undissociated form is not specified, then we are referring to the total concentration of undissociated acid plus dissociated conjugate base.

The distinction between the total concentration and the concentration of undissociated carboxylic acids is important because only the undissociated form inhibits pathogens (Butkus et al., 2011; Royce et al., 2013; Harroff et al., 2017). We previously produced carboxylic acids from HFM, but the pH remained nearly neutral throughout the experiment. Therefore, the fraction of undissociated carboxylic acids form remained relatively low, while the total carboxylic acid concentrations were promising (Harroff et al., 2017). Co-fermentation of HFM and food waste may present a solution to this problem. Many studies have shown that anaerobic fermentation or co-fermentation of carbohydrate-rich food waste results in rapid lactic acid production and pH decline (Rajagopal et al., 2014; Wang et al., 2014; Liang and Wan, 2015). In a series of recent studies, mixed food waste and HFM were co-fermented specifically to increase concentrations of undissociated carboxylic acids to inactivate pathogens, but these studies did not focus on chain elongation or report concentrations of individual carboxylic acids (Riungu et al., 2018a,b). Co-fermentation with food waste may be challenging because low pH and increased undissociated carboxylic acid concentrations can inhibit the bacteria responsible for fermentation and chain elongation, in addition to inactivating pathogens (Agler et al., 2012; Riungu et al., 2018a). Therefore, pH reduction must be carefully balanced with maximizing carboxylic acid production and chain elongation.

The primary goal of our study was to better understand how HFM can be fermented to produce undissociated carboxylic acids that cause pathogen inactivation in real sanitation and waste treatment systems. To accomplish this, we collaborated with Sanergy, which is a sanitation enterprise in Nairobi, Kenya, that maintains a network of container-based, urine-diverting dry toilets (UDDTs) and converts the collected waste to valuable reuse products. Carboxylic acid fermentation may be useful as a pre-treatment step in treatment facilities similar to Sanergy's facility, because the output has an adequately reduced pathogen load and has already undergone the often rate-limiting stages of biological degradation. It can then be used as the input for further processing into value-added products such as biogas from anaerobic digestion or livestock feed from black soldier fly larvae production. Here, we used real HFM collected from Sanergy's UDDTs, which we refer to here as UDDT-solid waste (UDDT-SW), and tested fermentation: (1) with 500-mL media bottles in the lab during Experiments 1 and 2; and (2) 45-L plastic barrels at the field-scale during Experiment 3. We tested three different inocula to determine if a specialized inoculum would be required to implement new treatment systems. We also

tested the addition of banana waste at different times during the fermentation process and at different ratios to maximize concentrations of undissociated carboxylic acids. Finally, we demonstrated pathogen inactivation from co-fermentation of HFM with banana waste using *Ascaris suum* eggs as indicators.

MATERIALS AND METHODS

Materials

HFM Samples

HFM was obtained from UDDTs that are located in informal settlements around Nairobi, Kenya. The UDDTs have separate containers to collect urine and solid waste. The UDDT solid waste (UDDT-SW) container is 30 L and contains a mixture of HFM and sawdust, which is added by users to reduce odor in the toilets. Sawdust is expected to constitute about 20% of the total mass of the UDDT-SW. Sanergy collects the urine and UDDT-SW containers each day and replaces them with clean containers. On the starting day for Experiments 1 and 3, samples of UDDT-SW were taken from several containers that had been collected from UDDTs that same day.

Banana Waste

The pre-consumer banana waste originated from Twiga Foods, which is a food supply platform that connects smallholder farms to market vendors. Sanergy receives fresh banana waste that is unsuitable for market every few days and stores it outdoors in a pile until it is used in waste treatment and recycling processes.

Chain-Elongation-Bioreactor Inoculum

The chain-elongation-bioreactor inoculum originated from a semi-continuous lab-scale bioreactor that chain-elongated ethanol-rich fermentation beer for 4 years (Ge et al., 2015). The effluent was collected from the bioreactor and centrifuged to concentrate the biomass. The bioreactor broth was discarded, and the biomass was re-suspended in an equivalent volume of liquid that was 50% (v/v) glycerol and 50% (v/v) fresh bioreactor media. The biomass was then frozen at -80°C for ~ 1 year until use. After thawing, the biomass was washed twice to remove the glycerol by centrifuging and replacing the supernatant with tap water. A new culture was then started by inoculating 10% (m/m) biomass into diluted UDDT-SW. This culture was kept in an airtight 1-L glass bottle. The headspace was sparged with nitrogen gas to create anaerobic conditions, and the bottle was incubated at 30°C in the dark for 24 days with periodic manual shaking to mix. Contents from this culture were used directly as chain-elongation-bioreactor inoculum for lab-scale experiments.

Rumen Inoculum

The rumen contents were selected for testing because considerable carboxylic acid production occurs in the rumen, and rumen contents were originally used to inoculate the bioreactor that was used for the chain-elongation-bioreactor inoculum (Agler et al., 2012). The rumen contents are also easier to obtain than chain-elongation-bioreactor inoculum, which would depend on successful implementation of carboxylic acid fermentation for waste treatment. The rumen inoculum was

TABLE 1 | Initial conditions for Experiment 1.

Inoculum type	Mass inoculum (g)	Mass HFM (g)	% Inoculum (m/m)	Moisture content (%)
1: Chain-elongation-bioreactor	65.4	584.9	10.1	84.0
2: Rumen	65.1	587.1	10.0	83.7
3: None (tap water)	65.3	586.1	10.0	84.7

Masses are total mass for the triplicate media bottles for each treatment.

collected from the rumen contents of a cow that had been freshly slaughtered at Dagoretti Slaughterhouse in Nairobi, Kenya. Next, the rumen contents were strained through a mesh screen and cheesecloth to remove large particulate matter before use.

Methods

Experiment 1: Determining the Importance of a Specialized Inoculum

Three inoculum treatments were tested to determine whether a specialized inoculum was necessary to achieve carboxylic acid production and chain elongation: (1) chain-elongation-bioreactor inoculum; (2) rumen inoculum; and (3) no inoculum. The chain-elongation-bioreactor inoculum and rumen inoculum were obtained as described above. For the third treatment, tap water was used in place of an inoculum, and therefore the fermentation in the media bottles for this treatment was dependent on the microbiome residing naturally in HFM. UDDT-SW was collected in the morning of the starting day of the experiment. The UDDT-SW samples were combined and homogenized in a kitchen blender before separating into treatments. Each treatment was prepared with ~ 65 g inoculum and 585 g UDDT-SW (Table 1). The treatment mixtures were manually mixed with a spatula, and 200 g was allocated to triplicate 500-mL glass media bottles. A 5-g sample of each treatment was also kept for pH and carboxylic acid analysis. The headspace in each media bottle was sparged with nitrogen gas for 1 min at a rate of 12 L min^{-1} . The media bottles were then sealed with a rubber stopper and plastic ring (Figure S1) and incubated in the dark at 30°C for 26 days. Additional mixing occurred only during sample collection. The fermentation gas was vented from the media bottles on Day 1 and 2 to avoid excessive pressure buildup before the first sampling point. Venting was performed by inserting a hypodermic needle into a septum that was located in the rubber stopper of each media bottle. The samples were collected from each media bottle on Day 5, 12, 18, 21, 25, and 26 to measure pH and carboxylic acid concentrations. The rubber stopper and plastic ring were removed from the media bottle, and the contents were manually mixed with a spatula before removing a small sample (~ 5 g) of contents. Finally, the headspace in the media bottle was sparged with nitrogen for 1 min at 12 L min^{-1} , and the rubber stopper and plastic ring were replaced. Carboxylic acid concentrations were measured using gas chromatography (Hewlett-Packard 5890 Series II; Wilmington, DE) with a method described previously, except nitrogen was used as the carrier gas instead of helium (Usack and Angenent, 2015).

Experiment 2: Co-fermentation With Banana Waste to Reduce pH, Increase Concentrations of Undissociated Carboxylic Acids, and Inactivate *Ascaris* Eggs

After Day 26 of the operating period, the media bottles from Experiment 1 were emptied. All of the remaining fermented UDDT-SW was combined, homogenized in a kitchen blender, and divided into three new treatments: (1) 0% banana-mash addition; (2) 10% banana-mash addition; and (3) 20% banana-mash addition, with all percentages on a mass basis. The banana waste had been collected from the outdoor storage pile 1 day prior to Day 0 and kept in the laboratory refrigerator overnight. The peels from the bananas were removed, and the bananas were homogenized in a kitchen blender. Finally, the resulting banana mash was combined with the fermented UDDT-SW to produce 360 g of each treatment. The mixtures were manually mixed with a spatula, and 155-g aliquots were distributed to duplicate 500-mL glass media bottles. We used duplicate treatments instead of triplicates based on limitations from the quantity of fermented UDDT-SW material available. The media bottles (**Figure S1**) were sparged with nitrogen, sealed, and sampled as described above, with samples collected on Day 1, 3, 6, and 15 to measure pH and carboxylic acid concentrations.

We tested *Ascaris* inactivation for the 0% and 20% banana-mash treatments using *A. suum* eggs. The eggs were collected from fecal material in the intestines of naturally infected slaughterhouse pigs and purified and stored as described previously (Harroff et al., 2017). We used plastic chambers (2.5 cm diameter, 2.5 cm height), which were sealed on both ends with 38- μ m metal mesh, to contain the eggs while exposing them to the conditions inside the media bottles. The plastic chambers were initially sealed on one end, and the open end was used to fill them with the 0 and 20% banana-mash treatment mixtures and to spike them with 10,000 unembryonated *A. suum* eggs. A soldering iron was then used to seal the open end of the plastic chamber with a second piece of mesh (**Figure S2**). Nylon mesh bags have frequently been used to contain *Ascaris* eggs in similar experiments (Johnson et al., 1998; Nordin et al., 2009; Manser et al., 2015; Fidjeland et al., 2016), but we found that the plastic chambers were more reliable. Prior to performing the experiment, we made several nylon mesh bags and sealed the seams with a thermal sealer, as has been done before, but we found that the seams separated over time or had incomplete seals. In a study that directly compared *Ascaris* egg recovery from nylon mesh bags vs. plastic chambers, plastic chambers were also found to perform better and more consistently (Nelson and Darby, 2002). The mesh bags often trapped gas inside, and the seams became unsealed, possibly due to the increased gas pressure. In contrast, the fixed volume of the plastic chambers seemed to force gas out of the chamber, and the more substantial seal held throughout the experiment (Nelson and Darby, 2002).

Eight plastic chambers were prepared, with two plastic chambers placed in each of the duplicate media bottles for the 0% and 20% banana-mash treatments. One plastic chamber from each media bottle was removed during sampling on Day 6 and 15. *Ascaris* eggs were recovered from the plastic chambers using

a method adapted from several methods published previously (Nelson and Darby, 2002; Bowman et al., 2003; Trönnberg et al., 2010; Amoah et al., 2018). First, the mesh from one side was peeled off, and a metal spatula was used to divide most of the contents between two 50-mL centrifuge tubes. The empty plastic chamber was then placed in a 200-mL wide-mouth cylinder with about 75 mL of 1.5 M ammonium bicarbonate (AmBic) solution. The cylinder was sealed and shaken for 30 s to dislodge any remaining solid material. The solution in the cylinder was poured into the two centrifuge tubes, and additional AmBic solution was used to rinse the chamber and cylinder into the tubes. The two centrifuge tubes were sealed and shaken for 1 min and then centrifuged at $1,000 \times g$ for 5 min. The supernatant was discarded, and the tubes were filled with $MgSO_4$ (specific gravity = 1.2), vortexed, and centrifuged again at $1,000 \times g$ for 5 min to float the *Ascaris* eggs. The supernatant was sieved through US 50 and US 200 sieves, before collecting the eggs on a US 400 sieve. Diluted H_2SO_4 (0.1 N) was used to rinse the sieve into a clean 50-mL centrifuge tube. The tube was centrifuged at $1,000 \times g$ for 5 min to concentrate the eggs at the bottom of the tube, and a pipette was used to remove supernatant to a final volume of 5 mL. Next, the tube was vortexed for a final time, the cap was loosened to allow oxygen flow, and the tube was incubated in the dark at 28°C for 3 weeks to allow any viable *A. suum* eggs to develop into larvae. After 3 weeks, at least 100 eggs were observed microscopically to determine viability. Fully developed larvae were considered viable, and all others were not.

Experiment 3: Field-Scale Trial

A field-scale fermentation trial was performed on-site at Sanergy's waste processing facility located southeast of central Nairobi in Kinanie. Six 45-L screw-top plastic barrels were used as batch bioreactors to test three treatments in duplicate (**Table 2**, **Figure S3**). For Treatments 1 and 2, UDDT-SW was fermented alone for 42 days before adding banana waste because we expected the banana waste to cause a low pH that would be inhibitory to carboxylic acid production. For Treatment 3, banana waste was mixed with UDDT-SW on Day 0, and no additional banana waste was added on Day 42 of the operating period. We used cow rumen inoculum for Treatments 1 and 3, and no inoculum for Treatment 2. We obtained rumen contents from a cow that was slaughtered the same morning, and we prepared the inoculum as described above. UDDT-SW was collected from two randomly selected storage containers that had been transferred to Kinanie within the previous 2–3 days. Each storage container held UDDT-SW collected from ~3–4 UDDTs. We collected banana waste from the storage pile onsite and roughly chopped the waste with the peels left intact. The required material for each barrel was combined and manually mixed with a shovel.

Data loggers (HOBO UX100-003, Bourne, MA and Lascar Electronics EL-USB-1, Erie, PA) were contained in sealed plastic tubes and placed in each barrel to record temperature every 15 min. The barrels were initially placed in a sunny area outside and covered with a black tarp to promote warmer temperatures. We conducted the field trial in May–July, which is the cool season for Nairobi. After 29 days of operation, the barrels were

TABLE 2 | Treatment summary for field-scale trial (Experiment 3).

Treatment number	Initial setup				Second stage (Day 42)
	UDDT-SW (kg)	Inoculum type	Inoculum mass (kg)	Banana waste (kg)	Banana waste (kg)
1	22.5	Rumen	2.5	0	6.5
2	22.5	None (tap water)	2.5	0	6.5
3	17.5	Rumen	2.5	5	0

Masses given are per barrel, with duplicate barrels used for each treatment.

moved inside a greenhouse to achieve warmer temperatures. Throughout the operating period, samples were collected every 2–6 days from the barrels. At sampling, the lid was removed, and the barrel contents were manually mixed with a large spoon. Small samples were collected from several points in the barrel and mixed together in a plastic tube. The composite sample was used to measure pH and carboxylic acid concentrations.

We also tested *Ascaris* inactivation in the field-scale trial. First, we placed *A. suum* eggs in plastic chambers as described above. Ten chambers were prepared for each barrel, with two chambers collected from each barrel at each time point. The chambers were attached to wires and sunk in the barrels to expose the *A. suum* eggs to the fermentation conditions. An additional 20 chambers were prepared and filled with autoclaved UDDT-SW instead of the UDDT-SW used in the barrels. Each of these chambers was sealed in a 50-mL plastic tube that was also filled with autoclaved UDDT-SW, and the plastic tubes were sunk in the barrels for Treatment 1. Duplicate chambers with the autoclaved UDDT-SW were collected at the same time points as the other chambers and were used to isolate inactivation that occurred for reasons unrelated to the fermentation process (e.g., temperature or compounds already existing in the UDDT-SW before fermentation).

RESULTS AND DISCUSSION

Experiment 1: Specialized Inoculum Was Not Necessary to Produce Carboxylic Acids in HFM

During 26 days of fermentation for the lab-scale trial, we found no benefit from either the chain-elongation-bioreactor inoculum or rumen inoculum compared to the no-inoculum treatment (Figures 1A,B). The *n*-butyric acid concentrations between the three treatments were nearly identical throughout the operating period with maximum average concentrations observed on Day 25 for all three treatments (Figure 1A). The average maximum *n*-butyric acid concentrations were 107, 111, and 107 mM for chain-elongation-bioreactor inoculum, rumen inoculum, and no inoculum, respectively, but the differences were within the standard deviations of the triplicate samples (Figure 1A). For *n*-caproic acid, the treatment with no inoculum actually outperformed the other two treatments with an average maximum concentration of 24.3 vs. 21.0 mM with

chain-elongation-bioreactor inoculum and 20.0 mM with rumen inoculum (Figure 1B). We also observed that the trends for *n*-valeric acid concentrations were similar to the *n*-caproic acid concentrations (Figure S4). The treatment with no inoculum consistently produced the highest *n*-valeric acid concentrations, and a maximum average *n*-valeric acid concentration of 35.0 mM was observed on Day 25 of the operating period (Figure S4). This *n*-valeric acid concentration is notably larger than the 11.3 mM observed in a previously published study (Harroff et al., 2017) and may be the result of competing pathways before entry into the chain-elongating pathway. Such pathways include the acrylate pathway, which converts lactic acid into propionic acid (Kucek et al., 2016). Chain elongation of propionic acid would then result in production of *n*-valeric acid instead of one of the even-numbered carboxylic acids. Although we did not measure lactic acid here, it is a common product of primary fermentation in addition to acetic acid, and is quickly removed while being produced in the anaerobic food web. We observed only a small difference in pH between the three treatments and throughout the time-course of the experiment. The average pH was 6.2 with a minimum of 6.0 and maximum of 6.3 (data not shown), which is similar to the previously published study (Harroff et al., 2017).

The promising performance of the treatment with no inoculum indicates that the gut microbiome, which is inherent to HFM, can be used to produce carboxylic acids. This finding is not surprising because the human-gut microbiome is already known to produce *n*-butyric acid and *n*-caproic acid within the digestive system (Cummings et al., 1987; Miller and Wolin, 1996). While not surprising, the finding is important for implementing the proposed treatment method in sanitation systems because the system becomes less complex and less expensive if a specialized inoculum is not required. However, human diet is known to affect the gut microbial community and resulting fermentation products (Flint et al., 2015). Therefore, variability in rates of carboxylic acid production and final concentrations should be expected when relying on the gut microbiome.

Experiment 2: Co-fermentation With Banana Mash and *Ascaris* Inactivation

Co-fermentation With Banana Mash Reduces pH

We observed only small changes in pH throughout the operating period when solely UDDT-SW was fermented for Experiment 1 (average pH of 6.2), which continued during Experiment 2 for the 0% banana-mash treatment (Figure 2A). A pH value of 6.2 causes only 4.2% of the total carboxylic acid concentration to exist in the undissociated form (Figures 2B,C, Figures S5, S6). After banana mash was mixed with the fermented UDDT-SW at a rate of 20% by mass, the pH dropped from 6.1 to 4.8 within 6 days of the operating period (Figure 2A). At such a pH value, half of the total carboxylic acid concentration was in the undissociated form (Figures 2B,C, Figures S5, S6). In the 10% banana-mash treatment, the pH decreased to 5.2 within 6 days (Figure 2A), resulting in 32% of the total carboxylic acid concentration in the undissociated form (Figures 2B,C, Figures S5, S6). A previous study co-fermented mixed food waste and UDDT-SW at a series

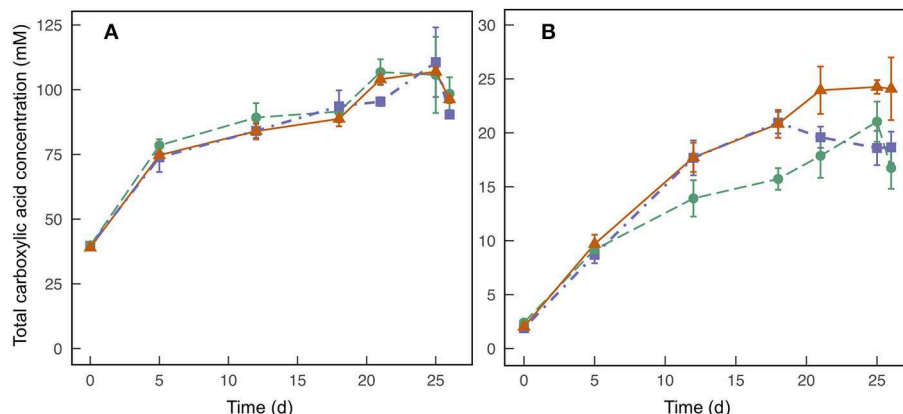


FIGURE 1 | Average total concentrations of *n*-butyric acid (A) and *n*-caproic acid (B) found in triplicate batch-fermentation media bottles for Experiment 1, which were started with inoculum from a semi-continuous chain-elongation bioreactor (●); inoculum from rumen contents of a cow (■); and no inoculum (▲). Error bars indicate standard deviations of the measured concentrations.

of substrate ratios and observed similar pH values compared to our study (Riungu et al., 2018a). When food waste made up 20% of the total mass, the pH decreased from 5.7 to 4.9 in 4 days (Riungu et al., 2018a).

Low pH Causes Inhibition of Carboxylic Acid Production

On Day 0 for Experiment 2, carboxylic acid concentrations were already 73.5–88.7 mM *n*-butyric acid, 23.3–27.3 mM *n*-valeric acid, and 15.4–17.1 mM *n*-caproic acid due to the pre-fermentation during Experiment 1. The addition of banana mash lowered these concentrations slightly due to dilution (Figure 2B, Figures S5A, S6A). When no banana mash was added, carboxylic acid concentrations continued to increase for the first 3 days of the operating period before tapering off (Figure 2B, Figures S5A, S6A). This result is similar to the result we would have anticipated if we had allowed the Experiment 1 treatments to continue fermenting. We observed maximum average concentrations for the 0% banana-mash treatment of 101 mM *n*-butyric acid, 31.4 mM *n*-valeric acid, and 21.7 mM *n*-caproic acid. The *n*-caproic acid concentration was similar to the maximum average concentration we observed in a previous fermentation experiment, but the *n*-butyric acid concentration was less than half of the concentration we observed (Harroff et al., 2017). Similar to Experiment 1, concentrations of *n*-valeric acid were higher than those seen previously (Figure S6), and this may partly account for the lower concentrations of *n*-butyric acid. In addition, a higher conversion efficiency of *n*-butyric acid to *n*-caproic acid may also have contributed to a relatively low *n*-butyric acid concentration.

For the 20% banana-mash treatment, no additional carboxylic acid production occurred (Figure 2B, Figures S5A, S6A), which can be attributed to a combination of inhibition from: (1) the low pH directly; and (2) the high concentration of undissociated carboxylic acids, which was considerably increased due to the low pH (Figure 2C, Figures S5B, S6B) (Agler et al., 2012; Riungu et al., 2018a). For the 10% banana-mash treatment,

production rates of carboxylic acids were slightly higher than those observed for the 0% banana-mash treatment (Figure 2B, Figures S5A, S6A). While the pH declined for the 10% banana-mash treatment, it remained above 5 (Figure 2A), which did not cause noticeable inhibition compared to the 0% banana-mash treatment. Therefore, the added banana mash provided a readily degradable carbon source that contributed to the increased production rates of carboxylic acids compared to the 0% banana-mash treatment.

A Low pH Must Be Balanced With Carboxylic Acid Production to Produce High Concentrations of Undissociated Carboxylic Acids

Although no additional carboxylic acid production occurred after banana mash was added in the 20% treatment, this treatment still resulted in the highest concentrations of undissociated carboxylic acids due to the lower pH value compared to the 0 and 10% treatments (Figure 2C, Figures S5B, S6B). The difference in undissociated *n*-caproic acid concentrations between the 20% treatment and the 10% treatment was minor (Figure 2C), but it was more pronounced for undissociated *n*-valeric acid and undissociated *n*-butyric acid concentrations (Figures S5B, S6B). The concentration of undissociated carboxylic acids is a function of the total carboxylic acid concentration and the pH value. The pH relationship is exponential and has a greater effect than the total carboxylic acid concentrations. This is clearly visualized by the much higher concentrations of undissociated *n*-caproic acid in the 10% banana-mash treatment compared to the 0% banana-mash treatment, while the total concentrations of *n*-caproic acid were somewhat equivalent between the two treatments (Figures 2B,C). However, comparing the 10% and 20% banana-mash treatments demonstrates that a high total *n*-caproic acid concentration is also important. The difference in undissociated concentrations of *n*-caproic acid between these two treatments was minor because the 10% treatment had a larger concentration of total *n*-caproic acid (22.3 mM in the 10% treatment and vs. 12.3 mM in the 20% treatment) (Figures 2B,C).

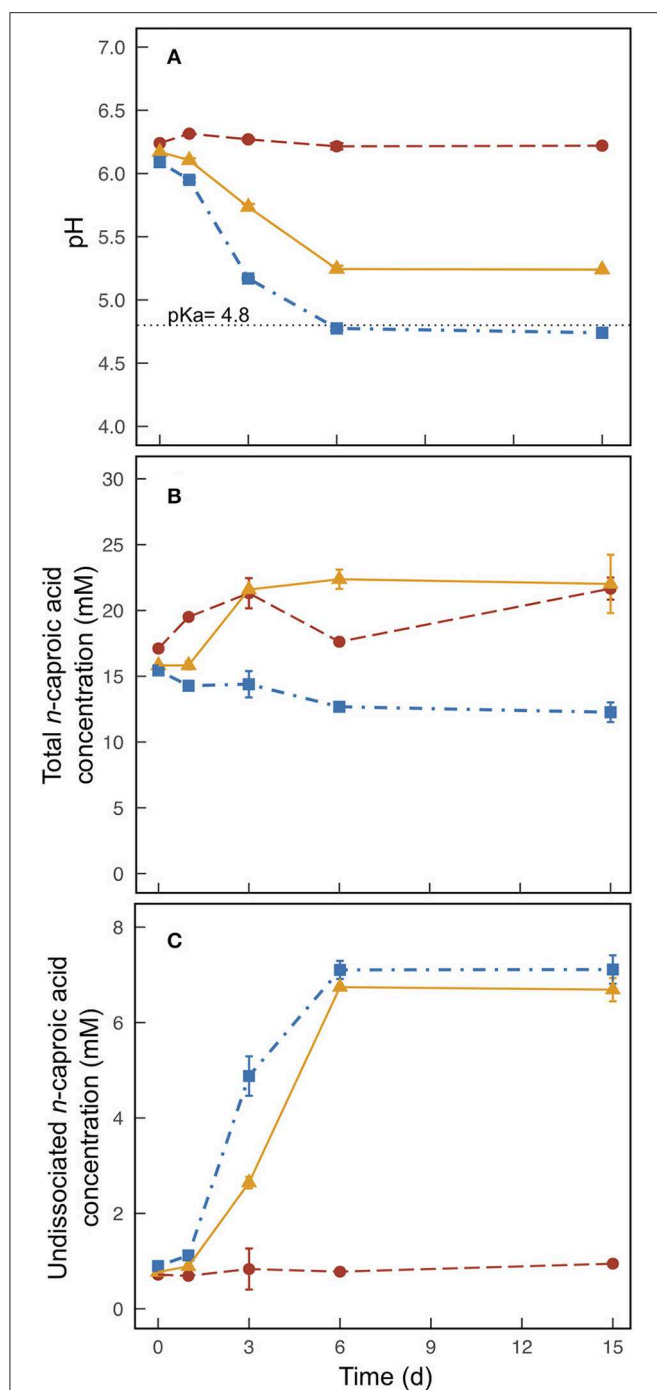


FIGURE 2 | Average pH (A), total *n*-caproic acid concentration (undissociated plus dissociated) (B), and undissociated *n*-caproic acid concentration (C) found in duplicate batch fermentation media bottles for Experiment 2 with banana mash added at 0% (m/m) (●), 10% (m/m) (▲), and 20% (m/m) (■). Error bars indicate the range of the measured values between the duplicate media bottles. The dotted horizontal line at pH = 4.8 indicates the pKa of carboxylic acids.

The baseline concentrations of carboxylic acids produced during Experiment 1 were also important here. Without it, the 20% treatment would have had very low total carboxylic acid

concentrations (and therefore low undissociated concentrations) due to inhibition from low pH after banana-mash addition. Therefore, 20% banana-mash addition may be appropriate if fermentation is completed in two stages, as was done here. However, it is simpler to conduct the fermentation in a single stage with UDDT-SW and banana mash mixed upfront. For this scenario, a lower fraction of banana mash may be desired, which would maintain a moderate pH (~5) that does not inhibit carboxylic acid production but still maintains a large portion of the total concentration in the undissociated form, as we saw in the 10% banana-mash addition treatment.

***Ascaris* Eggs Are Inactivated Faster When Banana Mash Is Used to Produce a Higher Concentration of Undissociated Carboxylic Acids**

We only compared *Ascaris* egg inactivation for the treatments containing 20% banana mash and 0% banana mash. For both treatments, we observed large decreases in *Ascaris* viability after 6 days and 15 days, but viability below detection was only achieved for the 20% banana-mash treatment after 15 days (Figure 3). The inactivation rates were also faster for the 20% banana-mash treatment compared to the 0% banana-mash treatment, with 1.4% viability observed after 6 days compared to 53% viability, respectively (Figure 3). The observed inactivation for the 0% banana-mash treatment was unexpected because the undissociated carboxylic acid concentrations were very low (Figure 2C, Figures S5B, S6B). However, similar inactivation rates have been observed previously for treatments with low undissociated carboxylic acid concentrations, and they were attributed to additional fermentation products that were not measured (Harroff et al., 2017). One fermentation product that we thought might be important, particularly with added banana waste, was lactic acid. However, during a preliminary experiment we exposed *Ascaris* eggs to concentrations of lactic acid up to 1 M (at pH 2, for 1–15 days at 30°C), and we observed less than a 10% decrease in viability for all treatments (data not shown). Previous studies that performed similar batch fermentations with food waste found lactic acid concentrations in the range of 0.1–0.2 M (Wang et al., 2014; Liang and Wan, 2015). Therefore, we can reasonably assume that lactic acid did not cause the *Ascaris* inactivation we observed in the present study.

Riungu et al. (2018a) also tested *Ascaris* inactivation related to fermentation of UDDT-SW and food waste. The results are difficult to compare because individual carboxylic acid concentrations were not measured in the Riungu et al. study; however, inactivation below detection limits was observed within 4 days for many treatments (Riungu et al., 2018a). These inactivation rates were even faster than in our study, which can be partly attributed to the higher temperature of 35°C for the Riungu et al. study. Indeed, temperature has been shown to have a significant impact on *Ascaris* inactivation rates by ammonia (Fidjeland et al., 2015). We anticipate a similar effect for carboxylic acids because both methods rely on the ability of the chemicals to cross the lipid membrane of the eggs (Fidjeland et al., 2015).

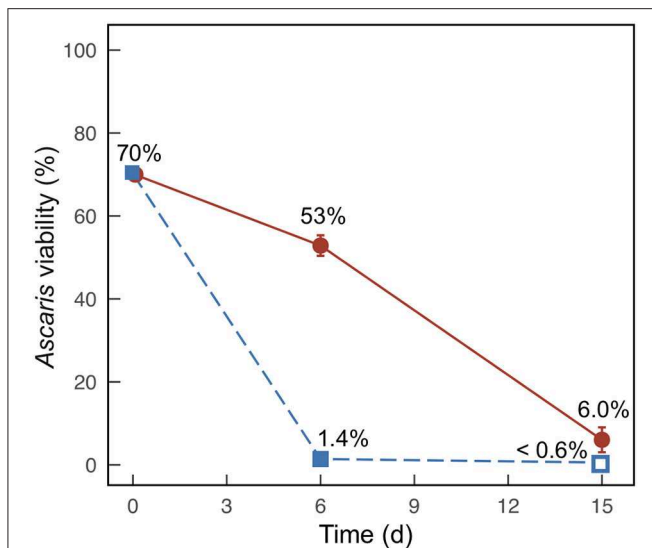


FIGURE 3 | Average percent viability of *Ascaris* eggs collected from duplicate batch fermentation media bottles for Experiment 2 with banana mash added at 0% (m/m) (●) and 20% (m/m) (■). Error bars indicate the range between the two samples. Open symbols indicate that viability was below the detection limit (0.6%).

Experiment 3: Field-Scale Trial

Cow Rumen Inoculum Did Not Affect Carboxylic Acid Concentrations

The effect of cow rumen inoculum was tested in Treatment 1 (rumen inoculum) and Treatment 2 (no inoculum). Although we already demonstrated that inoculum was not necessary in Experiment 1 (Figure 1), these results were not available when we started Experiment 3 because Experiment 1 was not fully completed. In Experiment 3, we further confirmed that the use of inoculum did not improve carboxylic acid concentrations (Figure 4A, Figure S8A).

Warm Temperatures Are Needed to Produce Carboxylic Acids

We used data loggers to measure and record the temperature every 15 min in each of the 6 barrels that were used for the field-scale trial. Two of the loggers failed during the experiment, and the data was not retrievable. However, we can assume that the temperatures in all barrels were the same because the temperatures measured from the remaining four loggers were not statistically different ($p = 0.235$) (Figure S7). During the first 29 days of the trial, the barrels were kept outside under a black tarp. The average temperature during this period was 22.1°C with a range of 17.7–29.0°C and standard deviation of 1.8°C. The observed temperatures were lower than we had originally anticipated, which resulted in very little carboxylic acid production during this period (Table 3, Figure 4, Figure S8). Previous fermentation trials that produced carboxylic acids from HFM or UDDT-SW, including the lab-scale trials here, were performed at higher temperatures of 30°C (Harroff et al., 2017) and 35°C (Riungu et al., 2018a). Therefore, we moved the barrels to a greenhouse on Day 29 of the operating period. From Day

29 until the end of the trial on Day 56, the average temperature was 26.9°C with a range of 18.7–34.5°C and standard deviation of 2.2°C. This temperature increase resulted in modest increases in *n*-caproic acid concentrations between days 29 and 42 (Figures 4A,B), but concentrations remained lower than seen previously, likely because the average temperature remained below 30°C.

If pH > 5 Is Maintained, Then Banana Waste Is Rapidly Converted to Carboxylic Acids, and High Undissociated Concentrations Can Be Achieved Without Experiencing Product Inhibition

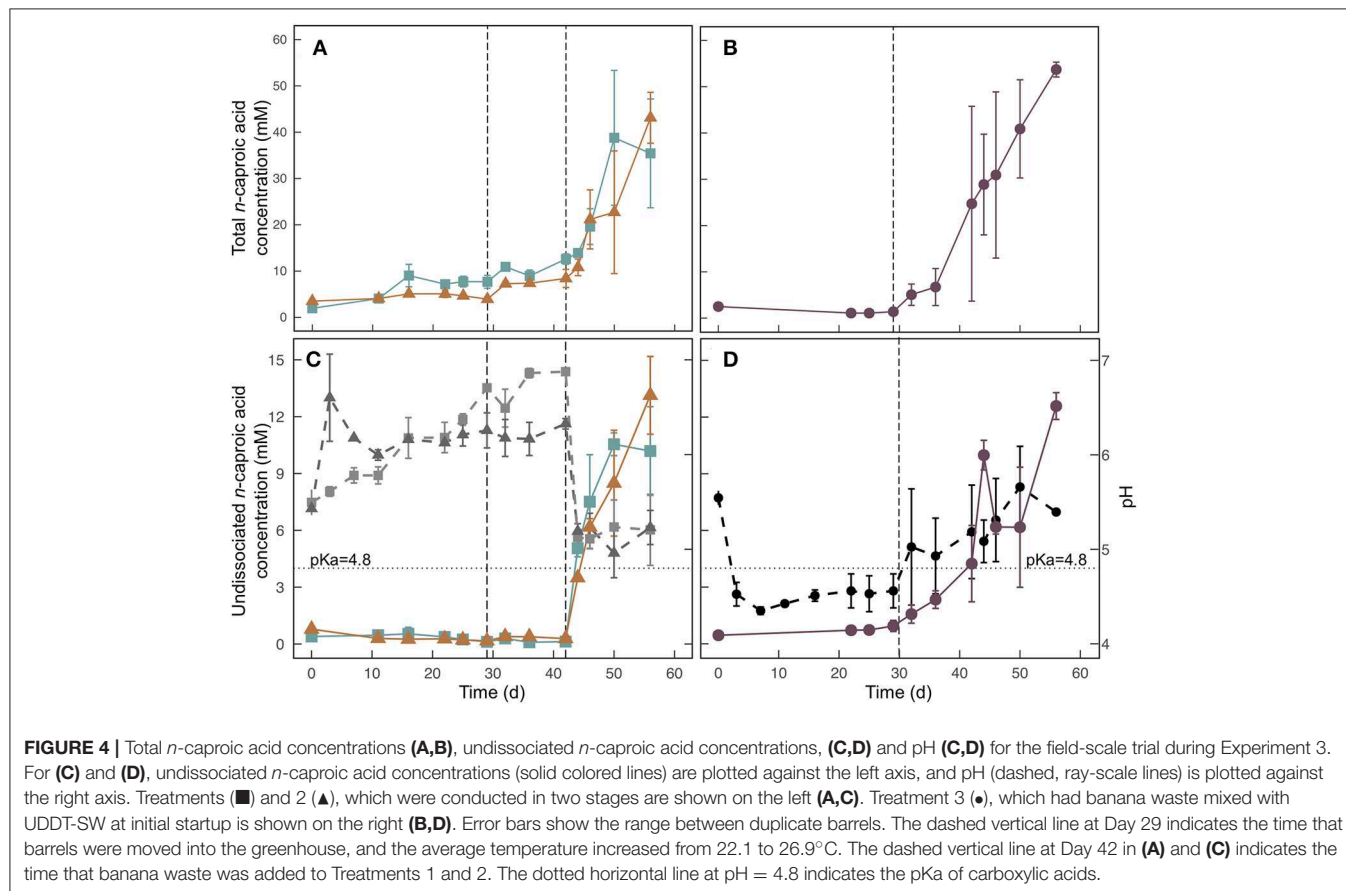
We added banana waste to Treatments 1 and 2 on Day 42 of the operating period as 20% of the total mass, which was after the temperature had been increased. As anticipated, the pH decreased within 2 days from 6.9 to 5.1 for Treatment 1 and from 6.3 to 5.2 for Treatment 2 (Figure 4C). However, the pH never decreased below 5.0 for either treatment. Based on the 20% banana-mash treatment during Experiment 2, we had anticipated a lower pH. We are attributing the higher pH for Experiment 3 to two possible reasons: (1) the different composition between banana mash without peel (Experiment 2) and banana waste with peel (Experiment 3); and (2) ammonia production from protein breakdown during the longer fermentation period compared to Experiment 2 (ammonia data not available), which would have caused a higher buffering capacity of the UDDT-SW (Procházka et al., 2012; Rose et al., 2015). For Treatment 3, we added banana waste from the beginning of the operating period (Day 0), and the pH was reduced to 4.5 within the first 3 days of the trial (Figure 4D), even though the temperature had been relatively low. The pH consistently remained below the carboxylic acid pKa of 4.8 until Day 29 of the operating period (Figure 4D). However, after moving the barrels to the greenhouse on Day 29, the pH for Treatment 3 increased slightly to above 5.0 (Figure 4D), likely due to increased ammonia production coinciding with increased microbial activity. However, ammonia concentration data is not available to confirm this theory. From Day 32 until the end of the trial, the average pH further increased, but remained below 6 (Figure 4D).

For all three treatments, *n*-caproic acid concentrations increased rapidly when the pH was >5.0 and banana mash was available as substrate. Similarly, Riungu et al. (2018b) found that co-digestion of UDDT-SW and food waste with a pH >5 resulted in higher total volatile fatty acid concentrations than when UDDT-SW was digested alone. Here, we achieved average final *n*-caproic acid concentrations for Treatments 1, 2, and 3 of 35.4, 43.1, and 53.7 mM, respectively (Table 3). If we had not stopped the trial on Day 56 of the operating period, the longer fermentation period would likely have resulted in even higher concentrations because the concentrations were still increasing. During Experiments 1 and 2, we observed a maximum average *n*-caproic acid concentration of 22.4 mM (Figures 1B, 2B), while the maximum average *n*-caproic acid concentration previously had been 27.1 mM (Harroff et al., 2017). The likely reason for the highest concentrations of *n*-caproic acid during Experiment 3, is the relatively high conversion

TABLE 3 | Carboxylic acid concentrations at transitional time points during the field-scale trial for Experiment 3.

Day	Treatment 1			Treatment 2			Treatment 3		
	<i>n</i> -Butyric acid (mM)	<i>n</i> -Valeric acid (mM)	<i>n</i> -Caproic acid (mM)	<i>n</i> -Butyric acid (mM)	<i>n</i> -Valeric acid (mM)	<i>n</i> -Caproic acid (mM)	<i>n</i> -Butyric acid (mM)	<i>n</i> -Valeric acid (mM)	<i>n</i> -Caproic acid (mM)
0	55.8 (±5.7)	5.6 (±0.3)	2.0 (±0.04)	70.4 (±3.3)	7.1 (±2.0)	3.5 (±0.7)	41.2 (±0.1)	4.6 (±0.3)	2.5 (±0.2)
29	69.8 (±1.0)	15.7 (±2.4)	7.7 (±1.4)	73.3 (±2.0)	15.5 (±0.7)	4.0 (±0.5)	43.1 (±1.8)	5.7 (±1.2)	1.4 (±0.3)
42	68.8 (±2.8)	19.2 (±0.5)	12.6 (±1.1)	75.1 (±1.3)	16.0 (±0.8)	18.5 (±2.0)	58.0 (±13.4)	18.1 (±12.2)	24.7 (±21.1)
56	85.0 (±0.5)	22.2 (±4)	35.4 (±11.7)	84.0 (±6.4)	27.4 (±1.3)	43.1 (±5.5)	63.6 (±2.7)	25.4 (±2.7)	53.7 (±1.6)

Barrels were moved into a greenhouse on Day 29; banana waste was added to Treatments 1 and 2 on Day 42; and Day 56 was the final day of the trial. Banana waste was already added at Day 0 for Treatment 3.



efficiency of *n*-butyric acid into *n*-caproic acid, explaining the lower concentrations of *n*-butyric acid during Experiment 3 than what we have observed previously (**Table 3**, **Figure S8**) (Harroff et al., 2017).

After increasing the temperature, we observed a larger difference in results between the two barrels per treatment than before for both the carboxylic acid concentrations and the pH (**Figure 4**, **Figure S8**). Particularly for Treatment 3, one barrel lagged behind the other one (**Figure 4B**). For one barrel, the pH immediately increased from 4.7 to 5.6 between Day 29 and Day 32, and the total concentration of *n*-caproic acid increased simultaneously. For the second Treatment 3 barrel, the pH increased more slowly after Day 29 and was not >4.8 until Day

44. Accordingly, the total concentration of *n*-caproic acid in that bioreactor increased from 3.6 mM on Day 42 to 18.0 mM on Day 44. The pH remained above 4.8 after that point, and the total *n*-caproic acid concentration increased steadily until it reached the same concentration as the duplicate barrel on Day 56 (**Figures 4B,D**). Regardless of the precise onset of carboxylic acid production, banana waste was rapidly converted to carboxylic acids when the pH was above 5.0 at the higher temperature (**Figures 4A,B**, **Figures S8A,B**).

Undissociated *n*-caproic acid concentrations were also higher than we have observed previously (**Figures 4C,D**). The average pH in all treatments was above the pKa by the end of this experiment, which caused <50% of the total

concentration to be in the undissociated form. However, a pH of 5 combined with the relatively high total concentrations of *n*-caproic acid resulted in undissociated *n*-caproic acid concentrations of 10.2, 13.1, and 12.6 mM for Treatments 1, 2, and 3, respectively (Figures 4C,D). The highest undissociated *n*-caproic acid concentration observed, thus far, was 7.1 mM in the 20% banana-mash treatment from Experiment 2. For Treatment 3, an unexpected trend for undissociated *n*-caproic acid was observed around Day 44 of the operating period because the total concentration of *n*-caproic acid steadily increased as anticipated, but the undissociated concentration increased between Day 42 and 44 and then decreased between Day 44 and 46 (Figure 4D). This inconsistency occurred because of non-homogenous conditions within the barrels, resulting in changes in the average pH between sampling days. The pH on Day 44 was slightly lower than on Day 46 (Figure 4D), and small differences in pH result in large differences in undissociated carboxylic acid concentrations due to the exponential relationship between the two. This result is noteworthy because it demonstrates that non-homogenous conditions within a bioreactor can result in large differences in efficacy for pathogen inactivation.

Additional Work Is Needed to Optimize Co-fermentation Conditions

The results here suggest that banana waste (or alternative food waste) co-fermentation rates should be optimized to result in a pH of about 5, which will maximize undissociated carboxylic acid concentrations by promoting a large fraction of the total concentration to be in the undissociated form, while avoiding inhibition that appears to result at pH values below 4.8. One goal of the current study was to determine whether a two-stage (Treatments 1 and 2) or one-stage (Treatment 3) process was preferred for accomplishing this goal. The final concentrations of undissociated *n*-caproic acid were similar between the three treatments, but they were also still increasing when the experiment ended on Day 56 (Figures 4C,D). With additional fermentation time, more differences may have been observed between the treatments. Therefore, it is difficult for us to make a firm conclusion about which treatment performed better. If the experiment is repeated with temperatures at 30°C during the entire experiment, fermentation should occur faster, and differences between the treatments may become apparent with less time required. When resulting performances in the one-stage and two-stage processes are determined to be equivalent, then a one-stage process is strongly preferred because it is simpler to operate and implement. Additional work should also be performed to determine optimal ratios of food waste to UDDT-SW that will result in a pH of ~5.

Ascaris Inactivation in Field-Scale Trial

We only collected *Ascaris* data at two time points (Days 11 and 29) during the field-scale trial and only during the low temperature period (Table 4). One of the duplicate Treatment 1 barrels caused lower viability

TABLE 4 | Average percent viability of *Ascaris* eggs collected for field-scale trial during Experiment 3.

Day	Percent viability (standard deviation)			
	Treatment 1	Treatment 2	Treatment 3	Autoclaved UDDT-SW
11	50.9 (17.9)	43.9 (9.1)	67.3 (3.1)	66.4 (5.0)
29	6.5 (4.9)	26.9 (12.0)	19.5 (8.8)	22.1 (8.9)

Viability is expressed as $\frac{\text{number of viable eggs}}{\text{total number counted}} \times 100$. Standard deviation of four samples (two *Ascaris* chambers in each of two replicate barrels) is given in parentheses. The baseline viability of *Ascaris* eggs used in this experiment was 69.9%.

than any of the others for unknown reasons. With the exception of this barrel, viabilities for the *Ascaris* eggs exposed in the barrels were similar to the eggs contained in tubes of autoclaved UDDT-SW. The similarity between the barrels and autoclaved UDDT-SW are supported by the slow fermentation rates observed during this time period. The barrels did not have high undissociated carboxylic acid concentrations until after Day 42 (Figures 4C,D, Figures S8C,D). Therefore, we expect that the conditions in the barrels were similar to the autoclaved UDDT-SW between Day 0 and Day 29.

The viability of *Ascaris* eggs in all of the barrels plus the autoclaved UDDT-SW decreased considerably between Day 11 and Day 29 (Table 4). Because undissociated carboxylic acid concentrations were still low at this time, we expect that the decline in viability was related to confounding factors in the UDDT-SW that were not measured here. Therefore, we did not collect additional *Ascaris* samples beyond 29 days because we recognized that we would not be able to identify the cause of inactivation or to determine the effect of undissociated carboxylic acid concentrations.

CONCLUSIONS

Several findings were made in this study that improve our understanding of how carboxylic acid fermentation can be used as part of a sanitation waste treatment system to inactivate pathogens:

1. Fermentation of UDDT-SW in combination with food waste can be used to inactivate *Ascaris* eggs in a real sanitation setting when the carboxylic acid product spectrum is shifted toward longer-chain carboxylic acids, including *n*-butyric acid, *n*-valeric acid, and *n*-caproic acid, rather than just acetic acid.
2. A specialized inoculum of carboxylic acid-producing bacteria is not necessary because the gut microbiome found in UDDT-SW is already capable of producing carboxylic acids with longer carbon chains.
3. Fermentation temperatures should be higher than 30°C to promote reasonable rates of carboxylic acid production. Lower

temperatures will result in longer required fermentation times or no carboxylic acid production at all. For waste treatment systems, long fermentation times require larger and more expensive bioreactors.

4. When easily degraded food waste, such as banana waste, is co-fermented with UDDT-SW in a way that maintains a pH between 4.8 and 5.2, we can accomplish our two main goals of: (1) producing high total concentrations of carboxylic acids; and (2) reducing the pH to cause a greater fraction of the total concentration to exist in the undissociated form. If the pH is below 4.8, then carboxylic acid production is inhibited. If the pH is above ~5.2, the fraction of carboxylic acids in the undissociated form is too low for the process to effectively inactivate pathogens.
5. The timing of food waste addition and ratio of food waste to UDDT-SW can both be optimized in a two-stage process to maintain an appropriate pH. However, for practical implementation of waste treatment systems, it is simpler to mix food waste and UDDT-SW when the fermentation is started. Therefore, an appropriate ratio of food waste should be found that could maintain the appropriate pH. This ratio will need to be adapted to individual systems based on composition and buffering capacity of the UDDT-SW and food waste used.
6. *Ascaris* eggs were inactivated faster when food waste was used to increase concentrations of undissociated carboxylic acids, but more investigation will be needed to determine appropriate operating conditions to inactivate pathogens, including *Ascaris* eggs.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

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AUTHOR CONTRIBUTIONS

LA directed the experimental design and overall concept and provided guidance to LH. DB and JL guided *Ascaris* methodology and experimental design. TE and EW assisted in design and implementation of the field-scale trial. LH designed and conducted the experiments, collected samples, and analyzed data. LH wrote the manuscript together with LA, with further revisions from DB, TE, EW, and JL.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2019.00139/full#supplementary-material>

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The Sprawling Planet: Simplifying the Measurement of Global Urbanization Trends

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Recent decades have been characterized by a rapid and steady urbanization of the global population. This trend is projected to remain stable in the future and to affect land use patterns in multiple ways. Monitoring and measurement concepts for urbanization processes have presented difficulties with the multitude of driving forces and variations in urban form as well as the assessment of outcomes of sometimes contradictory objectives for economic, social, and environmental policies. The monitoring frameworks that are employed with the aim of assessing the land use changes related to urbanization break down this complexity into singular dimensions that can be measured with individual indicators. Such monitoring allows planners and policy analysts to assess new urban growth against sustainable development criteria. Examples include compact city policies that allow urbanization to happen in suitable locations rather than laissez-faire urbanization that can happen regardless of environmental impacts and resource efficiency. In this context, we note that monitoring methods are most often designed for case studies in Europe or North America where urban structures are rather mature and consolidated. However, such monitoring can provide crucial information on urban development at a phase at which structures are currently evolving and can potentially still be modified. This is frequently the case in developing countries. Given this background, this paper presents an approach to simplifying the measurement of the land use changes related to urbanization with a new methodology. This paper condenses the needed measurement components into two dimensions: land use inefficiency and dispersion. The method can be used globally based on the newly available *Global Human Settlement* (GHS) layer that is available from the European Commission at no cost. In an initial application of the method to over 600 cities worldwide, we show the land use trends related to urbanization by continent and city size. In summary, we observe a consolidation of urban centers worldwide and continued sprawl on the outskirts. In European cities, a consolidation phase of urban structures began earlier, and cities are more mature and develop less dynamically compared to those in other regions of the world. More in-depth analyses of case studies present results for Paris, France, and Chicago, United States. In the case of Paris, the method helps to illustrate the growth pressures that led to massive

urban sprawl on the outskirts with a continued densification of the inner city. In the case of Chicago, we observe a type of urban sprawl that goes along with the waves of suburbanization with population loss in the inner city and continued urban sprawl on the outskirts that are consolidated over time.

Keywords: urban land use change, urban sprawl, density, dispersion, global human settlement

INTRODUCTION

Urbanization has been a megatrend of global land use change that can be observed in all parts of the world. By 2050 close to 70% of the global population will live in cities (Eurostat, 2016). The reasons that explain why the global population is more attracted to city life than ever have been widely discussed in the literature (see for example Adli, 2017). Many lines of argumentation suggest the so-called “urban advantage” that drives urbanization: city life is associated with better prospects for prosperity and progress, access to services, education, and amenities, as well as a richer cultural life. However, from a sustainability point of view, this promise may only hold true for the most attractive inner city locations where the distances between destinations are walkable, public transportation is good, and there is a density of people and activities result in what is perceived as urbanity. As a matter of fact most urban growth happens in the surrounding areas: We are living on a “suburban planet” and are trying to “make the world urban from the outside in” (Keil, 2017). These processes are not uniform though, the dichotomy between spatial categories has increasingly been contested over the last years. Suburbanization can have functional subcenters of high urbanity, peri-urban areas can consolidate over time to dense urban fabric, rural areas have centers with urban cores, and a high level of centrality and service quality (Hugo, 2017). The continuum between urban and rural areas becomes even more blurry in polycentric regions, where networks of cities form large metropolitan areas that exhibit a diverse set of land uses and urban functions (Danielzyk et al., 2016). It is increasingly becoming difficult for applications in urban land use monitoring to delineate functional urban areas in order to report on land use policies and related objectives. One such objective is to combat excessive forms of urban land take. This can be frequently observed in suburban areas, where the influx of people and the building activities that are needed to accommodate increased residential, industrial, and business land uses give rise to what is often labeled urban sprawl. The term “urban sprawl” has not yet been clearly defined. The term basically describes a form of urban growth whereby residential areas and social classes are highly segregated and dispersed over space, distances to amenities and workplaces can be comparatively long, and architecture lacks diversity (Galster et al., 2001; Wolman et al., 2005; European Environment Agency, 2006; Soule, 2006; Couch et al., 2007; Frenkel and Ashkenazi, 2008; Wei and Ewing, 2018). Urban theory concerned with sustainable planning has identified this form of urban growth as problematic. Expansions of urban areas should not be subject only to demand factors such as the housing preferences of the urban population or land

market conditions. Planning regimes must manage urbanization with more foresight before unsustainable and irreversible urban sprawl structures are established to prevent the impacts of inefficient and environmentally harmful urban land use (Soule, 2006). Examples of such planning include urban containment policies or regional planning approaches that work with spatial concepts to optimize land use change (i.e., transit-oriented development or nodal growth, see Calthorpe and Fulton, 2001). Evidence-based policy aims to increase urban densities along development axis and nodes of good public transport accessibility and high quality service provisions, strengthening the links between the urban core and subcenters in such concepts. Favoring urban development in such a way is complemented by development restrictions in the interspaces between development axes. The objective of spatial planning in this line of thinking is to minimize the environmental impact of land conversions to sealed surfaces and ensure good outcomes for resource efficiency, accessibility and mobility and at the same time to prevent social imbalances with mixed-use urban design and affordable housing areas. The monitoring of these processes, however, is complex, since it is almost impossible to assess the causalities of land use change between the effects of spatial planning policies and global trends. Another difficulty is that such functional relationships are difficult to assess when analyzing land use change. Dedicated indicators that measure different aspects of dispersion, density, concentration, and other features of urban land use structures are typically employed to overcome this problem (Tsai, 2005; Herzig et al., 2018).

Early attempts to conceptualize sustainable urban development have identified three main characteristics, which are referred to as the three D's of urban development (diversity, density, and design, Cervero and Kockelman, 1997). Other researchers have enhanced this view and presented measurement concepts for urban sprawl. The work of Siedentop and Fina (2010), for example, takes a similar approach and focuses on the state and trends of land use in terms of surface features (urban land use change), patterns (urban land use structure), and density (population or business density). To date, the implementation of such measurement concepts has mainly been conducted in study areas in the Global North, using advanced geodata structures and population registers to calculate indices. Most scholars agree that there cannot be one combined index to measure urban sprawl. One must approach the topic with multiple criteria and indicators that capture the complexity of the different aspects that drive the land use change related to urbanization (Frenkel and Ashkenazi, 2008; Fina, 2013).

Global measurement concepts for urbanization have been studied in a meta-analysis by Seto et al. (2011). The authors look

at 326 studies and applied four indicators of land expansion and population growth. In the results they identify the countries with the highest urbanization rates and analyzed correlations with factors like GDP growth or locational aspects. Zhang and Seto (2011) present an innovative way to display urban expansion in their research by using multi-temporal nighttime light data. This method allows for the differentiation between stable and highly dynamic urban areas, potentially defining urban growth rates for different countries. A more recently published way to present global urban growth is the *Atlas of Urban Expansion* (2019). Scientists from the New York University, the Lincoln Institute of Land Policy and UN Habitat developed an online visualization of urban land use for 200 cities worldwide. The project website contains information and visualizations of indicators suitable to differentiate between different types of urban form based on extent, density, and the composition of newly developed area. In our study we would like to conceptually advance and test such measures of urban form with a simplified approach that can be applied for multi-temporal data worldwide. Our main interest is to contribute toward monitoring concepts that detect and evaluate trends in urban development for global and regional land use dynamics.

In this context, this paper aims to use the knowledge generated by urban sprawl researchers in selected study areas and extend it to a global dataset that has only recently become available for a time series from 1975 to 2015. We use the global human settlement layers (GHSL) provided by the European Space Agency to calculate representative indices to measure urban growth worldwide. Based on these data, we establish a large database for each country with more than 5 million inhabitants. We apply the processing procedures for indicator calculations to the catchments of the cities with the highest urban land expansion rates in the observation period. To determine a valid logic for comparative analysis, we structure our sample by the selection of the fastest growing city regions in the classes of large, medium, and small cities (in terms of population size). We can thus assess some of the worldwide trends of land use change related to urbanization for growing cities. City borders are modeled using network analysis, taking travel times of up to 1 h in 15 min intervals as threshold values. This approach allows us to model catchments without arbitrary delineations of urban areas based on administrative boundaries. Monitoring applications for the largest urban regions in Germany conducted by the authors have shown that such delineation would not be realistic given the increasingly diverse mobility patterns of commuters. This is particularly true for polycentric city regions where the diversity of urban functions is increasingly becoming detached from theoretical conceptualizations of an urban-rural dichotomy (Fina et al., 2019). This methodology enables us to analyze the resulting land use characteristics in a comparative way, although some data inconsistencies explain deviations from the general logic.

These data and indicators provide the possibility of comparing the indicator results for world regions, planning regimes, or any other grouping logic in which urban researchers might be interested. However, the wealth of information is difficult to communicate and present. This paper therefore suggests

a simplification procedure for urban growth assessments that concentrates on the dynamics of two dimensions: land use inefficiency and urban dispersion. We explain how the logic of this method was inspired by the discussion of existing quantification methods for urban sprawl represented in the literature. Subsequently, we apply the methodology in global observation and in two sample implementation areas (Paris, France, and Chicago, United States), including descriptions of the city selection procedure and indicators used. For the first applications of our methodological approach, we focus on the growth of cities and portray how the urban land use change has progressed in growing cities. We discuss interpretation prospects, methodological potentials and limitations as well as concepts for further research. By doing so, we hope to initiate discussion on the value of such a monitoring approach. In addition, the suggested approach could be a contribution to the spatial monitoring. Our two dimensional approach simplifies the measurement of urban form and provides policy makers and planning practitioners new information about regional and urban development trends, especially in terms of sustainable city development (United Nations, 2018).

BACKGROUND

The growth of urban land use is a worldwide trend that threatens a range of ecosystem functions through the loss of vegetation and biodiversity, habitat functions, agricultural resources, and soil (Hasse and Lathrop, 2003; Haase et al., 2018). Such growth conflicts with climate change mitigation strategies in coastal locations that are susceptible and vulnerable to the effects of sea level rise and the higher frequency of extreme events (McGranahan et al., 2007). The actual impact of these effects varies; geographic conditions and specific sets of drivers of urban growth on global, regional and local scales as well as regulatory planning each play a role. There is a large consensus in the research community that some cities are more successful in terms of urban growth management than other cities (Fregolent and Tonin, 2015). In this respect, Tosics et al. (2010) classified the regulatory regimes of Europe in terms of land use controls and related it to the topic of urban growth. The authors basically consider the governance system to be either fragmented or consolidated and the planning policy system to have either strong or weak control at the regional or national governance levels. The findings of that study are instructive in comparing current planning systems across Europe but do not relate the findings to actual assessments of land use change in terms of urban growth. The influence of planning thus remains unknown, with some authors arguing that it may not be sufficiently effective to remedy the power of other driving forces in the context of urban sprawl. In this context, Angel et al. (2011) found that despite all planning efforts to control urban sprawl, such as compact city policies and urban growth management initiatives, urban land use change will continue to increase worldwide. The main reason for this increase is a rise in living standards that goes along with more land consumption per person. It is very rare that citizens dispense with less space for living and activities over time, although quite

a few initiatives promote this way of thinking where high inner city density is proclaimed to be a factor for quality of life (see for example the OECD compact city policies, Organization for Economic Cooperation and Development, 2013). Besides the problems of decreasing densities in growing cities, Wolff et al. (2018) detected this process also in shrinking urban areas. As the demand (e.g., for infrastructure) decreases, shrinking cities are more challenged with dedensification than their growing counterparts. These efforts, however, are outmatched by a strong global trend for more land consumption. The most recent population prospects published by the United Nations shows that the main driver for more urban land, population growth, is expected to bring the global population from 7.7 million people in 2019 to 9.7 million in 2050, a 26% increase. This growth is expected to occur with varying rates across world regions, ranging from 99% increase in Sub-Saharan Africa to only 2% in Europe and North America. As a matter of fact, more than half of the projected growth is expected in only nine countries: India, Nigeria, Pakistan, the Democratic Republic of the Congo, Ethiopia, the United Republic of Tanzania, Egypt, and the United States of America. In contrast, 27 countries or areas are already experiencing population decline today, including the most populous country, China. Fertility rates are decreasing worldwide, so that population growth can actually reverse over the generations. The increase of life expectancy compensates for some of this dynamic, and some countries will see a high increase in elderly people over the next decades (United Nations, 2019).

With regard to the consequences of population growth for urbanization, any regulatory approach must be based on robust information about the current state and development trends of urban land use change. Beginning in the 2000s, a line of research focused on this topic with studies that have given considerable research attention to land use dynamics. Cervero and Kockelman (1997) showed how to identify the influential factors of urban form with a view toward travel demand. The landmark study by these authors on the influence of the “density,” “diversity,” and “design” of urban neighborhoods (the so-called “three D’s”) inspired subsequent studies to work with multiple indicators to quantify urban land use patterns. In this line of thinking, authors such as Galster et al. (2001), Wolman et al. (2005), and Ewing and Rong (2008), established theories and measurement frameworks for urban sprawl assessments in the United States. These studies consider the drivers of urban sprawl and acknowledge the fact that the assessment of urban areas is very complex in terms of urban land use change. For example, Galster et al. (2001) identify eight criteria (density, continuity, concentration, clustering, centrality, nuclearity, mixed uses, proximity) related to land use and its distribution over an urban area for urban sprawl assessment in the United States. The criteria can be measured individually and are used in that study as inputs for a factor analysis to apply a form of multicriteria assessment for the ranking of US cities. Such rankings are useful for comparative analysis of development paths between cities but provide no normative information on the success of planning interventions or the criticality of urban sprawl in terms of its impacts. For such purposes, time series in monitoring frameworks dedicated to the testing of policy interventions are

needed. In this context, the European Environment Agency inspired further research with a technical report on urban sprawl in 2006 in which it identified the challenges of measuring urban sprawl with a matrix of drivers that are differentiated by topic (land, transport, governance, economy, society) and scale (global, regional, local) (European Environment Agency, 2006). Subsequent state-of-the-environment studies adopted these ideas by applying a so-called pressure-state-response model to the assessment of undesirable land use changes, resulting in rather alarming qualitative assessments of urban sprawl perspectives for the near (five-plus years) and more distant (20-plus years) future (European Environment Agency, 2011, 2015). The measurement approach considers the interrelationships among the current state of land use, the driving forces that exert pressure on the system (for example, population growth), the actual impacts on ecosystem functions (in a normative assessment), and the effects of policies. Other work that was later taken up by the European Union to report on landscape fragmentation with a view toward urban sprawl adopted measurement methods based on landscape metrics. The aim was to assess the configuration and patterns of urban land use using geographic information systems (Siedentop, 2005; Jaeger and Bertiller, 2006; Jaeger et al., 2009; Siedentop and Fina, 2010; European Environment Agency, 2011; Fina, 2013; Behnisch et al., 2018).

Our interpretation of the literature is that the US literature seems to focus more on economic urban functions and their resource efficiency within cities (e.g., Galster et al., 2001) whereas the European literature is more concerned with the environmental impacts of urban sprawl (e.g., European Environment Agency, 2006). However, there is no clear research agenda that explains this observation, and there are certainly many overlapping areas. A range of indicators are presented in the literature to measure the degree of urban sprawl that are applied to geographic datasets on different scales, from a binary view of urban vs. non-urban land, to the study of the building blocks of different land uses, to analysis at the level of street addresses and types of households and enterprises. There are too many indicators to discuss in this paper. However, the common denominator of most measurement concepts is the use of selected indicators that represent different dimensions of urban land use change. In this context, the study by Galster et al. (2001) mentioned above was able to operationalize eight dimensions based on census block data in the United States. Such data structures are not available for international comparative analyses on such a detailed level. Some authors have therefore employed simplified measurements, grouping the dimensions with the most essential indicators. A research group from Switzerland worked with only three indicators to determine the amount of urban structures in a study area (“degree of urban permeation”), their locational setup (“degree of urban dispersion”) and the intensity of use that might justify a higher urbanization level (“sprawl per capita”; Jaeger et al., 2010). These authors combined the indicator values for their study area to only one measure (“total sprawl”). Frenkel and Ashkenazi (2008) work with three dimensions of urban sprawl (“density,” “scatter,” “mixture of land uses”), to which they refer as configuration and composition parameters. The indicators to operationalize these

dimensions are as follows: population density, irregularity of the shape of the central built-up area boundary, fragmentation, land-use segregation, and land-use composition. Both of these studies require datasets that are not available everywhere. Similarly, a study from an environmental government institution in the German state of Baden-Württemberg suggested a measurement based on three dimensions that can be translated as density, efficiency, and quality of urban land use. This concept places explicit emphasis on indicators that detect changes over time in addition to indicators that measure the state of urban land uses at the beginning of an observation period (Landesanstalt für Umwelt, 2007).

Another example is the theoretical framework by Siedentop and Fina (2010) (see **Supplementary Figure 1**). This framework differentiates three dimensions of urban sprawl as being subject to singular indicator assessments. These dimensions include (1) surface characteristics, such as the amount of urban land use and its increase over time, the sealing degree of different urban land uses or the amount of urban green spaces and mixed land use functions. The resulting land use pattern (2) is the second dimension and addresses the configuration and position of different land uses toward one another as being dispersed or compact, fragmenting open space or forming planned and optimized structures, such as transit-oriented development or nodal growth. Urban density (3) considers the number of users in a given residential population, for example, based on their reliance on certain urban land uses, such as public transportation. In that case, more users would create a higher demand for such services, which would improve cost-efficiency.

The framework attributes certain impacts of urbanization to the sphere of influence of these dimensions (e.g., the loss/degradation of farmland, urban heat islands). Subsequent attempts to operationalize this concept have resulted in a challenging demand on data availability and time series consistency. The indicators that were implemented as representative are urban density; change in urban density; greenfield development rate (dimension: density); effective share of open space; patch density; mean shape index; openness index (dimension: pattern); and share of urbanized land and new consumption (dimension: surface). A full description would exceed the scope of this paper; however, references can be found in Siedentop and Fina (2010). Nevertheless, it is important to note that the pattern indicators are especially complex study objects in themselves. For example, the effective share of open space as a measure of landscape fragmentation is employed to reflect the value of habitat size for the health of flora and fauna. The resulting value is higher for large habitats and smaller for small habitats based on complex procedures to geographically extract habitat sizes among urban areas for a given study area and to assess the remaining connected size (Ackermann and Schweiger, 2008). The important aspect to note here is that many of the indicators presented in these studies can only be operationalized in administrations with advanced capacities to provide detailed geographic objects on urban land use and statistical data (e.g., on population development) on small-scale urban units. The results are convincing and promising; however, they cannot be extended to other regions with limited data

availability. From this viewpoint, it is regrettable that monitoring methods are being designed in regions of the world with advanced data structures in rather mature and consolidated environments that cannot be easily applied where they are needed most, namely, in regions where urbanization is still very dynamic and information on the alarming trajectories of land use change could be used in crucial decision-making processes and adjustments to land use policies. In this context, Wei and Ewing (2018) recently published a call for new research efforts on the topic with a view toward capturing the variety of urban land use change worldwide. The following sections present a dataset that we tested in this context. These are worldwide data, and they provide a consistent time series from 1975 to 2015.

MATERIALS AND METHODS

Land Use and Population Data

We use the GHSL as our main source for the analysis. This source, which is based on Landsat satellite and census data, is a dataset that is available worldwide. This dataset covers built land areas (built-up layer) and population data (population layer) for 1975, 1990, 2000, and 2015 (Pesaresi et al., 2013). The GHS built-up layer, with a high resolution of 38 m, provides the opportunity to monitor changes in urban land use and the resulting patterns using indicators that are applicable in a global context. In addition, we have information on population development from the GHS population layer on a 250 m cell size so that we can report on the demographic impact on urban growth. This data is available for 1975, 1990, 2000, and 2014 and stems from population estimation and disaggregation models (Freire et al., 2016). There are some caveats that come with the use of the dataset, although we generally assess it to be a unique source for the observation and analysis of global urban developments in the future. In their research, Pesaresi et al. (2016) described challenges and problems with the GHSL and its validation. A relevant issue for our study is that, due to a lack of available image data for 1975, there are problems in identifying built-up areas for this year. As the information of population is a result of the census data and the built-up layer, we also expect inaccuracies in the population dataset (see for the example of the German city of Pirmasens in **Supplementary Table 25**). We documented such inconsistencies found during data processing in the population (**Supplementary Table 25**) and in the remote sensing data (**Supplementary Figure 2**). This led to an exclusion of possible cities to analyse and to a drop in sample size (see **Supplementary Table 3**).

City Selection Procedure

To find a representative dataset of the most growing cities from different world regions, we defined a specific selection procedure. In a pre-analysis step, we used a 60 km buffer around every city centroid as a typical catchment for cities. The source is a point dataset provided by Esri and Garmin (Esri World Populated Places), which contains a centroid for the administrative boundaries of every city with more than 50,000 inhabitants. This distance can generally be managed in 1 h travel time by car, which represents a maximum commuting

time in Europe (Eurostat, 2019). We are aware that this not applicable for every country or city, but we need equal distances for our selection procedure. In comparison to the delimitation of functional urban areas of the urban audit, which includes employment and population data, we could identify similar catchment areas for the larger cities (Eurostat, 2013). As the research focus is set on cities with increasing urbanized areas, we concentrate on two kinds of city: those with most absolute growth and most relative growth in built-up land in city regions. The calculation of growth makes use of the GHS change layer of built-up areas and identifies the time period with the largest values from 1975 to 2015. Considering the fact that the most populated cities are often also the most growing cities, we decided to divide cities into three size classes: category A (over 500,000 inhabitants), category B (100,000–500,000 inhabitants) and category C (50,000–100,000 inhabitants). The information about the population classes is also part of the Esri world populated places with 2017 as reference year.

We also applied two filtering steps. The first approach eliminates the dataset entries for smaller countries, giving effect to the assumption that the growth of cities in small countries, such as Liechtenstein or Luxembourg, is highly influenced by neighboring metropolitan regions in other countries and does not provide the number of cities to present a solid selection base (e.g., small island country states, such as Samoa or Cape Verde). As a result, we excluded countries with fewer than 2 million inhabitants from this part of the research. To avoid agglomeration and overlap effects in polycentric urban regions—such as in the Ruhr area in Germany or the east coast of China, we implemented the second filtering rule: the cities of category A are not subject to any special restrictions. The cities of category B cannot be in a radius of 60 km from a city of category A. We dropped the cities of category C from the sample if they are in 60 km proximity to a larger city from categories A and B. Excluded cities remain part of the larger city region. Subsequently, the sample for a specific city category is filled with the next entry in the ordered list of growing cities in its category. For example, if a city of category B is part of the catchment of a city from category A, it will be part of the analysis for the larger city and will no longer be considered individually. Then, the city that is next in rank in category B is taken into consideration for spatial analysis. In its final state, the database contains a maximum of six cities per country, with the exception of smaller countries where many cities are geographically close to one another and affected by the application of filtering rules. As a result, some city categories are missing in such countries. For a total overview of all of the selected cities (see **Supplementary Table 4**).

Table 1 shows the resulting cities for the example of France. Of the largest cities, Paris had the highest absolute growth in hectares (4,614 km² from 1975 to 2015), which is certainly due to its extraordinary size compared to the next-largest cities. However, in relative terms, Reims had a higher growth rate in percentage terms (150% from 1975 to 2015). For cities with 100,000–500,000 inhabitants, Lille had the highest absolute growth rate, Avignon had the highest relative growth rate. In category C (under 100,000

TABLE 1 | Cities with the highest urban development dynamics in France, by population class (in brackets: absolute and relative change of urban land).

City/growth	Absolute	Relative
A (more than 500,000 population)	Paris (4,614 km ²)	Reims (150%)
B (between 100,000 and 500,000 population)	Lille (3,554 km ²)	Avignon (221%)
C (under 100,000 population)	Saint Quentin (1,208 km ²)	Beziers (271%)

inhabitants) Saint Quentin had the highest absolute growth and Beziers the highest relative growth.

Processing and Analysis

Our logic for delineating catchments for cities uses travel time areas from the city centers as its core element. This approach reflects the functional relationships between the city center and its surroundings better than linear rings or squares. Processes of urbanization (e.g., sub- or re-urbanization) and related phenomena (i.e., commutersheds) rely on street networks. Indicators that measure such processes are therefore better calculated in alignment with network geographies. In particular, the analysis of urban growth will provide more realistic and comprehensible results in this context. Topographic features such as mountains, water surfaces, forests, or conservation areas are usually not accessible by road and were therefore intentionally excluded from the research area. If frozen boundaries were used, inaccessible or unconnected areas would also be part of the analysis. However, defining the catchment areas by travel time polygons also contains some deficiencies. Although indicators for cities can be compared, they are based on different geometry sizes in contrast to static squares or circles. We have to consider this in the interpretation of the results.

The resulting polygons represent so-called isochrones (polygons of equivalent travel time for the centroid) or spatial units such as those shown in **Figure 1**. The travel time polygon for the city of Innsbruck in Austria runs through the valley locations to the east, south, and west. Northern parts in the alpine valleys are more difficult to reach and thus take longer. Another example is shown on the right-hand side, which is located in the desert. The isochrones of Hafar al Batin in Saudi-Arabia span the first two rings, the outer rings are singular radial roads, which are officially labeled as highways, and other parts are not accessible by sealed roads (excluding dirt tracks). Unaffected by any topographic restrictions, the city of Dusseldorf in Germany approximates circular travel time rings, albeit with some deviations.

For our analysis of individual cities, we calculated isochrones for car travel times of 15, 30, 45, and 60 min to the city center. Data basis for the analysis is the ESRI ArcGIS Online network analysis services, which delivers “up-to-date” road data by HereMaps. With an almost worldwide available routing network, this source ensures our requirements for the analysis with a given reference year of 2018 (Esri, 2019). We use the resulting isochrones for all observation years to make the results comparable. For the application on the global level in section

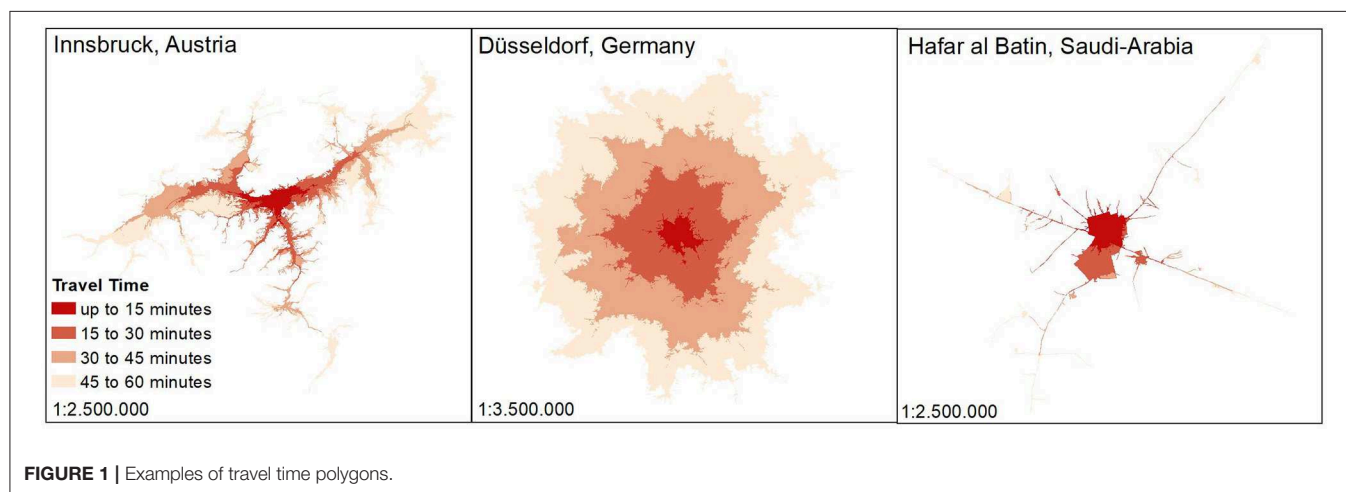


TABLE 2 | Overview of indicators and their calculation (gray = main indicators).

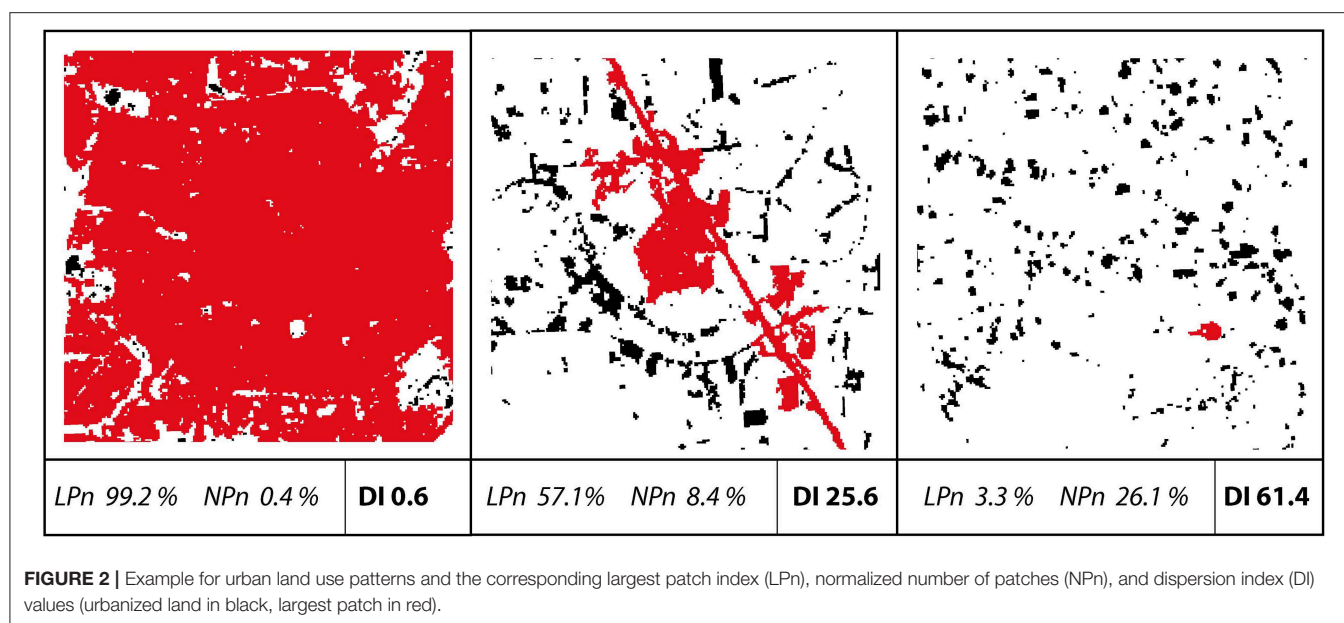
Indicator	Range	Calculation
Growth rate of built land per year (n) (BuiltGR)	$(-\infty)$ to $(+\infty)$	$BuiltGR = \left(\sqrt[n]{\frac{Start\ Built}{End\ Built}} - 1 \right) \cdot 100$
Growth rate of population per year (n) (PopGR)	$(-\infty)$ to $(+\infty)$	$PopGR = \left(\sqrt[n]{\frac{Start\ Population}{End\ Population}} - 1 \right) \cdot 100$
Land use inefficiency (LUI)	$(-\infty)$ to $(+\infty)$	$LUI = BuiltGR - PopGR$
Dispersion index change (DIC)	-100 to $+100$	$DIC = End\ DI - Start\ DI$
Dispersion index (DI)	0 to 100	$DI = \frac{NPn + (100 - LPn)}{2}$
Largest patch (normalized) (LPn)	0 to 100	$LPn = \frac{LP - \frac{\sum_{j=1}^n a_{ij}}{1}}{100 - \frac{\sum_{j=1}^n a_{ij}}{1}} \cdot 100$
Largest patch (LP)	0 to 100	$LP = \frac{i=1}{\sum_{j=1}^n a_{ij}} \cdot 100$
Number of patches (normalized) (NPn)	0 to 100	$NPn = \frac{NP - 1}{(\sum_{j=1}^n a_{ij}) - 1} \cdot 100$
Number of patches (NP)	0 to $(+\infty)$	$NP = NP\ (absolute)$

Global Observation, we simplify and aggregate the database by reducing the research area to a single catchment ring per city category. The travel time used to create the rings relies on city size. For the cities of category A (over 500,000 inhabitants), we assigned a travel time of 45 min; for the cities of category B (between 100,000 and 500,000 inhabitants), we assigned a travel time of 30 min; and for the cities of category C (<100,000 inhabitants), we assigned a travel time of 15 min. Restrictions such as toll roads or unpaved roads are ignored for this purpose because they prevent general accessibility and would have yielded unrealistic and complex isochrones, including islands, and possibly disconnected polygons. Such restrictions are therefore set to the minimum in the software we used, the ESRI ArcGIS Online network creation tool. Excluding toll roads or unpaved roads also leads to better results in the US and rural

parts of Asia and Africa, where a large number of roads are actually unpaved. The only restriction we considered is the use of ferries to avoid areas that are inaccessible by car. Despite its good usability, there are some limitations in the use of *Esri* service areas. In general, there are problems in countries, such as North Korea, Afghanistan or Yemen, where the street network data do not seem to be very reliable and yielded inconclusive results (mainly visible in the form of the resulting polygons). In addition, there are potential problems when calculating drive time areas in smaller cities in Asia, Africa and South America, most likely also due to deficiencies in the available street network data. Countries and cities with inconclusive results had to be excluded from the city selection to avoid unrealistic indicator calculations. It is important to note that especially smaller cities in the named continents have outer rings with smaller areas. We suspect that the definition problems in street hierarchy and connectivity in rural areas are responsible for this issue. In the interpretation of the results, it is crucial to check the forms of the catchments to reflect the topographic conditions in the area. It is also important to note that the isochrones are valid for the recent past when the street network we used was in place. Therefore, for the previous years of our observation period, we may have potentially overestimated accessibility. From a methodological point of view, this is a necessary specification and provides a solid framework for the analysis of trends from the past. For future monitoring purposes, such fixed spatial units could impose restrictions when excessive growth renders these polygons outdated.

Indicators

As shown in the literature review the measurement and analysis of urban land use trends requires a multitude of indicators. According to the framework depicted in **Supplementary Figure 1**, multiple indicators are selected to represent the three dimension of growth (see **Table 2**): change of land surface characteristics (dimension 1), change of urban density (dimension 2), and change of land use patterns (dimension 3). The indicators we selected for our study can be implemented with the GHSL. With this background, we



condensed the three dimensions of urban sprawl to only two dimensions: the dimensions of land use (in-) efficiency and dispersion.

- Land use inefficiency: we borrowed this concept from a range of reporting tools on urban form but could not identify who published the idea first¹; however, it is a simple comparison of population growth vs. urban area growth. If the population growth is higher, the urban footprint will become denser. If the population growth is much lower, the urban structure will become less dense, which is typical for urban sprawl. This dimension therefore measures the economic use of land resources over time in light of population development. This approach is sometimes labeled as land use efficiency, a term we adopt but invert the logic in terms of land use inefficiency to make our results more accessible in light of the indicator values. Land use efficiency is also an indicator in the United Nations Sustainable Development Goals and therefore highly relevant for policy formulation (Number 11, see United Nations, 2018; Florczyk et al., 2019). Put simply, land use inefficiency covers the dynamics of the surface and density dimensions.
- Dispersion: we monitor and assess the pattern dimension with the changes observed for the so-called “dispersion index.” The methodology for this indicator was first presented by Taubenböck et al. (2018) with a very simple binary analysis of settlement and non-settlement areas. Dispersion index derives two spatial metrics from these land use classes, the largest patch, and the number of patches (McGarigal, 2015), and it positions these metrics in relation to one another. The share of the largest settlement patch in the entire area represents

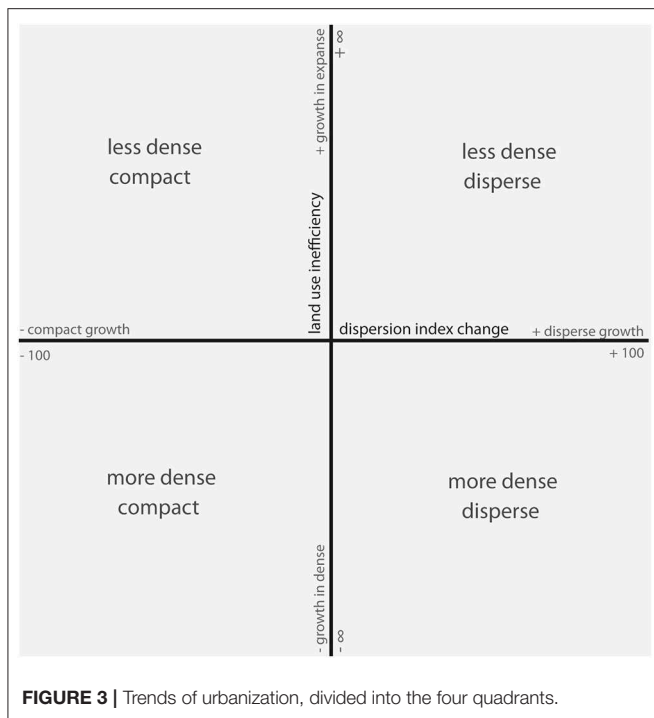
the dominance of one patch in a landscape. The number of patches equals the total number of all patches in the landscape. By normalizing the values of the number of patches and the largest patch, we obtain equal ranges from 0 to 100 (see Figure 2). Low values indicate a compact settlement structure, and high values indicate dispersion.

Based on these indicators, we can portray the development of a city in a two-dimensional matrix. On the y-axis, we translate the surface and density dimension of our measurement concept into a value for the land use inefficiency as a result of land consumption and population growth in a given time period. If the population and urban land use grow at similar rates, the land use inefficiency remains constant (see Figure 3). Urban land use change that exceeds population growth considerably is an indication of wasteful land use management and a decrease in urban density (“less dense” in Figure 3, upper two quadrants). If the population grows much more than the urban land use, we obtain densification and higher efficiency (“denser,” lower two quadrants).

In other words, land use inefficiency measures the difference of built-up area growth in relation to population growth and indicates whether urban density is increasing or decreasing. The x-axis, in contrast, is defined by the development of the dispersion index over time. If urban areas grow from a very patchy (or “sprawling”) condition to more compact structures, the values will be negative (“more compact,” two quadrants toward the left). Accordingly, if new patches of urban land are built in isolation from existing urban areas, the dispersion (and urban sprawl) will increase (positive values), placing the value of the dispersion index change further to the right (two quadrants on the right-hand side).

Based on this idea, we can now depict the development of urban areas in terms of land use inefficiency and the change in the dispersion index in combination. A positive land use inefficiency

¹See for example the planning tool “Vitalitätscheck” [vitality check] for rural settlements in Bavaria, Germany at <http://www.stmelf.bayern.de/landentwicklung/dokumentationen/059178/index.php> (March 31, 2019).



value refers to less dense growth with a higher consumption of land; a negative land use inefficiency value refers to denser growth in the particular period. The objective of this approach is to portray the urban land use changes for the three time periods from 1975 to 1990, 1990 to 2000, and 2000 to 2015. To present the results on the full time series (1975 to 2015), we use the geometrical mean as the average of the growth rates between single time periods (e.g., 1975–1990, 1990–2000, 2000–2015). The complete mathematical definitions of the single indicators required to fill the land use inefficiency and dispersion index change matrix is shown in **Table 2**.

RESULTS

In the first step, we apply the introduced methodology on a global observation level. For this purpose, we work with only one catchment ring, simplifying outputs to one value for a city per year, categorized by city size classes.

Global Observation

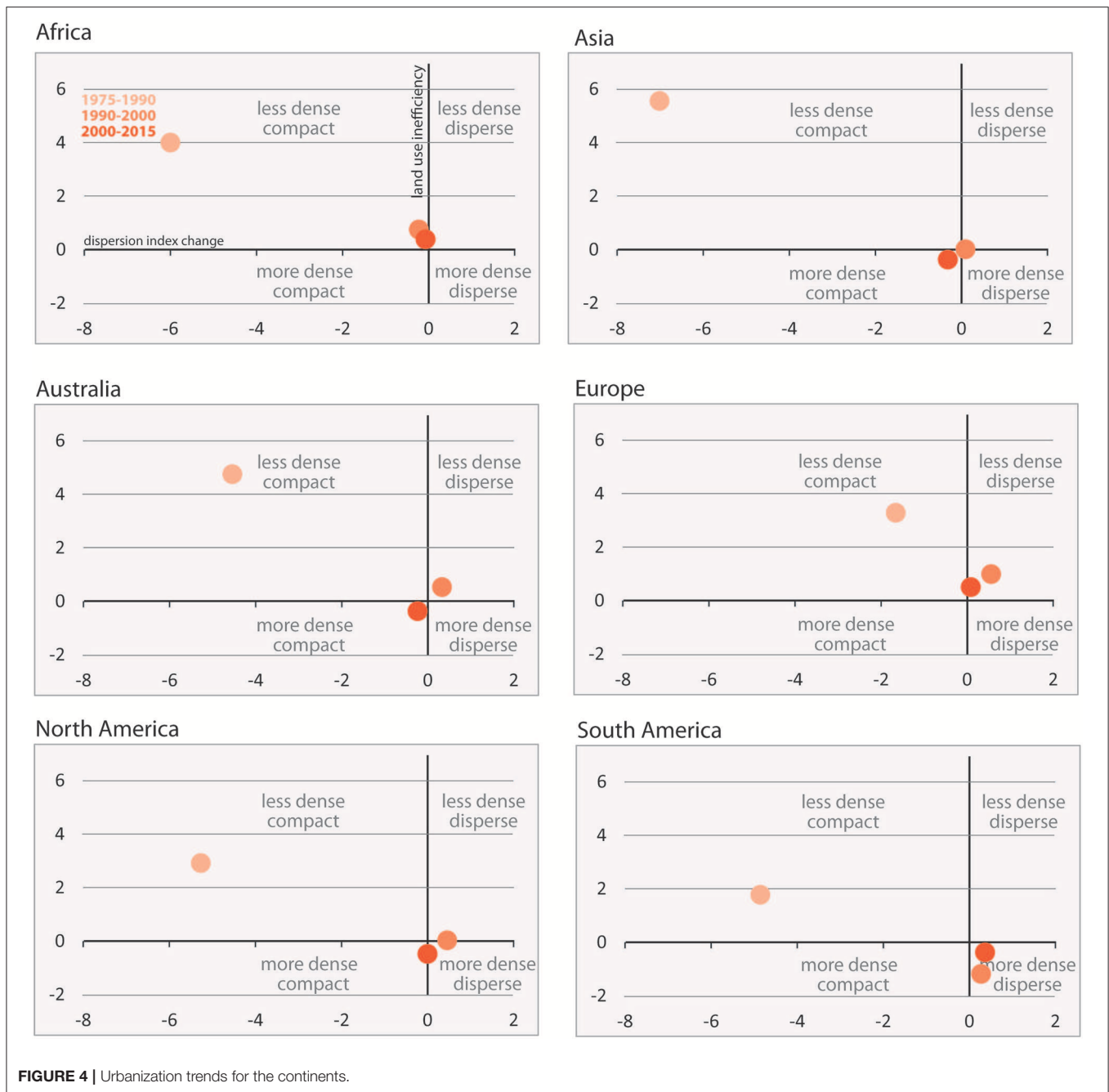
The overview in **Figure 4** shows the average values for urbanization trends by continent, taking the mean indicator values for each city type. The general development trajectories of urban land use change are comparable. Every continent exhibits a highly dynamic, less dense, and compact growth in the first period with some specific deviations. The growth development in Asia is more intense than, for example, in Europe, Australia and Africa. North and South American cities are characterized by a development compaction where the existing sprawling structures have been filled with new built-up areas over time; thus, urban footprints consolidate over the observation period

and become denser. The time periods from 1990 to 2000 and 2000 to 2015 suggest that the growth dynamics on every continent have accelerated. Differences can be observed when examining the location of the darker red points in the quadrants of **Figure 4**. The growth dynamics in African cities do not lead to the same densification as on other continents, i.e., the land use inefficiency increases on the y-axis. At the same time, the growth curve in Asia moves across the quadrants to the 3rd quadrant, illustrating a compaction and densification of the urban footprint. In Australia, built areas change from being less dense and dispersed to denser and more compact. European urbanization since 1990 is characterized by a starting point that is already quite consolidated, i.e., closer to the zero points of the x- and y-axis in **Figure 4**.

The above findings are typical for mature urban development with comparatively slow dynamics; however, the results show urban areas steadily becoming less dense and more dispersed due to fewer people on average using and increasing amount of urban land. In North and South America, the growth patterns can be described as becoming denser but dispersed over time, with a slowing dynamic in the most recent observation period from 2000 to 2015. In this context, it would be of great value to enhance this analysis with ring structures, which we demonstrate in further analyses for Paris and Chicago in the next section. The presence of rings around the inner city allows for a differentiation of urban structures from the historical core to the latest phases of urban extensions and for the monitoring of the land use inefficiency and dispersion dimensions for each ring. This analysis could be conducted for all of the cities in the sample if the validity of the measurement concept can further be substantiated in future studies.

For the time being, we would like to highlight that the interpretation of the results presented here follows the movements of points from paler shades of red to the location of the darkest red point. This dark red point symbolizes the end of our observation period. The values very close to the zero point of both axes show a decreasing dynamic that we interpret as a mature level of consolidation for urban development. The analytical value of this observation lies in the future monitoring of these points across the four quadrants. Once policies for urban containment and land saving densification are in place, one can detect progress toward policy objectives using this method.

In order to examine the difference of indicator results between the continents we apply the Kruskal-Wallis-Test (Vargha and Delaney, 1998) to all city regions in the sample, grouped by continent. The calculations were done in the SPSS statistical software package. Complete tables for the summary results presented here can be found in **Supplementary Tables 5–24**. If the value of the asymptotic significance p of the test is <0.05 , there is a difference in the central tendency, otherwise the sample is homogenous. We computed these numbers for the various time periods and the two indicators. If there is indeed a difference, a *post-hoc*-test determines which continents exhibit significantly different values. In such cases we use the Dunn-Bonferroni-Test df (Cohen, 1992; Dinno, 2015). The results are presented in **Tables 3, 4**, including the Qui-Square values as context information.



In the first time period the values for both indicators in the sample are not significantly related, the urban development paths are therefore heterogeneous. The pairwise comparison in the *post-hoc*-test shows that this result is mainly due to the large difference between urban development paths of South America in comparison to Asian city regions. The medium effect size of $r = 0.45$ shows that the land use inefficiency deviates significantly, the data shows that it is Asian cities that densify over time, South American cities much less. The heterogeneity of the dispersion index change can mainly be attributed to the difference between Asia and Europe ($r = 0.32$,

medium effect size) and Asia and Africa ($r = 0.32$, medium effect size). In contrast, the continents in the subsequent periods are homogenous with regards to the dispersion index change. For the land use inefficiency we can also identify differences in the central tendency in the more recent observation periods. For 1990–2000, we see a multitude of relations that emphasize the inequality of urban development paths across continents. The effect size is small and varies between 0.23 and 0.29. In the last period the inequality can mainly be attributed to the difference between Europe and North America ($r = 0.23$, small size effect), Europe and South America ($r = 0.29$,

TABLE 3 | Results of the Kruskal-Wallis-test (continents).

	1975–1990		1990–2000		2000–2015	
	Land use inefficiency	Dispersion index change	Land use inefficiency	Dispersion index change	Land use inefficiency	Dispersion index change
Qui-square	17,566	32,185	41,513	2,223	29,345	2,149
Dunn-bonferroni-test (df)	5	5	5	5	5	5
Asymptotic significance (p)	0.004	0.000	0.000	0.817	0.000	0.828

TABLE 4 | Results of the Kruskal-Wallis-test (city-size).

	1975–1990		1990–2000		2000–2015	
	Land use inefficiency	Dispersion index change	Land use inefficiency	Dispersion index change	Land use inefficiency	Dispersion index change
Qui-square	22,873	4,245	29,605	5,759	28,131	14,173
Dunn-bonferroni-test (df)	2	2	2	2	2	2
Asymptotic significance (p)	0.000	0.120	0.000	0.056	0.000	0.001

small size effect) as well as Europe and Asia ($r = 0.21$, small size effect).

In addition, we can aggregate the data on the city size level with the same methodology as above. **Figure 5** presents the results for the three city categories A, B, and C. The results are similar to the comparison by continent. From 1975 to 1990, the growth dynamic was higher in the smaller cities. The cities in categories B and C develop in a less dense and compact fashion on average at this point as well as in the succeeding time periods. The largest category, cities over 500,000 inhabitants, moves from the quadrant with less density but a compaction of development (upper left) to the denser but dispersed quadrant on the lower right. We interpret these effects as maturing urban development where urban sprawl in the 1980s was followed by a densification and consolidation of suburban areas in the more recent observation periods.

Similar to city region groupings by continent we also conducted the Kruskal-Wallis-Test for city region groupings by city category. For the dispersion index change we identified homogeneity in the sample for the first two decades. However, the asymptotic significance value p for 1990–2000 is close to the level of significance and in the last period it falls under the threshold. This is due to major differences between cities of city category A and the smaller city categories, effect sizes are small. In contrast, we see a continuous heterogeneity between the size classes in terms of land use inefficiency. In all time periods we determine differences in the central tendency between major cities (City A) and the smaller cities (City B and C). Checking for the position of the city size. A symbol in the matrix of **Figure 5** which is closer to the x and y axis we can conclude that large cities over 500,000 population have experienced less dispersion and dedensification than smaller cities in the observation period.

Urbanization Trends for Selected Cities

To apply this new methodology for individual cities, we selected Chicago and Paris. Both cities have similar populations (city

category A), and they show the largest increase of total urbanized land in their respective countries, the United States of America and France (for more detailed information on city statistics see **Supplementary Tables 1, 2**). In addition, we can reflect on the urbanization trends for these two cities based on a rich body of literature on their historical development and influential factors (e.g., Dear, 2001; Hudson, 2006; Angel et al., 2010). Such knowledge is valuable in testing new measurement concepts such as the one we present here.

Urbanization Trends in Chicago

With over 2 million inhabitants in the core city and over 9 million in the metropolitan area, Chicago is one of largest cities in the United States. Located at the Western edge of Lake Michigan, Chicago is subject to specific growth conditions that have been a prominent subject of research in urban theory for a long time (Dear, 2001). Waves of suburbanization and reurbanization have changed and enlarged the outskirts of Chicago and have placed pressure on land resources and changes in land rents (McMillen, 2003; Hudson, 2006). To measure the resulting growth patterns since 1975, we have delineated the expansion area toward the west with travel time rings formed as semicircles around the city core, not covering the water area of Lake Michigan. **Figure 6** shows that the main areas of rings 1 and 2 were covered by built-up land before 1975. Urban land built between 1975 and 1990 closed the gaps in rings 1 and 2. Urban land extended toward the existing built-up areas in rings 3 and 4. New urban land that was developed after 1990 is mainly located in the outer two rings. Some parts extended new settlement areas in ring 2.

Figure 7 illustrates urban land use change in Chicago for the three time periods subdivided into the different rings (graphs on the left). To put the values into context, additional graphs on the right show the development of built-up areas (green graph) and population growth (red graph) since 1975. The values are indexed and normalized for the starting value in 1975, which is set to 80. Subsequent developments show the deviation from 80

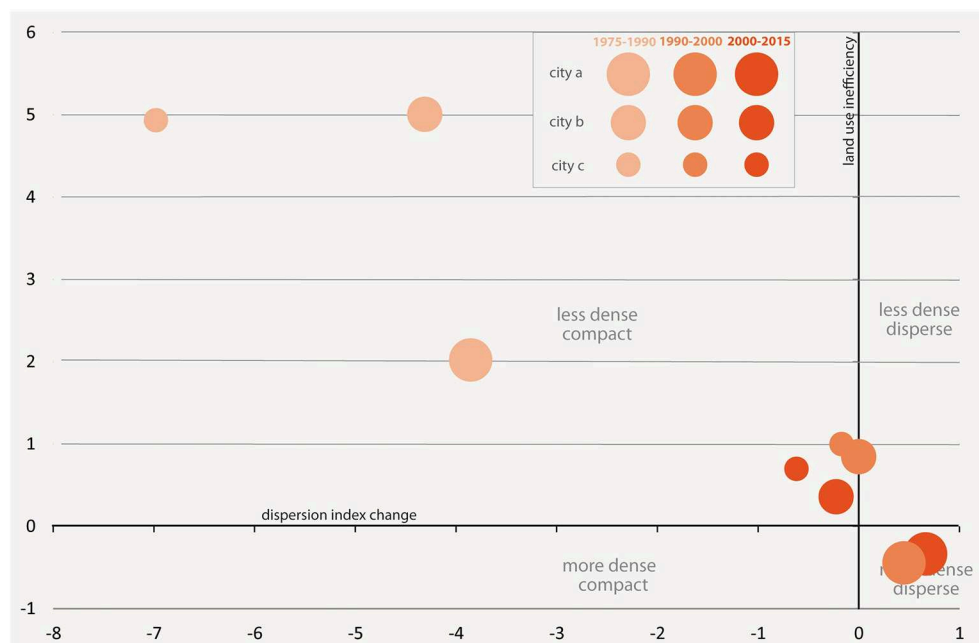


FIGURE 5 | Urbanization trends by city size.

for the different years after 1975 on the x-axis. This information is complemented by the absolute change of the dispersion index (dark yellow graph) from 1975 to 2015. It is important to note that all of the values represented here are calculated as change rates based on the starting point in 1975.

The resulting graphs on the left-hand side show that the inner core ring represents high growth dynamics in the first time period from 1975 to 1990. This trend reflects a compaction of urban form with a loss of urban density and can be seen as a consequence of the strong suburbanization in this time period during which large shares of the affluent population fled the inner city problems of industrial pollution and crime in deprived neighborhoods (also known as “white flight”) (Boustan, 2010; Boustan and Margo, 2013). The following two periods indicate a further consolidation of the built-up area in the first ring with a decreasing value for the dispersion index change. The decrease in population in the city core continued, leading to a less dense settlement structure during the whole research period. Similar trends can be seen in ring 2. From 1975 to 1990, the total built area increased by ~50 %, while we find no significant changes in the population numbers and a strong decrease in the dispersion index change. Development is found to be less dynamic in the following time periods. We can see a form of stagnation from 1990 to 2000 and decreasing urban density in the last time period due to a shrinking population base. The exterior rings 3 and 4 are similar in their development paths.

Overall, we determine an increasing population in these zones, extraordinary growth rates of built-up land that are typical of suburbanization (graphs on the right-hand side) and shrinking dispersion from 1975 to 2015. Here, the development path during the first period was less dense and very compact.

This development is a reflection of continued development in formerly dispersed land use patterns that are typical for the outskirts of large cities. In both outer rings, the second time period from 1990 to 2000 shows a temporal increase in dispersion until 2010, probably a result of newly developed built-up land in isolated locations. However, this trend reversal does not continue in the last given period from 2010 to 2015, where we find a decreasing dispersion index and, therefore, a compaction of urban land use patterns. In this sense, the indicator values in **Figure 7** suggest a convergence of the urbanized land in the outer rings.

In summary, we can characterize the urbanization in Chicago as a form of urban consolidation with compact development in the inner rings, and a subsequent compaction of dispersed land use patterns in the outer rings. This is a typical form of suburbanization. The example of Chicago lends itself to the application of this new monitoring concept that provides information about dispersion and land use inefficiency for such urban sprawl conditions. The trend analysis for Chicago shows that the growth in urban land was accompanied by a continuous decrease in urban density over all rings in the research area. We attribute this result to a massive migration of the inner city population to the outer rings in the wake of suburbanization, largely surpassed by the tremendous growth of urbanized land in rings 3 and 4.

Urbanization Trends in Paris

In contrast to Chicago, our second test case, the city of Paris, has no substantial topographic or natural restrictions for urban growth around the city. This lack of restrictions is why Paris has equal travel time rings in a radial growth pattern around

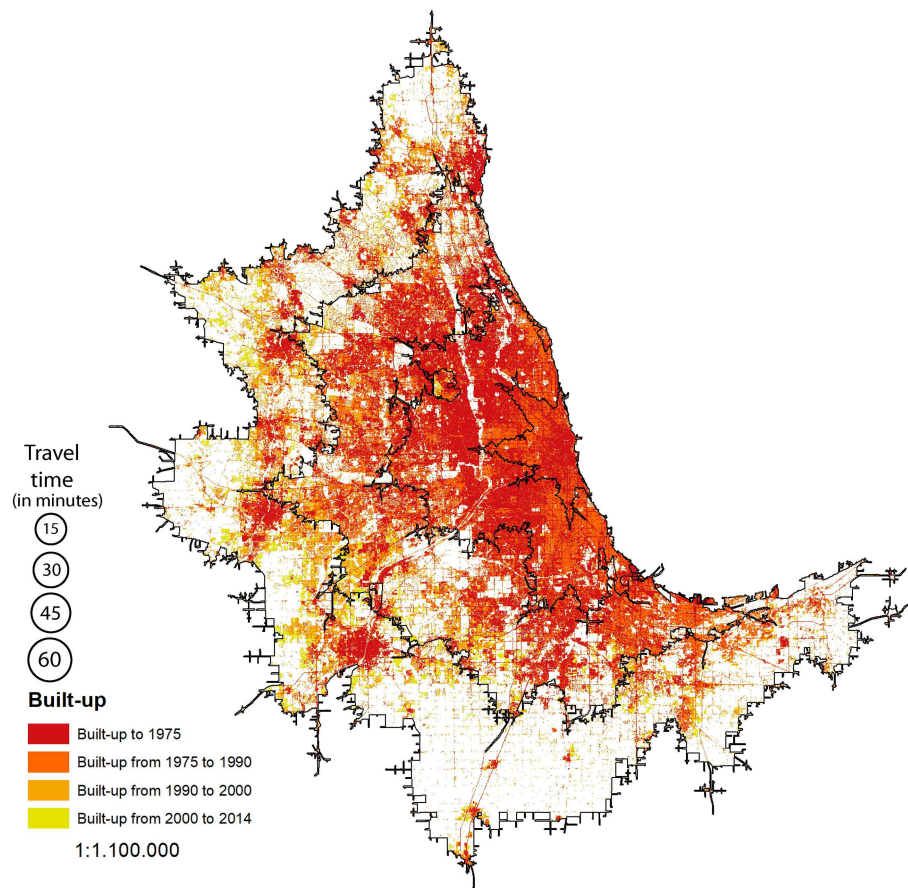


FIGURE 6 | Urbanization periods in Chicago, based on the GHSL.

the city core, with strong concentrations of newly built urban land along the major transport routes into the hinterland. As the capital of France, Paris has historically attracted urban growth with a concentration of government and business functions for centuries, dominating spatial development with strong transportation linkages to second-tier cities in France. It is therefore not surprising that **Figure 8** shows the inner rings as already vastly built-up at the beginning of our observation period in 1975. Newer urban growth mainly occurred in the third ring along the main transportation axis. Ring 4 contains some smaller separated settlement areas in 1975, which we identify as formerly self-contained cities that attracted new growth in our observation period. The urban areas that were created after 1990 extend existing settlements, visible here in the orange and yellow patches of new urban land in rings 3 and 4.

In the city core, the existing urban layout did not allow for much compaction in our observation period; it was already built-up. Our measurement concept reflects this observation accurately: the built-up land and subsequent dispersion remain constant, and the added area of 1.1 km² of urban land in 39 years of our observation period is marginal considering the area. Nevertheless, we identify continuous population growth in the first ring and therefore a denser settlement structure. The

popularity of Paris for city dwellers obviously led to new infill development or perhaps denser forms of living arrangements on average. The second ring, also part of the inner city of Paris, is characterized by dense and compact growth based on the negative land use inefficiency (e.g., population growth exceeding the growth of urban land) and development trends for the dispersion dimension. **Figure 9** presents a shift over the time periods from 1975 to 1990, 1990 to 2000, and 2000 to 2015 along the x-axis to the zero point of the dispersion index change dimension.

We interpret these changes as a form of spillover effect from the first ring to the second ring where continued population pressure led to a compaction of neighboring suburbs. In contrast to the urban center of Paris, the third ring exhibits a different development path. We can observe increasing values of population and urbanized land but also a growing dispersion in the first two periods. Because we find a higher growth in built-up areas than in population in ring 3, we can assume a form of sprawling suburbanization with less dense and dispersed growth in the first two time periods. However, this trend was reversed in the third time period where population growth exceeds the built-up area growth. The land use inefficiency shows the effect along the y-axis, and the result is a gain of urban density. This minor

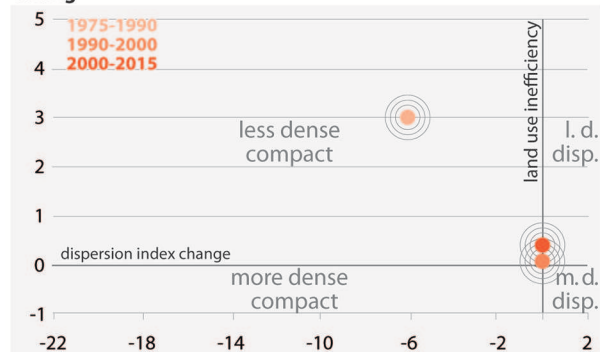
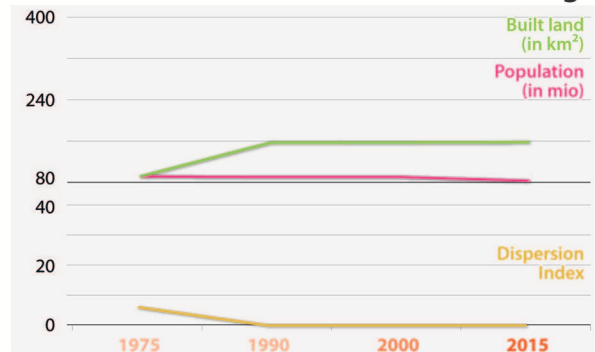
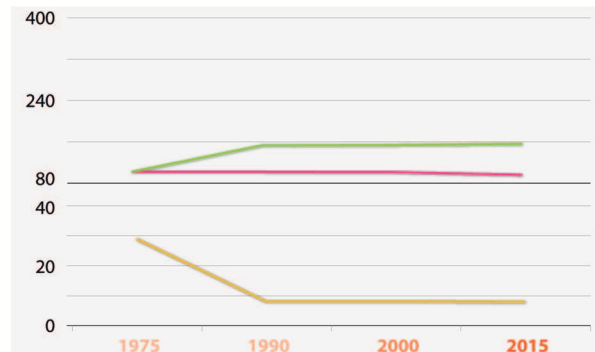
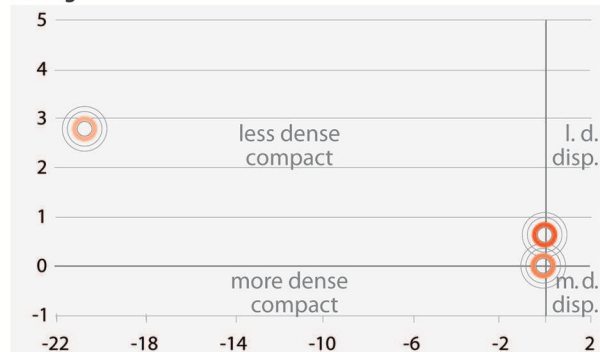
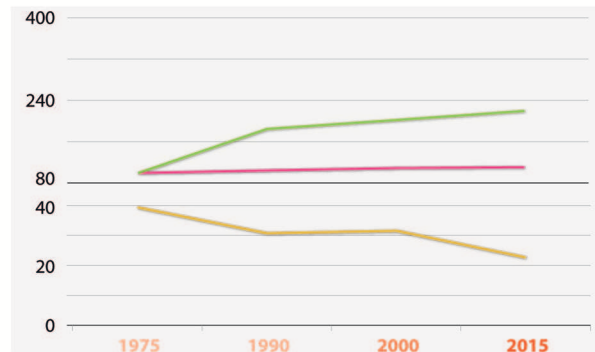
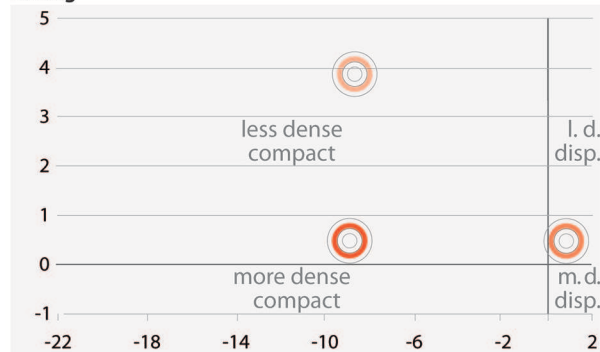
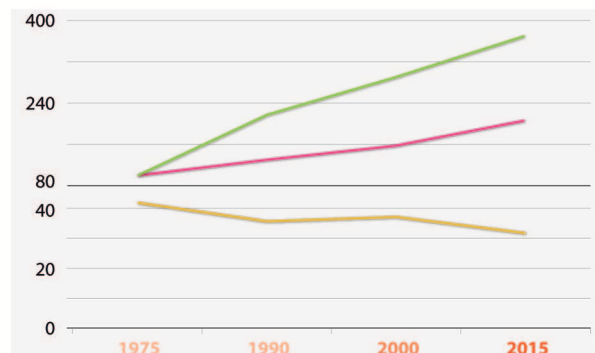
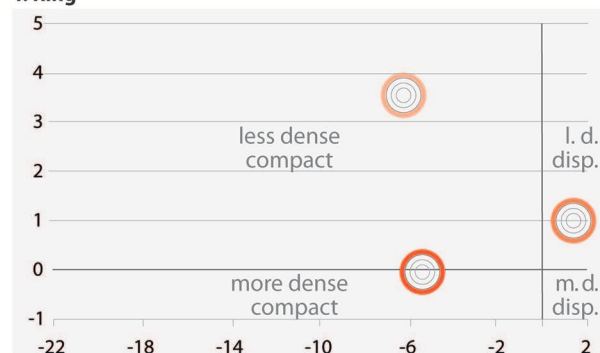
1. Ring**Chicago****2. Ring****3. Ring****4. Ring**

FIGURE 7 | Urbanization trends for Chicago divided into the four rings. The line charts illustrate the corresponding development of built-up land, population, and dispersion index over time.

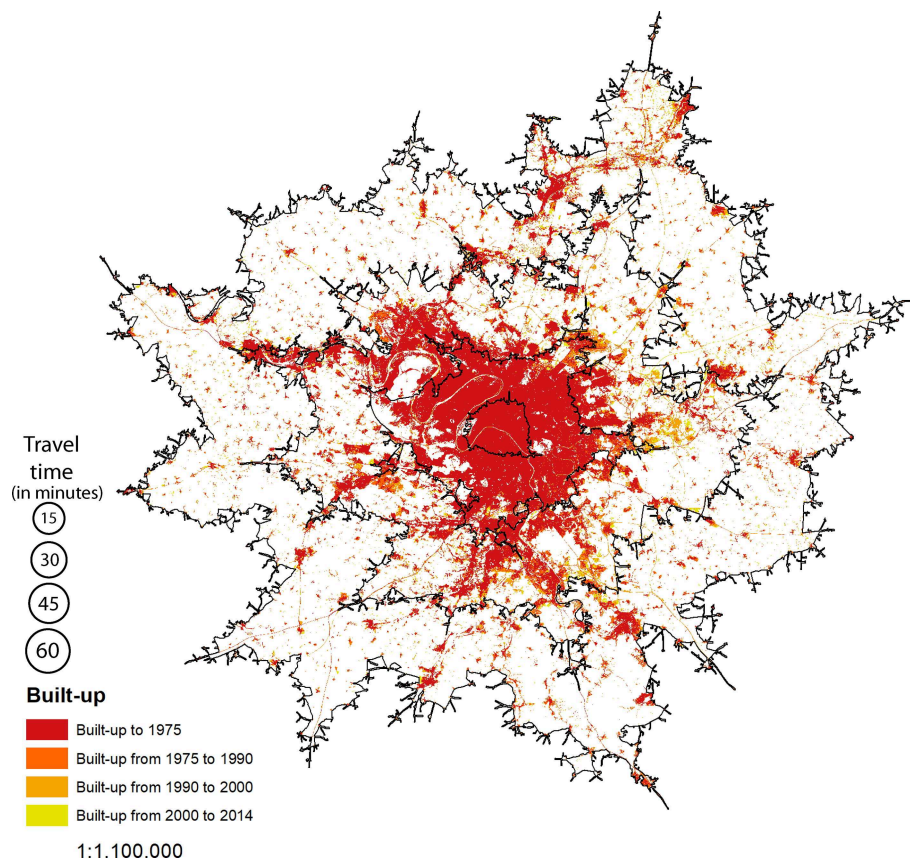


FIGURE 8 | Urbanization periods in Paris, based on the GHSL.

trend of densification and dispersion in the last period could be a result of densification policies in the city-region (Touati-Morel, 2015). The exterior ring follows this trend with higher dynamics. Notable is the change in the urbanized land, which increases by a factor of 2.5 since 1975 and promotes the dispersion and the decrease in urban density in the fourth ring. We suspect that advantages in land rents and accessibility for commuters shifted the suburbanization from the city core further out to rings 3 and 4 of the research areas. This phenomenon is a typical process for European cities where historically compact cities have seen massive forms of urban sprawl on the outskirts. This is an observation that is often overlooked in theories on urban form that falsely idealize European cities as blueprints for compact city policies (European Environment Agency, 2006).

In summary, in Paris, we can identify different trends from rings 1 through 4. The city core is very compact and can only grow in density; ring 2 experiences a consolidation of urban form as a consequence since 1975. The low value for the dispersion index change in 2015 suggests that this process is largely completed, and the urban form is fully built-up. The developments in rings 3 and 4 have been subject to massive processes of suburbanization and urban sprawl in our observation period, with less dense and disperse growth of urban land and very high land consumption rates.

DISCUSSION

The main results of our study inform about global urbanization trends and new monitoring methods based on remote sensing data. In terms of selected examples on urbanization we show that the highest growth dynamics occurred in the period from 1975 to 1990. In most world regions, this result is typical for the expansive building policies in the wake of the automobile-oriented suburbanization that dominated urban development from the 1950s onward for some decades. This trend is global, although with variations in timing and scale (MacLean, 2008). Our results deliver additional evidence for such variations for selected cities and city groupings in this time period. In the following periods, from 1990 onwards, we see a general compaction of urban development. We attribute this compaction to a consolidation of suburban developments over time with new built-up areas. However, the results of densification or land use inefficiency are not as clear. Most of the cities in our sample show densification in the inner rings and later in the outer rings. Generally, cities exhibit a time lag between the growth of built-up areas and the influx of inhabitants that leads to higher densities. The dichotomy between urban and rural areas becomes blurry.

The additional results of our analysis indicate the following urbanization patterns in the study areas:

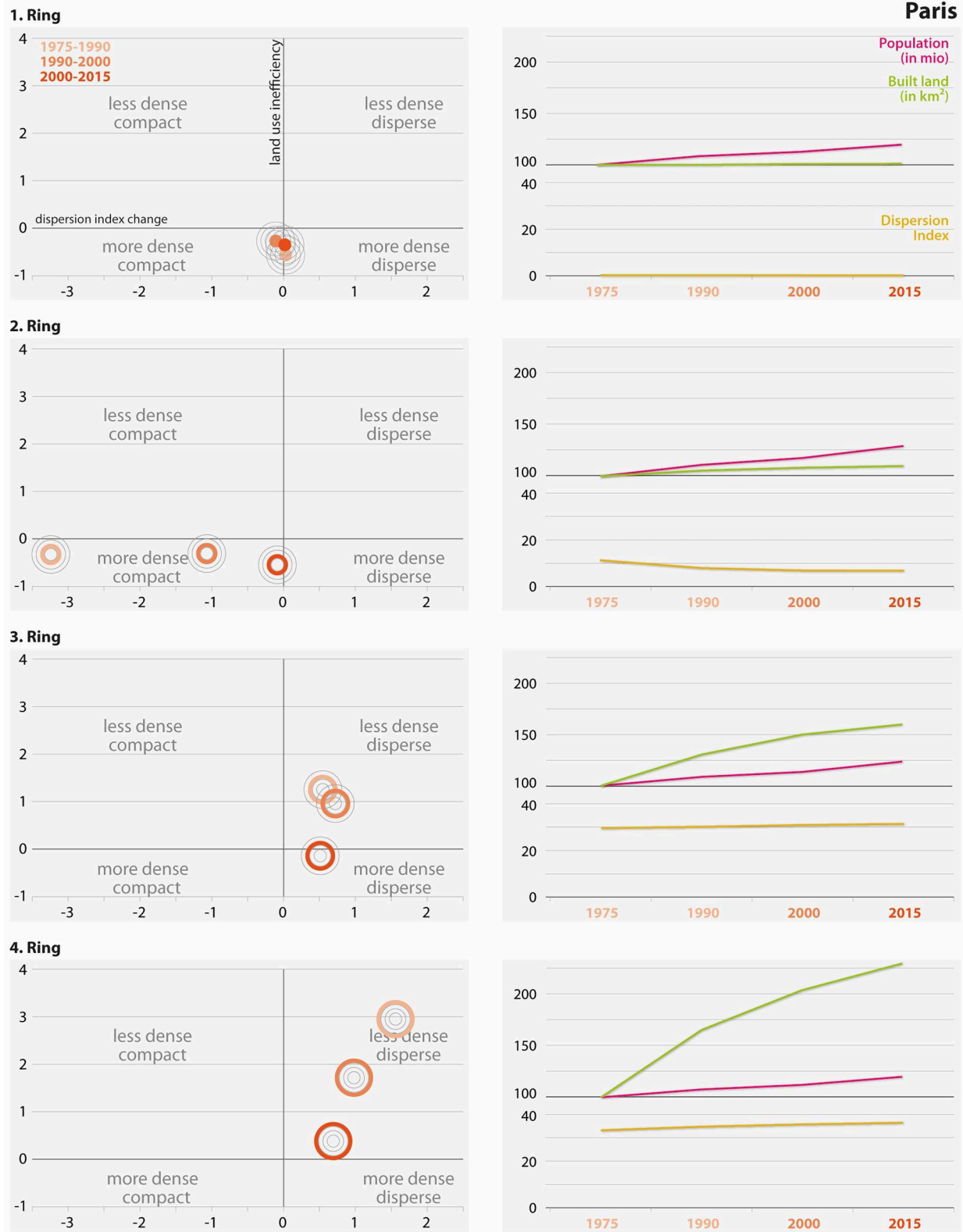


FIGURE 9 | Urbanization trends for Paris divided into the four rings. The line charts illustrate the corresponding development of built-up land, population, and dispersion index over time.

- Chicago shows a decreasing density that is in line with the results obtained by Angel et al. (2010) from the early 1950s onwards. According to the results of that study, the share of urbanized land increased from 1970 to 2000 from 50 to 100%. We can verify this result with the urbanization shown in **Figure 7** that illustrates a steady trend of increasing land consumption per inhabitant.
- Due to the historically established high density in the urban core of Paris, the growth of urbanized land occurred in the outer rings, although the inner city is still growing in population. As a typical characteristic of this process, the settlement patterns are most compact in the city core, and they become more dispersed in the outer rings, following the main transport routes. In the outer rings, the settlement density decreases as a consequence of the high land consumption rate, which is typical for urban sprawl.
- Angel et al. (2011) already showed that the growth of urbanized areas in European cities had already peaked before 1975. In contrast, cities in Asia, Africa and South America are still experiencing high growth rates, after 1970 and continuing until the last year of data availability in 2015. These trends were also identified by Seto et al. (2011). **Figure 4** shows these differences in land use inefficiency and dispersion dimensions. The results of the statistical analysis present a major difference in the dispersion index change between the continents for the first time period. Since 1990 the sample seems to be more equal. In comparison, the differences of the land use inefficiency for the continents are tremendous for the complete observation time.

From a methodological point of view, our findings show that monitoring methods need to be complemented by validation procedures to test for data reliability. Future analysis must work with ground truth data and more in-depth case studies to assess the accuracy of monitoring results. This aspect is important due to doubts about data quality. As explained in the section on data, we cannot rule out that the limited data quality for the 1975 data is responsible for the large deviations of this time period compared to the trends of the whole observation period. This warning especially applies to the population data presented in section Land Use and Population Data. In terms of land use we expect an improvement of data reliability for more recent years based on the introduction of higher quality sensors and classification procedures. Due to our city selection procedure, our interpretation results on the global level refer only to growing cities. Therefore, we cannot comment on shrinking or stagnating cities. Other methodological findings are:

- The differentiation of growth trends according to city size is affected by the cities' catchment areas. For cities with more than 500,000 inhabitants, we used a 45 min travel time polygon as the research unit, which may also include suburban and rural areas in cities with a high density gradient. This area is mostly where urban sprawl happens, namely, at the outskirts along motorways, often as a result of suburbanization processes. In these cities, the urban density can increase in the core. On the outskirts, the urban patterns become more sprawled due to newly developed settlement structures. This

effect is evidenced by major differences in land use inefficiency and dispersion between city size categories. Statistical analysis shows that large cities with more than 500,000 inhabitants have consolidated dispersed and inefficient sprawling conditions much more over time than smaller sized cities.

- Our analysis of cities by continent does not obtain clear differences for interpretation. Grouping cities by continent obviously mixes too many specific city types with unique development paths, and the resulting average values disguise the analytical power of a single portrayal of development trends such as those we presented for Chicago and Paris. To extract more analytical value from city classes, we aim to concentrate on planning regimes and other characteristic properties of cities for future groupings and the inclusion of different rings of observation.

In essence, we used this study to test a new methodology for a rich database. Our initial results show that the results can be conclusive and reflect global urbanization trends in a new and simplified way. In comparison to other global assessment methodologies (e.g., Seto et al., 2011; Zhang and Seto, 2011) that utilize more measures, we manage to combine the dimensions of population, land expansion, and urban sprawl in one measurement approach. The strength of this methodology is in the analysis of the individual cities and their different catchment areas, which can be adapted for further analysis. Thus, we can assign the development in the individual city rings to different trends of urban land use change. The delineation of cities and their catchments remains a problem for monitoring applications. Our approach to use network analysis and the most recent street network to model multiple rings around an urban core provides the flexibility to analyze urban development phases over time. Based on this approach we can capture land use changes in the rings without relying on the stability of administrative areas. In addition, we do not have to differentiate arbitrarily between urban fringes, suburbia and the rural hinterland, categories that have changed highly dynamically in many city regions of our observation period. In addition, we can customize our methodology. With far-reaching methodological changes of our previous dataset, we are able to exchange the GHSL with similar data sources. The minimum requirements include small-scale information about population and built-up land. Such requirements also apply to our indicators as long as the new indicators are based on the same theoretical and methodological foundations. However, we have also identified limitations in the methodology. To interpret and compare the results additional information such as the initial absolute values of the indicators are useful. Further, our selected indicators also have strengths and weaknesses. As already determined, the application of the dispersion index does not consider the intensity of urban land use or functional centrality (Taubenböck et al., 2018). The indicator focuses on adjacent cells, where minimal inaccuracies in the dataset can break topological adjacency. However, we assess this problem as a minor inaccuracy that is acceptable with a view toward capturing general trends. It is also clear that aspects like urban density could be more accurate when using construction volume or 3D-building data. However, our approach is designed

to work with data that is available worldwide, which is not the case for 3D data (e.g., Jahn et al., 2015; Krehl et al., 2016). For future analysis, it would be interesting to compare the results of our simplified analysis to a more complex and detailed measurement. For the time being we can validate the results based on the literature on urban development trends. Future analysis on drivers and impacts will certainly demand additional indicators and qualitative assessments.

We are aware that this idea initially requires some effort to conceptualize. However, once it has been understood and established as a monitoring concept, we expect benefits from its continuous use. In addition, this idea can be a relatively accessible and easy-to-communicate approach for the measurement of urban land use change. The advantage we envisage is that the data requirements are by far not as demanding as in most other measurement concepts. We also expect that further research is needed to make the analytical power more accessible to planning practitioners. Such research must include working with advanced visualization techniques and incorporating other reference data to validate the results. For example, further research could consider planning regimes or economic conditions to obtain information about the development paths of land use change. Such analysis will be left to future endeavors once we have established, communicated, and received feedback on the explanatory value of our methodological approach.

CONCLUSION

This study was designed to extend measurement methods on urban land use change for worldwide assessments of cities by city size. To achieve this goal, we explored several methods and produced a simplified framework based on the previous studies in Western countries. The idea of this framework is to condense the dimensions of urban land use change suggested in the literature without compromising the analytical depth. Our new approach combines the dimension of urban area, land use patterns, and urban densities into a unified measurement model on two axes: land use inefficiency (built-up area growth divided by population growth) and dispersion of urban patches in the research area.

Overall, the portrayal of global development based on this method provides a first glimpse into the analytical potential of both the method and dataset. Indicator results can be used as single measurements or in the combined analysis of the land use inefficiency and dispersion matrix. Future analysis will provide more insight into the actual development trends by using several rings and other groupings of cities, for example, with a view

toward the planning culture or other specific dynamics. For the time being, we introduce this new analytical concept for discussion in the research community. Ideally, we would like to see such analysis used in monitoring applications in planning practice to inform decision makers about urban development paths. Put simply, our recommendation is to capture the starting point of the urban footprint in terms of density and compactness, formulate appropriate objectives, and then begin to monitor development paths with this new method.

The understanding of how cities have grown is an essential facet of the spatial sciences. Newly available, small-scale population and built-up area data serve as the basis for innovative but conceptually challenging analysis options. It is up to the scientific community to improve planner's abilities to effectively employ such data. In this sense, the results presented here deliver on the call of Wei and Ewing (2018) for the development of new monitoring methods for urban land use change.

DATA AVAILABILITY STATEMENT

The datasets analyzed for this study can be found in the Global Human Settlement Layer data repository of the European Commission at <https://ghsl.jrc.ec.europa.eu/datasets.php> (last accessed March 29, 2019). The dataset for the city selection procedure can be found in the world populated places layer package by Esri and Garmin International at <https://www.arcgis.com/home/item.html?id=587c838521864164acd245ea03315006> (last accessed August 29, 2019).

AUTHOR CONTRIBUTIONS

CG conducted the analysis and data processing for this paper. The concept and methodology was established by all authors.

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Jutta Rönsch, cartographer at the Research Institute of Urban Development, assisted in the preparation of **Figures 4, 5, 7, 9**.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2019.00140/full#supplementary-material>

Tables with indicator values for Paris and Chicago, excluded countries, list of selected cities per country and the results of the Kruskal-Wallis-Test are provided as **Supplementary Material**.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Targeting Extreme Events: Complementing Near-Term Ecological Forecasting With Rapid Experiments and Regional Surveys

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Ecologists are improving predictive capability using near-term ecological forecasts, in which predictions are made iteratively and publically to increase transparency, rate of learning, and maximize utility. Ongoing ecological forecasting efforts focus mostly on long-term datasets of continuous variables, such as CO₂ fluxes, or more abrupt variables, such as phenological events or algal blooms. Generally lacking from these forecasting efforts is the integration of short-term, opportunistic data concurrent with developing climate extremes such as drought. We posit that incorporating targeted experiments and regional surveys, implemented rapidly during developing extreme events, into current forecasting efforts will ultimately enhance our ability to forecast ecological responses to climate extremes, which are projected to increase in both frequency and intensity. We highlight a project, “chasing tree die-off,” in which we coupled an experiment with regional-scale observational field surveys during a developing severe drought to test and improve forecasts of tree die-off. General insights to consider in incorporating this approach include: (1) tracking developing climate extremes in near-real time to efficiently ramp up measurements rapidly and, if feasible, initiate an experiment quickly—including funding and site selection challenges; (2) accepting uncertainty in projected extreme climatic events and adjusting sampling design over-time as needed, especially given the spatially heterogeneous nature of many ecological disturbances; and (3) producing timely and iterative output. In summary, targeted experiments and regional surveys implemented rapidly during developing extreme climatic events offer promise to efficiently (both financially and logistically) improve our ability to forecast ecological responses to climate extremes.

Keywords: ecological forecasting, adaptive monitoring, anticipatory science, disturbance, climate change, climate extremes, extreme climatic event, drought

INTRODUCTION

The frequency and severity of climate extremes such as drought, floods, and heat waves are projected to increase with global climate change (de Coninck et al., 2018; Hayhoe et al., 2018). These climate extremes can trigger rapid ecosystem responses (i.e., extreme climatic events; Smith, 2011), including widespread tree die-off (Allen et al., 2010, 2015), algal blooms (Havens et al., 2016), wildfires (Moritz et al., 2010), plant invasions (Sheppard et al., 2012), and extensive soil erosion (Coppus and Imeson, 2002). This has created a need for rapid anticipatory science and management to increase ecosystem resistance to and/or recovery following extreme climate events (Ummenhofer and Meehl, 2017; Bradford et al., 2018). Yet forecasting not only the climate extremes, but also the ecological responses (Smith, 2011), is a key prerequisite toward conducting anticipatory science and management in the face of climate change (Dietze et al., 2018). Near-term ecological forecasting has been developed to make iterative predictions of ecological responses to inform management action and has the potential to transform our ability to rapidly manage natural resources during and following extreme climatic events (Clark et al., 2001; Dietze et al., 2018). In this Perspective, we propose that near-term ecological forecasting can be enhanced by targeted experiments and regional surveys implemented rapidly during developing extreme climatic events to improve our ability to forecast, and ultimately manage, ecological responses to climate extremes.

Near-term ecological forecasting has already been used to iteratively forecast wildfires (Chen et al., 2011), influenza outbreaks (Shaman and Karspeck, 2012), algal blooms (Stumpf et al., 2009), and more (see also Dietze, 2017; Dietze et al., 2018) and can be coupled with adaptive management to further improve predictions and guide anticipatory management (Bradford et al., 2018). Successful near-term ecological forecasting can be achieved by continually making predictions, taking measurements, observing results of those predictions, and integrating data with models (Kalnay, 2002; Dietze, 2017)—in essence “learning by doing” (Shuman, 1989). These steps can be further refined using the scientific method to propose alternative models that test appropriate hypotheses by comparing observations to specific, quantitative predictions rather than the conventional null hypothesis. These improved models are then used to forecast, observe, analyze, and refine hypotheses, and the iterative forecast cycle continues (Dietze et al., 2018). Coupled synchronously with this ongoing iterative forecast cycle is the adaptive management cycle, wherein monitoring data can be used not only to assess previous management decisions but can also be assimilated into forecasts that explore alternative management scenarios moving forward (Gregory et al., 2012; Ketz et al., 2016). Performed in tandem, these two cycles will accelerate our capacity to predict and respond to extreme climatic events.

We see an opportunity to complement the near-term ecological forecasting framework to more effectively address rapidly developing, ecologically significant extreme climatic events that are projected to increase with global climate change. At present, most examples of near-term ecological forecasting have generally relied on long-term data from a

single site (e.g., Hobbs et al., 2015), a network of sites (e.g., Kuikka et al., 2014; Thomas et al., 2017), or near-real time data through satellites (e.g., Stumpf et al., 2009). Yet speedily implemented natural and manipulative experiments provide a way to target transient and progressive spatially-heterogeneous extreme climatic events (e.g., algal blooms, exotic species invasions, disease and insect outbreaks, drought-induced tree mortality and dieback) while simultaneously increasing understanding of these events and harnessing the predictive power of long-term datasets. As highlighted by recent efforts to increase adaptive monitoring whereby monitoring efforts adjust overtime to more efficiently capture spatiotemporal dynamic ecological processes (e.g., Hooten et al., 2009; Krause et al., 2015), we posit that targeting the event as it develops is an informative way to study these phenomena. Manipulative experiments, a core tool in ecology (Hairston, 1989; Peters, 1991; Sala et al., 2000; Scheiner and Gurevitch, 2001; Weltzin and McPherson, 2003), can identify the mechanisms underpinning responses and the potential thresholds that are difficult to identify *post-hoc*, and have successfully been used to improve ecological forecasting (Jiang et al., 2018). Additionally, regional surveys and associated summaries are effective for change detection associated with extreme climatic events (Hughes et al., 2018; Ruthrof et al., 2018; Fettig et al., 2019; Flake and Weisberg, 2019), and are especially useful given the often spatially heterogeneous nature of many ecological disturbances (e.g., Lybrand et al., 2018). There are a range of remote sensing technologies that can be used to augment regional field surveys and forecast changes at a broader spatial scale. These include an array of satellite imagery, including not only traditional multispectral imagery (Landsat, Sentinel-2, MODIS, VIIRS), but also lidar (GEDI), thermal (ECOSTRESS), radar (PALSAR, NISAR), microwave soil moisture (SMAP), gravimetric (GRACE), high temporal resolution geostationary imagery (GOES), and commercial cubesat constellations (e.g., Planet Labs). There are also emerging opportunities to leverage airborne and drone technologies to augment field data and/or satellite imagery and ultimately improve near-term ecological forecasting. We propose that adding rapidly implemented experimental and regional studies during developing extreme climate events can complement existing near-term ecological forecasting efforts to ultimately improve our capacity to forecast ecological responses to climate extremes.

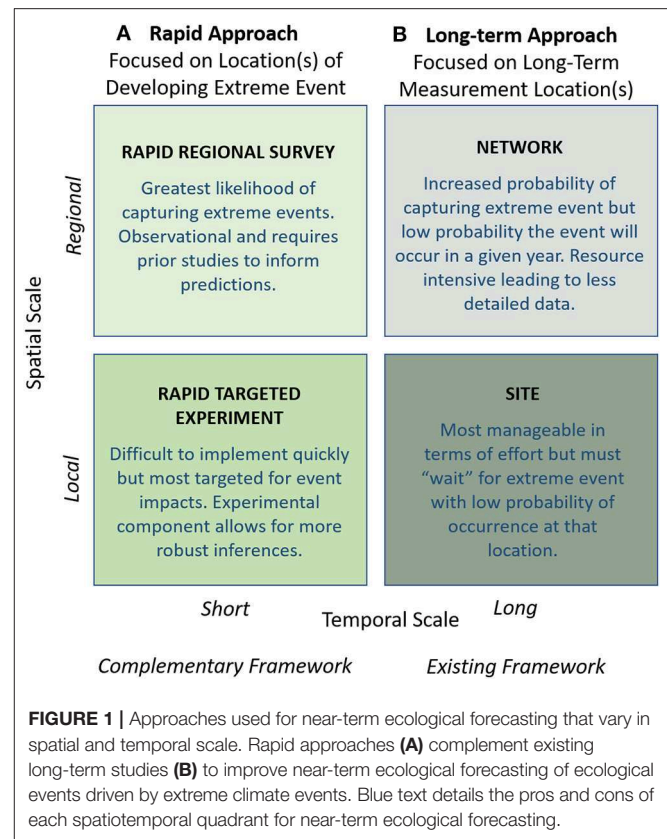
Below we: (1) provide a framework for expanding near-term ecological forecasting by incorporating targeted experiments and regional surveys implemented rapidly during developing extreme climatic events; (2) illustrate our points with a case study, “chasing tree die-off”; and (3) provide examples of where this approach can be used to improve our ability to forecast other critical ecological processes.

EXPANDING NEAR-TERM ECOLOGICAL FORECASTING TO OPPORTUNISTICALLY EXPLOIT EXTREME EVENTS AS THEY ARE DEVELOPING

A key aspect of near-term ecological forecasting to date is that it is generally based on ongoing long-term measurements

at a single site or across a network of sites at regional or larger scales (**Figure 1B**). This is practical because there is substantial effort needed to launch an iterative and highly automated near-term ecological forecasting framework. The advantages of such a framework are sound and can aid management decisions (Dietze, 2017; Dietze et al., 2018). However, ecological changes are increasingly being driven by climate extremes, which are infrequent in time, variable in space, and therefore require a rapid-response capacity to capture observations or implement experiments. Some of these ecological changes, such as vegetation and biogeochemical responses to extreme drought (e.g., Ciais et al., 2005; Schlesinger et al., 2016) can potentially be anticipated during the development of the extreme event. Given the importance of extreme climatic events, we propose that rapidly implemented studies at local and regional scales can be a useful complement to other approaches based on ongoing long-term data collection (**Figure 1A**). A developing extreme climatic event can become the focus of an experimental study implemented rapidly within the impact location to increase return on investment, such as by implementing water additions as treatments during a developing drought (Jentsch et al., 2007). This type of effort is most likely to provide rapid useful information on the impacts of climate extremes, particularly concerning ecological thresholds. Similarly, opportunistic surveys at a regional scale can provide added insight on extreme event impacts. An excellent example of this is the rapid monitoring of an emerging influenza outbreak in 2009 to document and ultimately improve forecasting of influenza spread (Ong et al., 2010). These targeted regional surveys are especially useful in documenting spatially heterogeneous disturbances. The rapid approach and long-term approach are highly complementary: long-term monitoring sites can be used to generate forecasts during developing extreme events (e.g., Diffenbaugh et al., 2017) and insights learned from the rapid approach can subsequently be used to improve forecasts at these long-term monitoring sites.

Within this proposed expanded framework of near-term ecological forecasting (**Figure 1**), there are pros and cons to each spatiotemporal quadrant. In the simplest type of near-term ecological forecast, ongoing long-term data at a site are used to iteratively update predictions (**Figure 1B**, lower). This type of approach is most feasible in terms of effort because it is focused on a single site, allowing for intensive, detailed measurements, and access to long-term time series, which makes the specific timing of initiating the forecasting perhaps less critical (Dietze, 2017; Dietze et al., 2018). However, in this type of forecast, the study site has a high probability of being located outside the area impacted by an extreme climatic event and much data may need to be collected before the timing and location of an extreme event impact that site. Moving to a regional scale, with a network of long-term ongoing data (**Figure 1B**, upper) the probability of capturing an extreme climatic event within the network increases, although this depends on network density. Networks of sites are beneficial in that they are more spatially extensive relative to a single-site but, in general, have less detailed data due to the greater expense of maintaining multiple sites. Further, there is still the issue, albeit of less concern relative to a single



site, of potentially waiting a long time for an extreme climatic event to occur at one of the sites within a network, particularly if there is a low density of sites. By observing progressive conditions for climate extremes such as a drought, efforts can potentially be rapidly deployed at a specific location within the area forecasted to be impacted by the extreme event (rapid targeted experiment; **Figure 1A**, lower). Similarly, at regional scales, a survey following an extreme climate event can document its impact (rapid regional survey; **Figure 1A**, upper), but this type of approach often relies on having preliminary data at a regional scale that can be built upon. This survey can be done using observations from on the ground field surveys and/or with remote sensing technologies to document ecological responses (e.g., Miller et al., 2006; Schepaschenko et al., 2019) and to also develop forecasts (e.g., Liu et al., 2019). These two types of efforts, can strongly enhance our understanding of the ecological consequences of extreme climate events and require less financial costs over-time, but can be challenging to implement because funding, site selection, experimental treatments (**Figure 1A**, lower only), and monitoring all need to occur quickly. Importantly, the rapid approach discussed here is most effective in climate extremes like drought that develop on slower time-scales. In summary, each of these four quadrats (**Figure 1**) can provide useful information for near-term ecological forecasting, and each has constraints. Furthermore, advances in statistical methods for iterative forecasting, and data fusion approaches for informing models with multiple data constraints, play a critical role when trying to combine information from these four quadrats (Dietze,

2017). We next illustrate how rapidly implemented local and regional scale studies provide useful insights into the left-hand column of the framework.

CHASING TREE DIE-OFF: A CASE STUDY

We began tracking predictive maps provided by the US Drought Monitor (The National Drought Mitigation Center, 2019) and local weather stations during the winter of 2017/2018. The cumulative evidence predicted 2018 to be an exceptionally dry year that could lead to regional tree mortality comparable to 2002–2003 (Breshears et al., 2005). We received funding from the NSF-RAPID program to implement a two-part study integrating an experiment and regional surveys into near-term ecological forecasting to evaluate piñon pine (*Pinus edulis*) mortality during a developing drought in the US Southwest during 2018. We coupled a watering experiment at two sites (i.e., **Figure 1A**, lower) with a regional survey (i.e., **Figure 1A**, upper) to forecast mortality based on over a dozen published equations to predict mortality at varying spatiotemporal scales (Breshears et al., 2018).

Rapid Target Experiment

In April 2018, two sites were established in a piñon-juniper woodland separated by approximately 300 m in elevation. To test previously published hypotheses on how tree size and age affect mortality (e.g., Floyd et al., 2009; Meddens et al., 2015), we identified 24 clusters of trees at each site that consisted of one reproductively mature individual in close proximity to a sapling and a seedling. We randomly assigned each cluster of trees to one of three treatments: ambient (drought), small watering, and large watering. Watering treatments were done on May 21, 2018 by imposing an artificial rain event of two magnitudes by slowly saturating either the top 10 or 30 cm of soil. This was done in an effort to vary soil moisture across trees to refine mortality thresholds. We tracked soil moisture using a combination of handheld and permanent soil moisture probes through the duration of the experiment. We sampled pre-dawn water potential, stomatal conductance, and canopy percent brown from late May to mid-September at ~2-week intervals to assess water stress and tree mortality, and continued to sample canopy percent brown monthly through mid-November, 2018 to obtain final estimates of tree mortality. Mortality forecasts were updated ~every two weeks during the duration of the experiment based on our water stress measurements and compared to mortality data.

Rapid Regional Survey

The regional survey of piñon pine mortality was stratified by elevation and soil available water capacity across a 700 km region of Colorado and New Mexico to target sites that were anticipated to have variable levels of mortality. In October, 2018, we measured the size, vigor, and survival of all trees at 32 sites. Further, we recorded microsite conditions of all juvenile trees to refine predictions of nursing effects on juvenile survival under varying climate conditions (e.g., Redmond et al., 2015). The landscape to regional-scale mortality predictions for piñon pine

focused on here can only be updated at an annual time-step (Breshears et al., 2018) and thus forecasts were done at the end of the study once all input climate data were available. Due to the short duration of this study (1 year), we were only able to perform iterative predictions for the finer spatiotemporal scale predictors assessed in the experimental survey and we were unable to assess whether mortality continued into the following growing season. Collectively, these two approaches (manipulative experiment and regional survey) allowed us to test previously published equations used to predict piñon pine mortality at varying spatial scales and refine future predictions.

Watering treatments in the rapid targeted experiment successfully increased soil moisture levels initially, with the small and large watering treatments resulting in a 4.7% and 11.6% increase in volumetric water content, respectively. This subsequently resulted in a trend of decreased tree water stress (i.e., less negative water potentials) at the low elevation site only. Yet the effect of watering on soil moisture rapidly declined—by 2 weeks soil moisture levels were equivalent between the treatments and there were no differences in mortality between treatments. The rapid target experiment was challenged by a 1,000-year rainfall event that occurred in July 2018 and resulted in very little (<3%) subsequent overstory mortality, despite predictions of high (>30%) mortality based on prior plant water potential thresholds identified in Adams et al. (2017). As a result, we documented the recovery of a population affected by drought following a substantial rain event despite exceeding previously established thresholds that closely linked extreme plant water potentials to mortality. Forecasting mortality based on data collected during the developing drought in the rapid target experiment (i.e., **Figure 1A**, lower) allowed us to refine previous predictions by providing data of where mortality was expected to occur, but ultimately did not occur. This highlights the importance of forecasting mortality during droughts rather than *post-hoc* investigations following known die-off events. Notably, the rapid regional survey (**Figure 1A**, upper) allowed us to sample areas where the developing drought continued to persist and ultimately lead to tree mortality (Wion et al., unpublished). Given uncertainties of forecasting climate extremes and the spatially heterogeneous nature of ecological disturbances, this two-part study reveals the benefit of integrating targeted experiments with regional surveys during an emerging drought to further improve ecological forecasting.

From our project, some of the more general insights that apply toward conducting rapid studies during developing extreme climate events (e.g., **Figure 1A**) include:

- (1) The need to track developing events and their potential timeline. This is more challenging for extreme climate events that occur and end rapidly, such as heat waves, relative to extreme climate events that develop over longer time periods, such as drought.
- (2) The ability to ramp up measurements rapidly and if appropriate install and initiate an experiment quickly. This includes challenges associated with quickly obtaining or redirecting funding and selecting sites on a tight timeline.

TABLE 1 | Examples of where near-term ecological forecasting can be enhanced by experiments and regional surveys implemented rapidly during emerging climate extremes to improve our ability to forecast extreme ecological events and mitigate risk through early detection.

Climate Extreme	Extreme Ecological Event	Forecasting Challenge	Opportunities for Rapid Approaches
Drought	Insect Outbreaks & Tree Mortality ^{a,b}	Determining mortality thresholds under varying levels of insect densities and water stress; efficacy of management options	Experiment: • Experimental management interventions (e.g., insect control techniques) • Precipitation manipulation treatments Survey: • Pheromone traps to quantify insect population densities • Tree mortality surveys (field and/or remote sensing based)
Extreme Temperature (Acute Heat Wave)	Coral Bleaching ^c	Determining expulsion thresholds, spatial extents, and cascading effects	Experiment: • Mesocosms to manipulate abiotic conditions (temperature, pH) and biotic communities Survey: • Rapid implementation of surveys before, during, and after heat events coupled with abiotic monitoring of currents, temperature, and other abiotic conditions.
Extreme Temperature (Sustained Heat)	Permafrost thawing ^{d,e} , peatland & alpine grassland drying ^f	Uncertainty in rate of thawing and drying; high spatial heterogeneity requires extensive sampling	Experiment: • Manipulation of temperature, moisture, or solar input Survey: • Carbon stocks and fluxes and changes to organic matter stoichiometry • Surveys of changes in vegetation species richness and biomass (field and/or remote sensing)
Extreme Precipitation (Flooding)	Infectious disease outbreaks ^{g,h}	Waterborne transmission of viral, bacteria, and parasitic diseases leading to disease spread and high risk areas with flooding	Experiment: • Experimental management interventions (e.g., sterilization of disease vectors) • Mesocosm experiments to manipulate density and diversity of hosts Survey: • Rapid response through population monitoring of vectors and disease agents and tracking outbreaks with social media
Extreme Wind (Hurricane)	Windfall ⁱ	Predicting changes in forest structure and understory vegetation following high wind; managing to promote recovery	Experiment: • Experimental silvicultural treatments, nutrient additions to couple biogeochemical changes with understory vegetation responses Survey: • Tree mortality surveys to test forecasts across species and size classes • Biogeochemical and understory vegetation sampling

^aAnderegg et al., 2015, ^bLiu et al., 2019, ^cLiu et al., 2018, ^dSchuur et al., 2015, ^eBrouchkov and Fukuda, 2002, ^fGanjurjav et al., 2018, ^gHunter, 2003, ^hWells et al., 2015, ⁱCooper-Ellis et al., 1999.

- (3) Accepting uncertainty in projected climate extremes and, when possible, altering the sampling design (i.e., adaptive monitoring) as the event progresses, especially given the spatially heterogeneous nature of many ecological disturbances.
- (4) Producing timely and iterative output under a short time period.

EXAMPLE OPPORTUNITIES THAT CAN RAPIDLY EXPLOIT DEVELOPING DISTURBANCES

We highlight five disparate examples of how coupling rapid experiments and/or regional surveys with near-term ecological forecasting can allow for efficient and effective investigation of extreme events (Table 1). These examples represent both aquatic and terrestrial responses to shifting climate drivers and are not an exhaustive list. We summarize some of the major challenges

related to predicting and understanding these ecological events, while cataloging some of the opportunities available for future study using our expanded framework (see Table 1).

CONCLUSIONS

Climate change is occurring at such a rapid pace that the Anthropocene falls outside of the typical range of natural variability (Smith et al., 2009), creating a need for iterative near-term ecological forecasting (Dietze et al., 2018). Generally lacking from these near-term ecological forecasting efforts is the integration of shorter-term, opportunistic data associated with developing extreme events such as targeted experiments or regional surveys. In this Perspective, we propose that studies implemented rapidly during developing extreme events such as drought can provide useful complements to near-term ecological forecasting. Implementing targeted experiments and regional-scale surveys increase the likelihood of capturing highly spatially heterogeneous

ecological disturbances and ultimately improve our ability to forecast the ecological responses to climate extremes. By drawing on advancements in adaptive monitoring whereby monitoring efforts adjust overtime to more efficiently capture spatiotemporal dynamic ecological processes, we posit that studies implemented rapidly during a developing extreme climate event can ultimately enhance our ability to forecast extreme ecological events. This approach has already been successfully used to study influenza outbreaks (Ong et al., 2010) and tree die-off in response to drought. Notably, this framework can be further expanded as near-term ecological forecasting models continue to be developed to not only target developing extreme climate events but to also use ecological forecasting predictions to target sampling efforts to test alternative hypotheses and ultimately refine hypotheses and predictions.

AUTHOR CONTRIBUTIONS

DB conceived the idea with input from AW, CC, DL, JF, MR, NM, and NC. MR lead the writing of the paper, and AW, CC,

DB, DL, and MR all wrote sections of the first draft of the paper. All authors contributed substantially to refinement of the ideas, examples provided in the text and in the table, and editing of the text.

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A Humboldtian Approach to Mountain Conservation and Freshwater Ecosystem Services

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Tackling complex environmental issues requires transdisciplinary solutions that cannot be achieved, unless we integrate scientific disciplines and communicate science directly with civil society actors, decision-makers, and stakeholders. Alexander von Humboldt offered an approach to integrate knowledge across disciplines aiming to broadly understand human-environmental issues, yet current scientific practices largely ignore that holistic transdisciplinary approach. Here, we develop a conceptual framework for sustaining mountain environmental integrity and securing their ecosystem services based on transdisciplinarity. We use headwater systems located in old tropical mountains as examples of meta-ecosystems because they supply water for multiple human uses and are home to disproportionately high levels of species richness and endemism. We describe the vulnerability of mountains to global changes in the Anthropocene and discuss difficulties in implementing sustainable development goals for them. Specifically, we indicate the importance of mountains to human water supplies and the conflicts between anthropogenic disturbances and riparian stream meta-ecosystems for providing ecosystem services particularly failures. We also argue that the interplay between academia and other sectors of society must improve and increase the permeability of scientific knowledge into policy- and decision-making, which is crucial for improving natural resource management and sustaining ecosystem services. We argue that the prioritization of tropical montane headwater systems is a feasible, desirable and strategic issue to be included in the global sustainability agenda. Failure to implement sustainable development goals in global mountains is likely to continue resulting in catastrophic events with harsh consequences for both humans and global biodiversity.

Keywords: biodiversity conservation, freshwater governance, global changes, transdisciplinarity, mountain conservation, freshwater conservation, Anthropocene, meta-ecosystems

INTRODUCTION

Humboldt Inspired Transdisciplinary Research and Management

Alexander von Humboldt was born 250 years ago in Germany, and his contribution to science is unparalleled (Walls, 2009; Körner and Spehn, 2019). He pioneered studies linking geodiversity and is considered a founder of biogeography and vegetation ecology (Nicolson, 1987). Humboldt was the first scientist to describe similarities in vegetation along altitudinal gradients and across different mountains, emphasizing the importance of understanding that all organisms, including humans, are interconnected and form a web of life (Pausas and Bond, 2019). His transdisciplinary studies were not limited to mountains. He also studied astronomy, geology, soils, climate (including anthropogenic climate change), geomagnetism, and ocean currents, including how they influenced human cultures and vice-versa. His advocacy for an integrative view of nature shaped the way we understand our world (Pausas and Bond, 2019), being a cornerstone for evolutionary biology, ecology, oceanography, geography, and science. After witnessing deforestation in Venezuela nearly 200 years ago, Humboldt described concerns about water provisioning for both humans and animals.

Mountain Headwater Streams and Water Governance

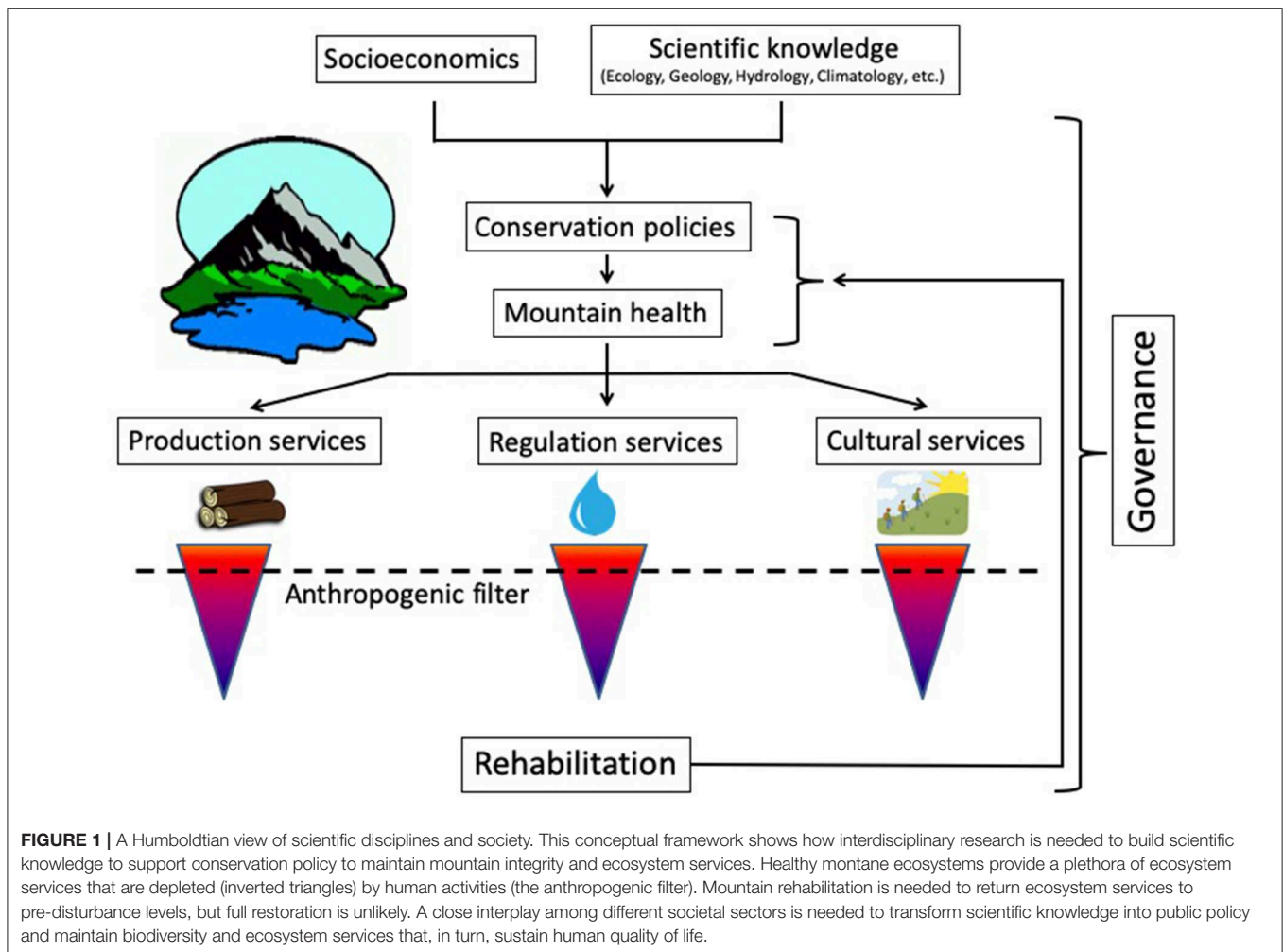
Headwater streams are excellent indicators of environmental change, analogous to the blood system, which indicates the state of human body health. Mountain headwater streams are intertwined systems, responsible for sustaining biodiversity as well as water recharge of rivers, lakes, and estuaries globally (Hoorn et al., 2018). Mountain degradation, therefore, affects the functioning of several ecosystems, also influencing lowlands and their human-production systems, which are dependent on water supply (e.g., agriculture, industry).

Considering the alarming rates of changes throughout the planet, many scientists agree that several environmental issues are a lost battle if fought as they have been traditionally (Barber et al., 2014). Several ecological papers present “results with policy implications,” concluding that those results will support more effective environmental policies and actions. However, very few studies provide clear recommendations and action plans that could be readily used by decision-makers, and even fewer are effectively implemented. Considering the urgency for evidence-based conservation actions, resource management needs much more than “research with potential applications.” Together with basic science, we also need studies that are designed toward specific conservation goals with evidence-based guidance. Hence, recommendations, guidelines and experimental scenarios must account for legal and socioeconomic aspects to be effectively applied and useful for decision-makers. The current scant resources for environmental research should advance our understanding of ecosystem functioning and services, as well as sustainable conservation goals (Costanza et al., 1997; Fernandes et al., 2017).

Therefore, what should our research priorities be? Inspired by von Humboldt’s transdisciplinary view of nature and human society, we advocate for multi-disciplinary research and management approaches that integrate all components of ecosystems and society (including the public, academics, and decision-makers) and that takes an integrated view of scientific sub-disciplines that are usually lacking in the ecological literature. We argue that reclaiming a Humboldtian holistic approach is fundamental for understanding and managing ecosystems and for securing sustainable resource quality and quantity. For instance, geological, hydrological, and climatological research must be merged with ecological research to build a robust scientific knowledge of mountain systems. In addition to this inter-disciplinary starting point amongst the natural sciences, socioeconomic research (e.g., Ringold et al., 2013) must be incorporated to develop and implement meaningful conservation policy for maintaining or restoring ecosystem health and ecosystem services (Figure 1). For example, management of federal forests in the U.S.A. Pacific Northwest has been driven since 1993 by a plan mandated by President Clinton that incorporated contributions from terrestrial and aquatic ecologists, wildlife biologists, botanists, foresters, fish biologists, hydrologists, geomorphologists, GIS geographers, economists, sociologists, and policy analysts [Forest Ecosystem Management Assessment Team (FEMAT), 1993]. Implementation of the Murray-Darling Basin’s (Australia) water resources is driven by a basin plan and an advisory panel with expertise in climatology, fluvial geomorphology, hydrology, aquatic ecology, cultural geography, water law, and economics [Murray-Darling Basin Authority (MDBA), 2019]. The U.S. Environmental Protection Agency’s National Aquatic Resource Surveys were developed in response to a Clean Water Act requirement to assess the status and trends of all U.S.A. surface waters. They incorporate inputs from statisticians, geographers, hydrologists, soil scientists, biogeochemists, limnologists, phycologists, botanists, wetland scientists, benthic ecologists, fish ecologists, and economists (U.S. Environmental Protection Agency, 2019). In the Brazilian state of Para, the Sustainable Amazon Network (Rede Amazonia Sustentavel, RAS) incorporates research by economists, sociologists, geographers, soil scientists, agronomists, fire ecologists, hydrologists, ecologists, botanists, mammalogists, ornithologists, aquatic and terrestrial entomologists, and fish biologists to guide sustainable land uses in Amazonia (Gardner et al., 2013; RAS, 2019).

Anthropocene Degradation of Nature

Our species is imposing unprecedented pressures on the planet (Díaz et al., 2019). Since the 1950’s we have accelerated several indicators of global change, such as human population size, gross domestic product, water use, carbon dioxide release, biodiversity loss, and pollution of terrestrial and aquatic ecosystems (Sachs et al., 2009; Rands et al., 2010; Steffen et al., 2015; Young et al., 2016; Díaz et al., 2019; Sánchez-Bayo and Wyckhuys, 2019). As a result, we have entered a new geologic epoch in Earth’s history: the Anthropocene (Crutzen and Stoermer, 2000;



Lewis and Maslin, 2015). Human impacts are so strong that our activities currently surpass the effects of natural events, for example, transporting more sediment than all natural forces combined (Hooke, 2000; Wilkinson, 2005). The detrimental effects of such global changes combined with social issues such as political-social ignorance and apathy are severely compromising our ability to maintain our natural world, our well-being and, ultimately, our very own survival (Rockström et al., 2009; Steffen et al., 2015).

Sociopolitical and Scientific Connections Are Needed

In 2015, world leaders adopted the 2030 Agenda for Sustainable Development at the United Nations. Its 6th Sustainable Development Goal is ensuring the availability and sustainable management of water and sanitation for all people, which requires inter-basin cooperation. Despite the necessity of integrating socio-political and environmental efforts, billions of people on Earth lack clean drinking water and 71% face a water security crisis along with increased frequency and severity of

droughts and floods (Vörösmarty et al., 2010; Díaz et al., 2019; Reid et al., 2019).

Regardless of widespread knowledge of human-water interactions, there is rising awareness that much of the available ecological research has been neglected by decision-makers (Giehl et al., 2017). In several countries, politicians ignore scientific evidence in decision-making and public policy, despite ecologists' claims to have solutions for environmental problems (Azevedo-Santos et al., 2017). This condition is evidently induced, and generally favored, by political-economic sectors less concerned with the maintenance of ecosystem quality than instant monetary gains, and continuous unsustainable economic growth; such as widespread unsustainable agribusiness and industry practices (Limburg et al., 2011; Czech and Daly, 2013). However, not only has society ignored scientific evidence, but ecologists and economists have also failed to understand the needs of society (Pilling, 2019; Weber and Ringold, 2019), creating many mismatches between science and policy (Karam-Gemael et al., 2018). This stems from poor communication and little mutual understanding between scientists and other sectors of society (Azevedo-Santos et al., 2017; Fabian et al., 2019).

Therefore, a vast portion of information that is generated is not read or used by non-scientists nor across scientific disciplines, thus preventing the development of economic, land, and governance systems capable of safeguarding water quality and distribution. This must be changed to achieve sustainable levels of population and consumption, and secure the life-support systems underpinning current and future human well-being (Guerry et al., 2015; Ripple et al., 2017; Wantzen et al., 2019).

Therefore, we have two objectives here. First, we offer a multi-disciplinary framework to study and understand ecosystems and ecosystem services with a focus on mountain headwater catchments. Second, we delve into the complex social networks that drive (or should drive) scientific knowledge, environmental management, and transdisciplinary approaches for sustaining ecosystem services, using mountain water management as examples.

DISCUSSION

Humboldt Inspired Transdisciplinary Research and Management

Freshwater ecosystems are best understood through a hierarchical organization divided into several spatial extents: hydrographic basin, segment, site, habitat type, and micro-habitat (Frissell et al., 1986). They are influenced by the surrounding landscape as well as human changes in their flow regimes (Poff et al., 1997; Fausch et al., 2002; Hughes et al., 2019). Thus, a Humboldtian transdisciplinary approach is needed to assess the aquatic biota and the physical and chemical indicators of ecological quality in landscapes and riverscapes that are driven by human activities. Fish and benthic macroinvertebrate assemblages are particularly suitable aquatic biological indicators of these impacts (Resh, 2008; U.S. Environmental Protection Agency, 2016).

Despite von Humboldt's influence, our current scientific practice, including much of ecology (Moret et al., 2019) and geography (Omernik and Griffith, 2014), are diversions from the integrative approach he pioneered and championed. Such disconnection challenges the development of integrative ecological theories, ecosystem conservation, ecosystem management, and ecosystem services. A remarkable case of such dissociation is the usual way ecologists and managers have disassembled terrestrial and aquatic systems from each other in their research and management. For example, by conducting a scientometric assessment of the major aquatic ecology and general ecology journals, we tracked the citation patterns of papers published in prominent journals specializing in aquatic ecology and journals devoted to general ecology. More than 150 years after the publication of the last volume of Humboldt's *Kosmos*, subareas in ecology rarely or weakly interact, thereby fragmenting and segregating knowledge into distinct modules (Modularity $Q = 0.36$, **Figure 2**). Therefore, aquatic and terrestrial ecology and resource management remain fundamentally disassociated.

The many ecosystem services provided by mountain streams exemplify the complexity of mountain ecosystems and their

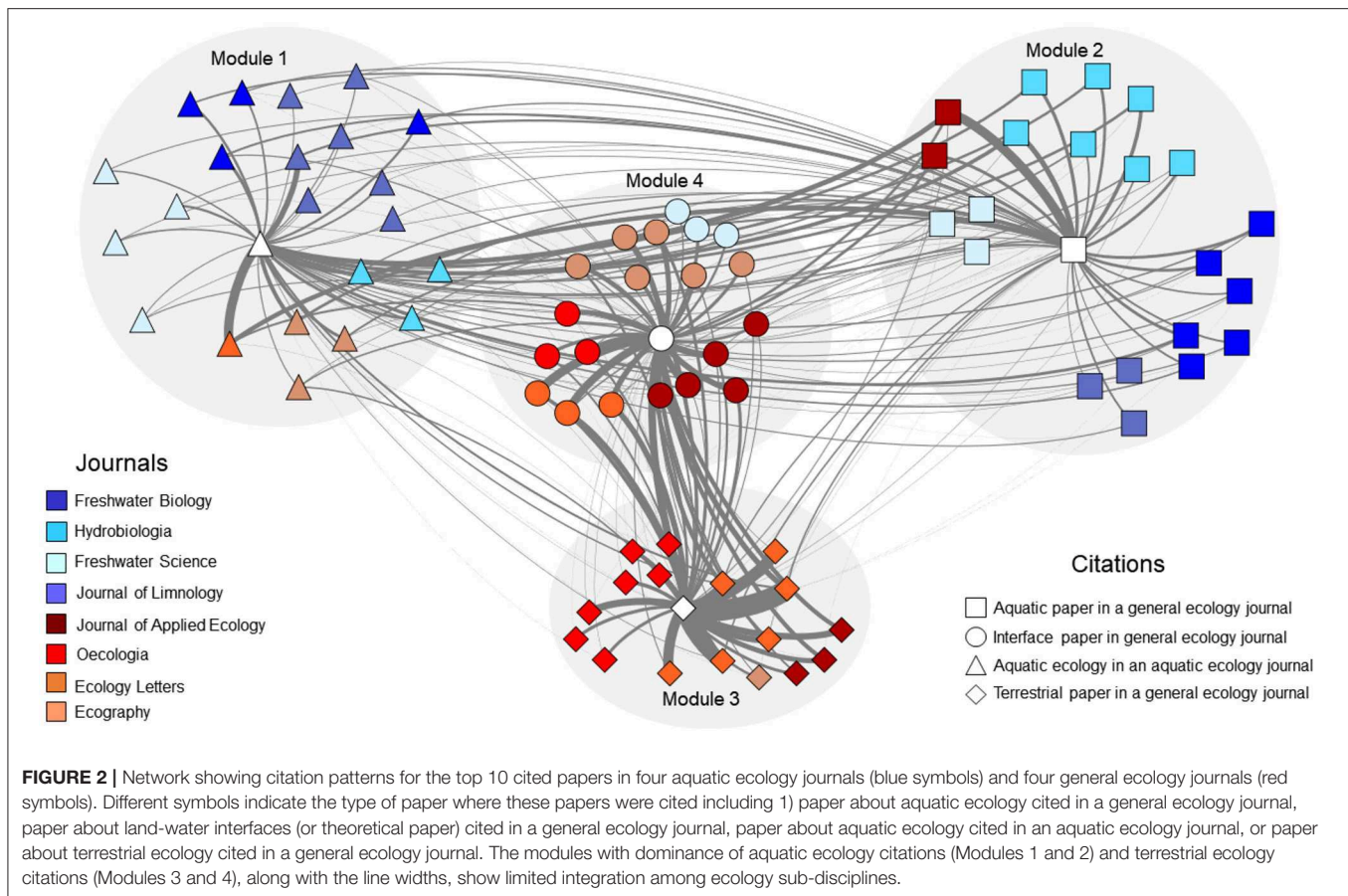
emergent properties. Thus, to understand ecosystems and manage their resources wisely, we need a transdisciplinary approach that combines natural science disciplines and socioeconomics rather than a reductionist approach focused on separate ecosystem components [Forest Ecosystem Management Assessment Team (FEMAT), 1993; Michael, 2017]. In other words, rigorous meta-ecology studies and management of mountain ecosystems need professionals from different fields (e.g., chemists, climatologists, hydrologists, biologists, engineers, landscape ecologists, geographers, economists, sociologists, political scientists, historians, and philosophers), thereby embracing the full complexity of mountains and their surrounding human-natural environments.

Mountain Headwater Streams and Water Governance

Mountains are spatially-defined territories in which complex social-ecological systems can be explored to provide quicker answers to the impacts of global change on ecosystems worldwide (Körner et al., 2011; Encalada et al., 2019; Malanson et al., 2019; Salick et al., 2019). Mountains are home to disproportionately high levels of biological and geological diversity and species endemism (Kier et al., 2005). Yet, they are especially vulnerable to global change drivers including land-use changes, biological invasions, and climate warming because several mountaintop species cannot migrate upwards to track climatic belts associated with cooler temperatures (Colwell et al., 2008; Hoorn et al., 2018). In addition, those species cannot easily migrate to other mountains because of innate dispersal limits and the impermeable intervening lowland environments between other mountaintops (e.g., de Castro Pena et al., 2017). In other words, mountaintops act as islands in a sea of lowlands, as proposed six decades ago by Janzen (1967) and Brown (1971). In the short distance that changes occur in altitudinal gradients we can better understand and predict the synergistic and additive effects of those environmental and social demographic changes, all of which affect biodiversity and ecosystem services (Alexander et al., 2016; Fernandes, 2016).

Being highly biodiverse and providing multiple ecosystem services (Díaz et al., 2019), yet still undergoing rapid changes, mountains are important natural laboratories for long-term studies of global change and ecosystem resilience (e.g., Körner et al., 2017). Many recent initiatives to establish mountain research networks arose from the recognition that mountains provide many ecosystem services essential to human well-being [including water supply (Díaz et al., 2019)]. However, most present-day research networks are focused on geologically young mountains such as the Alps (Steck and Hunziker, 1994) and Cascades (Kaylor et al., 2019). Therefore, we know less about managing ancient tropical montane ecosystems for securing ecosystem services in those ancient, bio-diverse and nutrient-poor landscapes (Hopper et al., 2016; Silveira et al., 2019).

The multitude of abiotic and biotic conditions found in mountains, together with the compression of climatic belts make mountains complex systems (Körner, 2004). Mountain topographies are of critical importance for species distributions,



biodiversity, and fundamental ecosystem services (e.g., Brown, 1971; Brown and Lomolino, 1998; Peters et al., 2019). The strong selective pressures imposed by the low nutritional quality of the derived soils drive the evolution of vegetation with profound effects on biodiversity and ecosystem services (Körner, 2004; Silveira et al., 2016).

The role of mountains in producing ecosystem services is tightly linked to species diversity and vegetation cover (Körner, 2004). In addition, the loss of some species has cascading effects across interaction networks (Barbosa et al., 2019). Therefore, as complex as mountains are, they should be considered as an intertwined system that varies spatially and temporally in the ecosystem services that they provide. Among the main ecosystem services produced in mountain environments, water provision stands out because of the abundance of springs and creeks. The headwater streams of many watersheds are found in their mountains, owing to their unique physiographic and hydrologic features. Beniston (2003) argues that mountains are the sources of over half of the planet's rivers. In fact, 26 of the 30 major rivers in the world (including the Amazon and the Nile) are born in mountain systems and 3.1 billion people live in these drainage systems [Center for International Earth Science Information Network (CIESIN), 2017].

Often the patterns that we observe over a given temporal period or spatial extent are not solely explained by ecological

mechanisms and evolutionary processes acting at the same time (Harding et al., 1998) or extent or even in the same system (Chase et al., 2018). For example, the community composition in a habitat patch of a lake or stream is not simply driven by the environmental characteristics within it, but also by extrinsic factors such as the dispersal of organisms and the connectivity with other suitable patches, including energy and matter exchanges. Thus, to understand human impacts on mountains, we need tools for assessing more extensive spatiotemporal patterns that encompass the connections among systems (Hughes et al., 2019). The meta-ecosystem approach tackles this issue by explicitly considering the interdependency among ecological systems resulting from the flux of organisms (i.e., meta-populations and meta-communities Levins, 1969; Leibold et al., 2004), energy, and matter (Loreau et al., 2003). Specifically, using a meta-ecosystem concept—a set of ecosystems connected by their ecological processes in larger spatial extents (e.g., watershed) (Loreau et al., 2003)—is a promising approach for understanding and conserving mountains. These complex systems offer clear examples of habitat mosaics of terrestrial (cliffs, plains, valleys) and aquatic systems (streams, ponds, wetlands) integrated by energy flow and matter cycling. Therefore, under the same theoretical framework, we understand that ecosystem services are not a function of a single scale or system; consequently, we should also consider

ecosystem-services generated at one place or time but delivered in others.

Because montane headwater streams permanently link highlands to lowlands and their surrounding landscapes, applying the meta-ecosystem framework to mountain headwater streams offers foundations for better conservation strategies across different spatial extents. The clearest example is the pivotal role of mountains as sources of freshwater for downstream lowland ecosystems. However, the flux of matter and energy in a mountain meta-ecosystem is not only downstream (Schiesari et al., 2019). For instance, salmonids seasonally migrate upstream for hundreds of kilometers from the ocean, and atmospheric currents transfer pollutants from lowland agriculture to mountaintops (Landers et al., 2010).

The latest estimates of Díaz et al. (2019) forecast a dire situation for biodiversity and ecosystem services worldwide, with 1 million species threatened by human activities. Ecosystem services are defined as ecosystems' capacities to support human well-being (Millennium Ecosystem Assessment, 2003; Ringold et al., 2013; Hughes et al., 2019). Those ecosystem services extend far beyond the physical boundaries of the stream riparian vegetation and strongly affect lowland ecosystems, including the provisioning of food, water, shelter, nutrient processing, and energy that are provided to hundreds of millions of people worldwide (Payne and Van Itterbeeck, 2017; Hoorn et al., 2018). The safety and well-being of one-fifth, and the water supply for almost half of all people, depend directly or indirectly on the functional integrity of mountain ecosystems (Körner, 2004). Ultimately, mountains play a pivotal role in promoting sustainable development (Glass et al., 2018), but their role in providing ecosystem services should be more rigorously assessed, especially in ancient mountains (Grêt-Regamey et al., 2012). Although it is undisputed that maintaining biodiversity and vegetation cover is essential to secure permanent water supplies (Körner, 2004), such mechanistic relationships remain unclear particularly for geologically ancient mountains. Such old mountains, some dating back to the Pre-Cambrian, are heavily eroded. Consequently, they have relatively low altitudes and soil fertility compared to young mountains, such as the Andes, Alps, Himalayas, and Rockies. It could be argued that drainage in such old mountains would not be paramount for water supply, because these are snow-free mountains whose streams lack contributions from glaciers or snow fields. Nonetheless, their very old, fractured metamorphic geology allows precipitation to seep into ground water and be stored there. These ground waters are important sources of water for lowlands, thereby providing ecosystem services for humans and ensuring the conservation of numerous species of in stream endemic algae, fungi, invertebrates, and macrophytes.

Montane land-water interfaces also help secure ecosystem functioning (Abelho and Descals, 2019), and subsequent ecosystem services, especially in terms of water quality and quantity. Riparian forests help protect water bodies from polluting sources and create essential microenvironments for maintaining ecological processes (Gregory et al., 1991). These processes include the use of riparian zones as biodiversity corridors, as well as critical foraging and nesting sites for

terrestrial wildlife [Forest Ecosystem Management Assessment Team (FEMAT), 1993]. Viewing the energy and matter movements that link riparian and aquatic habitats within the meta-ecosystem approach helps us produce more effective conservation strategies and more sustainable ecosystem services [Forest Ecosystem Management Assessment Team (FEMAT), 1993; Hanna et al., 2018; Tiegs et al., 2019]. In addition, riparian vegetation provides other meta-ecosystem services, such as reducing siltation, soil loss, streambank erosion, surface runoff, and light penetration [Forest Ecosystem Management Assessment Team (FEMAT), 1993]. Likewise, riparian vegetation helps maintain microclimate conditions, biomass and carbon storage, biological diversity, pollinators, and pest controls. Mountains are emblematic examples of natural, cultural and economic landscapes (Messerli, 2012). Therefore, ecologists and environmental scientists must undertake a broad view of sustainable development in mountains and include social scientists in research and management aiming of mountain resources.

Anthropocene Degradation of Mountains

Although mountain ecosystems, particularly headwater streams, provide many services for human populations, they are constantly threatened by a series of unsustainable activities. Among the major pressures to mountain stream biodiversity and ecosystem services, road fragmentation, mining, agriculture, climate change, and diffuse pollution clearly stand out (Hoorn et al., 2018).

Roads on mountains are common consequences of mining, agriculture, silviculture, recreation, and connections between cities. During road construction and use, erosion occurs, increasing sediment loads and petrochemical pollution from vehicles. Roads also facilitate biological invasions (Barbosa et al., 2010) and poorly constructed road crossings on small streams act as barriers to fish movement, leading to riverscape fragmentation (Nislow et al., 2011; Evans et al., 2015; Leitão et al., 2018).

Mining generates pollution, sediments, and hydrologic alterations (Woody et al., 2010; Hughes et al., 2016). Recent collapses of tailings storage facilities in southeastern Brazil and British Columbia (Canada) reveal the catastrophic effects that mining can have downstream (Fernandes, 2016; Hughes et al., 2016). As a consequence of two recent dam failures in southeastern Brazil (2015 and 2019), people have been killed, biodiversity has been eroded, ecosystem services have been compromised or eliminated entirely, and water supplies and the quality of life for tens of thousands people have been degraded (Silveira et al., 2019). Such tailing dam failures are recurrent tragedies in mountains worldwide [Bowker and Chambers, 2017; Santamarina et al., 2019; World Mine Tailings Failures (WMTF), 2019].

Agriculture occurs in numerous mountain systems and areas adjacent to them. The main problems for headwater streams from agriculture are siltation, agrochemicals, channel homogenization, and hydrological alterations. Livestock grazing in mountainous regions is common and has particularly negative effects on riparian vegetation, stream channels, and flow permanence (Beschta et al., 2013). These effects propagate along

river systems, from upstream to downstream, as well as through channel incision and stream bed lowering that erodes from downstream to upstream (Beschta et al., 2013).

Climate change alters hydrological cycles and water temperatures (Isaak et al., 2018) with clear implications for biodiversity and fisheries (Winfield et al., 2016). An example of how climate change threatens water supply recently took place in the ancient mountains of South Africa. The combination of three consecutive dry years, poleward migration of moisture corridors (Sousa et al., 2018), and economic and population growth resulted in a water crisis known as “Day Zero” (Maxmen, 2018). Nearly 3.7 million people experienced unprecedented water shortages. Other episodes of water crisis in major cities such as São Paulo (Brazil) and Barcelona (Spain) suggest urban areas must move from crisis responses to effective management of the water that is essential to lives, livelihoods and environments (Muller, 2018). Globally, we need to embrace governance models that include the interests of the public, governments and companies, as well as biodiversity and ecosystems, when dealing with water issues.

Diffuse pollution is one of the most complex process to understand among the global stressors on mountains. Despite frequently occurring in legally protected areas, mountain ecosystems act as sinks for invisible airborne compounds as indicated by the high levels of pollutants that reach mountaintops of national parks in southeastern Brazil (Meire et al., 2012) and the western USA (Landers et al., 2010). The sources of pesticides are extensive croplands thousands of kilometers from the mountains. These pesticides are carried by wind and condense on moisture particles under the lower temperatures above mountaintops, then fall as precipitation. Similarly, phosphorous is carried as atmospheric dust and reaches minimally disturbed reference sites in the USA (Stoddard et al., 2016) in the same manner that acidic deposition affects mountaintops far from coal-fired power plants (Baker et al., 1991). Nutrient runoff from land use in mountains degrades freshwaters, leading to losses of sensitive species, dominance of tolerant organisms, reduced species diversity, poor assemblage condition (U.S. Environmental Protection Agency, 2016; Jacobson et al., 2019), and establishment or dominance of invasive non-native species (Hughes and Herlihy, 2012; Egan, 2017; Ruaro et al., 2018; de Carvalho et al., 2019; Linares et al., 2019).

Sociopolitical and Scientific Connections Are Needed

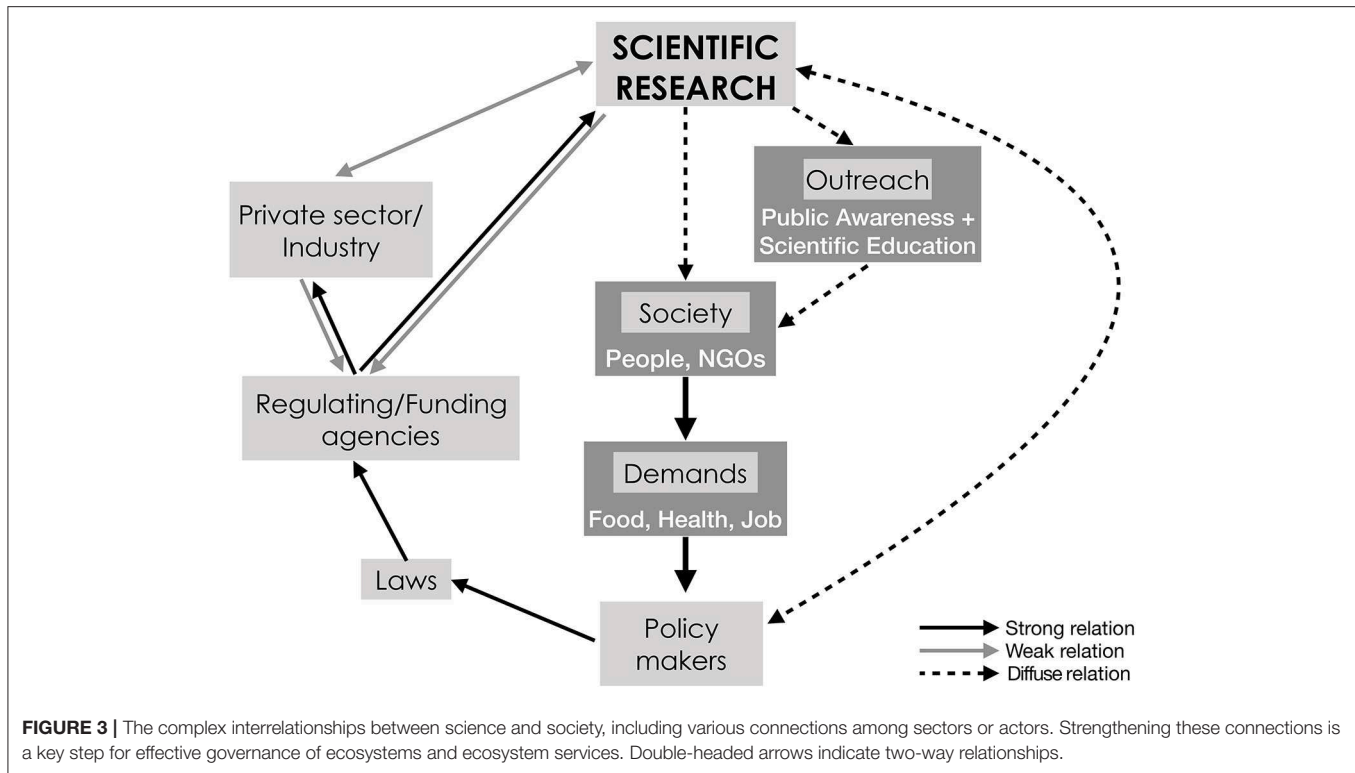
Scientific information is generated at an increasingly rapid rate and at no other time in history has humanity had as much access to information as it does today. However, the abundance of information has not altered the behavior of people who base their decisions on opinions or beliefs. At a time when there is strong political polarization, high quality, trusted information is key to rational environmental governance. In this sense, access to inaccurate information leads us to a situation analogous to the Middle Ages, in which myths thrive, conspiracy theories arise, and much of the public believes that governments, media, and universities are trying to manipulate them (Scheufele and Krause,

2019). Such a situation causes widespread disbelief in science and scientific knowledge. Instead, people support information that suits their current perspective, which is considered cognitive bias and is not necessarily linked to educational or economic level (Lazer et al., 2018).

In addition, politicians and managers have struggled to gather information in a systematic way that is useful for decision making (but see <https://www.conservationevidence.com>). Much information produced by academics is not understood by the public or used by practitioners, hindering environmental solutions (Fabian et al., 2019). Also, during periods of serious economic restrictions, environmental management is seen as a luxury compared with other demands, such as jobs, public safety, or health care. However, in the longer run, environmental negligence has dreadful economic, health, and criminal consequences. But again, such long-term consequences must be made clear to both managers and the public, because managers and the public respond to their most immediate demands (Fabian et al., 2019). Clearly, it is important for scientists to create new strategies of disseminating information to better reach the public, policy makers and managers through alternative media or technologies (da Rocha and da Rocha, 2018).

As with science in general, but especially in ecology, improved education and nature appreciation are lacking or even declining in many nations. In addition, scientists assume that their information is of paramount importance, yet they claim to be constantly ignored by policy makers. Why is that? We believe that there are widespread misunderstandings, disconnects or weak links between the multiple components of education and environmental management (Figure 3). One of the roots of those mismatches lies in society's expectation of immediate solutions from their leaders for their current concerns and needs—without thorough understanding or appreciation of the complex nature and basal causes of those problems. As a result, those short-term solutions often create substantial, negative external effects as discussed above. In addition, scientists generally overlook or are unaware of the many other components that drive sociopolitical decision-making.

How might we best resolve these dilemmas? We need leaders and a public that know and agree with science and that also care about nature, ecology, and ecosystem health (Wood, 2014). We also need scientists who are willing to engage with the society and policymakers at multiple levels of government and society. To achieve that belief and engagement, we need improved formal and informal public education, both in schools and in all types of media. And the foundation of that education originates in universities and their products, including the traditional path of scientific publications, but also effective actions promoting open debates and the dissemination of scientific knowledge with the general public (i.e., outreach activities). Although certainly much more is needed in terms of scientific publications related to ecological processes involved in meta-ecosystems and the associated ecological theories and applications, we believe this kind of product is well done by researchers. On the other hand, there is an evident failure to go beyond the walls of the universities and the pages of scientific journals to effectively communicate our science to the public (Figure 3). Therefore,



it is important to improve university extension programs by having university administrators recognize and reward extension research and education that directly meets stakeholder and public information needs (Lewinsohn et al., 2015). In addition, extension publications and other forms of information must be better translated so that they are understood by society and elementary and secondary school teachers. Such information might include field exercises, teacher training workshops, webinars, and hands-on assistance from university students and professors (e.g., Stapp, 1978; França et al., 2019). Improving research and information exchanges with NGOs, civil society, industries, and multiple local, state and federal agencies also leads to more socially useful research and better educated societies (Allen et al., 2019).

Assuming that scientifically and ecologically literate leaders will eventually result from the public education process, we presume that much more ecologically and environmentally comprehensive and protective statutes will be developed as they have been in some nations [e.g., United States of America, 1972; Forest Ecosystem Management Assessment Team (FEMAT), 1993; Whittington et al., 2001; Minas Gerais, 2008; European Community, 2017]. Those, in turn, should lead to better agency management, stakeholder practice, and environmental conditions. Clearly, such changes are directly and indirectly expensive. But consider how much international money and expertise are dedicated to understanding interstellar space and seeking bacterial fossils on Mars, instead of knowing our own planet (Mayr, 2004). Therefore, ecologists, the media, and writers must do a better job of exciting the public and leaders, in the same way that science fiction movies and books have functioned to stimulate funds for space science and exploration. Certainly, if

the society and leaders can become interested in funding studies of the infinity and timelessness of the universe, they can become interested in global ecological concerns and the world we are creating for future generations.

There is a widespread expectation that government leaders can provide jobs and money to meet public demands. Although citizens dislike taxes, politicians have the legal strength to stimulate sustainable enterprises that could create such employment. But there are direct links between economic and population growth and environmental degradation (Czech and Daly, 2013; Hughes, 2014; Green Party US, 2019; Pilling, 2019). This is true throughout the whole society, including basin committees, financing entities, regulatory agencies, universities, and professional scientific societies. Being reductionist, we focus on parts—not von Humboldt's whole natural and social systems. However, the complex nature of such relations allied with the weak links among all sectors and actors hinder translating ecological research and its benefits to society, as well as translating managers' and citizens' needs to scientists (Figures 2, 3). Strengthening those links is critical for effective governance of ecosystems worldwide, including mountain headwater streams.

CONCLUSIONS

The re-emergence of a post-truth society in recent years has significantly contributed to the environmental crisis we experience today. However, looking back to achievements can inspire current generations, driving us toward better environmental governance. Humboldt taught us the value of integrating environmental and social sciences to understand the nature of the world and human societies. To solve our

current and future environmental and social problems, we must follow his iconic example and integrate multiple disciplines such as ecology, geography, engineering, mathematics, anthropology, economics, sociology, and political science to create transdisciplinary solutions. Therefore, we call for a multi-disciplinary and transdisciplinary, science-public strategy to safeguard worldwide biodiversity and its associated ecosystem services. By adopting this strategy, we believe that we can shift environmental concerns from their current high-risk zones to a safe-operating zone.

AUTHOR CONTRIBUTIONS

All authors conceived the rationale of the study and wrote the manuscript.

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Current Status of Wetlands in Srinagar City: Threats, Management Strategies, and Future Perspectives

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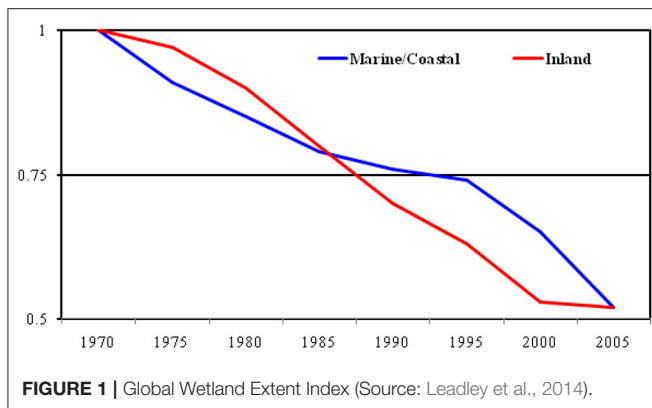
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Wetlands are the most diverse, highly dynamic, productive, and ecologically sensitive areas in Earth. In Kashmir Himalaya, Srinagar city is bestowed with a large number of picturesque wetlands. These wetlands are important in regulating ecosystem services such as providing fresh water supplies, food products, fisheries, water purification, harbor biodiversity, and regulation of regional climate. These are also important as socio-economic support systems for the city inhabitants and valued as habitats of migratory birds that visit Kashmir valley from different continents of the world. Owing to the increased rate of anthropogenic activities and anthropogenically driven changes in natural processes, these wetlands are degrading at an alarming rate, seriously affecting their health and water quality. The major threats to wetlands include pollution, land use and land cover changes, urbanization and encroachments, and climate change. The intensive agricultural practices, introduction of exotic species, and changes in hydrological flows during the past few decades have resulted in degradation of wetlands over this region. Sustainable management of wetlands is crucial as these ecosystems offer an array of ecological functions that sustain livelihoods all over the world. This review provides special insights about the significant changes in spatial scale, land use and land cover changes, and water quality of major wetlands in Srinagar city.

Keywords: wetland ecosystems, land system changes, water quality, management strategies, Srinagar, Kashmir Himalaya

INTRODUCTION

Wetlands are ecosystems intermediate between aquatic and terrestrial systems, which are permanently or seasonally covered with shallow water (Mitsch and Gosselink, 1986). They occupy ~6% of earth's land surface (Maltby, 1988). Wetlands are productive (Ghermandi et al., 2008) and biologically diverse ecosystems (Keddy et al., 2009). They provide numerous socio-economic and ecosystem services (Prasad et al., 2002; Ramsar Convention Bureau., 2002) including wildlife habitat, maintenance and conservation of biodiversity (Mitsch and Gosselink, 2007; Whitehouse et al., 2008), water purification (Brown et al., 2000), fisheries and recreation (Keddy, 2010; Junk et al., 2013), flood control (Penatti et al., 2015), water supply (Lemly, 1994), nutrient removal (Raich and Schlesinger, 1992), carbon sequestration (Turner et al., 2000), and environmental restoration (Fink and Mitsch, 2007; Moreno et al., 2007). Wetlands serve as a means of livelihood for rural



populations (Turyahabwe et al., 2013; Lamsal et al., 2015), particularly in developing nations and are greatly valued by many cultures (Ghermandi et al., 2010; Maltby and Acreman, 2011). Owing to the high potential of wetlands for agricultural productivity, fisheries, and water supply, many of the wetlands of the world have been historically relied upon by human civilizations. In spite of the ecosystem functions and sustenance of human livelihoods, 30–90% of the wetlands of the world are strongly modified or lost (Junk et al., 2013; Reis et al., 2017) and many remain threatened and degraded due to high population pressure and urbanization (Central Pollution Control Board, 2008; Bassi et al., 2014). Davidson (2014) reviewed 189 reports and estimated wetland losses as 64–71% in the twenty-first century. There was a decline of 69–75% in the extent of inland wetlands and 62–63% decline in the extent of coastal wetlands. Wetland losses continue in the twenty-first century. Leadley et al. (2014) found the Wetland Extent Index and estimated ~40% decline in coverage of both inland and coastal/marine wetland ecosystems during last 40 years due to fragmentation and degradation (Figure 1).

Presently, the wetland ecosystems are under tremendous stress due to massive land system changes and infrastructure development (Pramod et al., 2011), as well as intensification of agricultural and industrial activities (Bassi et al., 2014), manifested by the decline in their areal extent resulting in a decline in the hydrological, economic, and ecological functions (Bassi et al., 2014). This has led to adoption of various policies and approaches for conservation, protection, and management of wetlands [Ministry of Environment and Forests (MoEF), 2006].

CURRENT STATUS OF WETLANDS IN SRINAGAR CITY

Srinagar city in Kashmir Himalaya has a rich natural heritage of magnificent lakes and picturesque wetlands (Figure 2) lying along the floodplains of river Jhelum, which are famous waterfowl habitats (Kaul and Pandit, 1980; Habib, 2014). Besides being a source of attraction for tourists from all over the world, these freshwater ecosystems of the Kashmir Himalaya have been playing a great role in the socio-cultural activity and economy (Kaul and Pandit, 1980; Pandit, 1982) of the valley since ancient

times. They are a great source of natural products like fish, fodder, vegetables, tourism, and a variety of economically important aquatic plants (Pandit and Qadri, 1990; Bano et al., 2018). However, over the last few decades, the deteriorating water quality (Verma et al., 2001; Rashid et al., 2017a) and land system changes (Romshoo and Rashid, 2014; Rashid and Aneaus, 2019) including encroachment of otherwise notified wetland areas and depleting stream flows (Mitsch and Gosselink, 2000; Showqi et al., 2014; Romshoo et al., 2015) have impacted their health (Iwanoff, 1998; Chauhan, 2010; Naja et al., 2010; Reza and Singh, 2010).

Nowadays, wetlands are being recognized as “wastelands” serving as grounds for a variety of waste materials (Khan et al., 2004; Bano et al., 2018). The increasing trend of conversion of agricultural lands into urban areas is currently one of the dominant patterns of land use change in the valley of Kashmir (Rashid and Romshoo, 2013; Rashid et al., 2017b). This pattern of land use change has the potential to alter the composition and functional processes of wetlands by changing the hydrological regimes and sedimentation processes besides the flux of nutrient materials. The ecological consequences of agricultural runoff and municipal wastewater discharges have resulted into widespread eutrophication (Khan and Ansar, 2005; Badar et al., 2013a). The conversion of forested and agricultural areas into built up areas has impaired the water quality (Rather et al., 2016) that has led to the extirpation of local populations of aquatic species. As a result, many freshwater wetlands have altogether vanished or are facing severe anthropogenic pressures. The harmful social, financial, and ecological impacts of declining biodiversity and degrading water quality are a matter of concern (Verma et al., 2001; Bassi et al., 2014). Most of these wetlands used to act as buffers soaking flood waters but the encroachment and infrastructure development within these wetlands has reduced their water holding capacity, increasing the vulnerability of people toward flooding (Romshoo et al., 2017). The central business hub of Srinagar, the capital city, is often affected during a normal precipitation event as the drainage channels that used to drain out storm water runoff have mostly been taken over by concrete surfaces (Rashid and Naseem, 2008). The changes in the spatial extent of lakes and wetlands in Srinagar are presented in Table 1. As a result of unplanned urbanization, encroachments, and population pressures, nearly 91.2 km² of wetland area has been lost between 1911 and 2004 (Rashid and Naseem, 2008).

Anchor Lake

Anchor is a semiurban, single basin lake situated between 34°07'–34°10' N latitudes and 74°46'–74°48' E longitudes at an altitude of 1,583 m above mean sea level (a.m.s.l.). The lake is situated about 14 km from Srinagar city on the northwestern part. The lake covered an area of 19.54 km² during 1893–1894 (Lawrence, 1895). Since then, the area of the lake declined substantially to 6.5 km² (Jeelani and Kaur, 2012). The current area of the lake is 4.26 km² (Sushil et al., 2014; Fazili et al., 2017). The water supply of Anchor Lake is maintained by Sindh, a tributary stream of Jhelum and Achan Nallah in addition to springs along the vicinity of lake. The lake has a vast catchment area that comprised a mixture of residential, forest, agricultural,

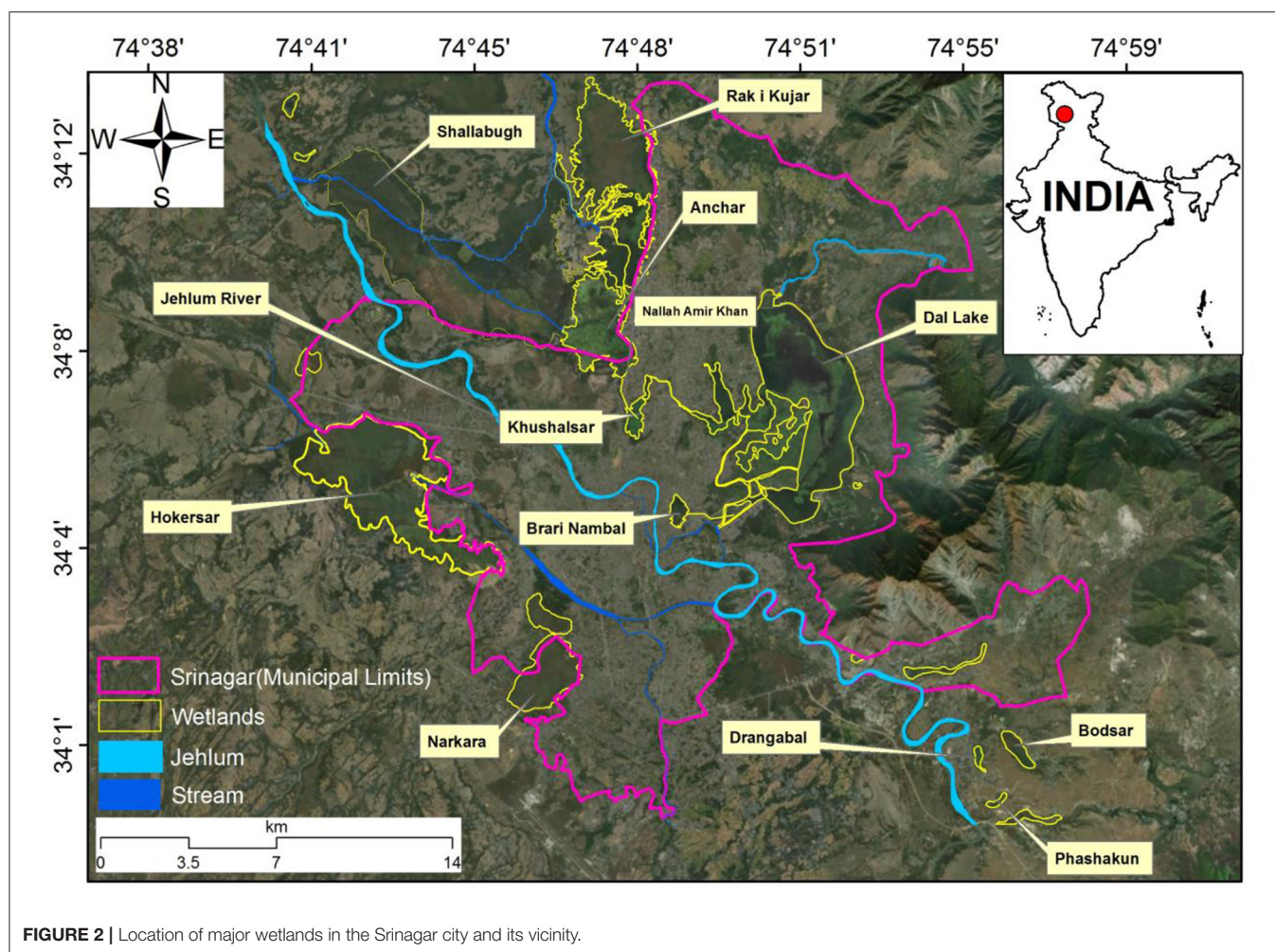


FIGURE 2 | Location of major wetlands in the Srinagar city and its vicinity.

TABLE 1 | Changes in the spatial extent of lakes and wetlands of Srinagar between 1911 and 2004 (Source: Rashid and Naseem, 2008).

S. No.	Class name	Area (km ²)	
		1991	2004
1	Open water surface	40.00	30.65
2	Wetland/marshy area	134.25	64.07
3	Built-up land	17.45	107.91
4	Others	505.05	494.13
	Total	696.77	696.77

and horticultural land (Jeelani and Kaur, 2012; Bhat et al., 2013). The last few decades have resulted in the decline of the water quality of the lake (Farooq et al., 2018; Table 2). The main causes of degradation of Anchar Lake are anthropogenic activities, encroachments, sewage, and dumping of domestic wastes including polythene, clothes, plastic bottles, and effluents from hospitals and wastewater treatment plants (Najar and Khan, 2012; Bhat et al., 2013; Fazili et al., 2017).

TABLE 2 | Long-term water quality changes in Anchar Lake (Source: Kaul, 1977; Kaul et al., 1978; Farooq et al., 2018).

Parameter	1970–1972	1975–1976	2018
pH	7.4–9.6	7.5–9.5	7.2–8.3
Dissolved oxygen (mg L ⁻¹)	6.88–12.32	4.2–10.85	3.5–6.5
Conductivity (μS cm ⁻¹)	132–385	388–555	200–475
Total alkalinity (mg L ⁻¹)	53–80	75–130	100–399
Ca (mg L ⁻¹)	16–30	22–24	48.5–74.5
Mg (mg L ⁻¹)	10–14	9–13	5.3–9.9
PO ₄ -P (μg L ⁻¹)	9–25	12–29	182–698
NO ₃ -N (μg L ⁻¹)	90–57	95–580	558–641
NH ₄ -N (μg L ⁻¹)	70–85	5–18	231–381
Total P (μg L ⁻¹)	-	92–666	550–910
Cl (mg L ⁻¹)	8–10	-	23.5–42

Dal Lake

Dal is an urban lake, situated between 34°5′–34°6′ N latitude and 74°8′–74°9′ E longitude at an altitude of 1,584 m a.m.s.l. The lake has been formed due to fluvial activity of river Jhelum

TABLE 3 | Land system changes within Dal lake from 1859 to 2013 (Source: Rashid et al., 2017a).

Class name	Area (km ²)								
	1859	1903	1962	1972	1979	1992	2001	2010	2013
Aquatic vegetation	2.91	1.35	3.85	8.23	9.42	7.75	8.75	10.40	8.64
Builtup	0.05	0.06	0.84	0.68	0.80	1.83	2.10	2.03	2.02
Floating gardens	0.78	0.82	5.66	1.1288	1.39	1.36	2.52	2.70	2.89
Marshy land	1.49	1.44							
Plantation	6.02	3.99	3.63	3.16					
Water	20.59	23.98	13.84	13.19	12.41	13.10	10.68	8.91	10.50
Total	31.84	31.64	27.82	26.40	24.02	24.04	24.04	24.04	24.04

(Rather, 2012) and is fed by Dagwan stream (Sabha et al., 2019). The lake is under high stress due to anthropogenic influences (Badar et al., 2013b; Khanday et al., 2018). During 1200 A.D., the extent of Dal Lake was about 75 km² (Wani et al., 2013). The lake covered an area of ~32 km² in 1859 and has shrunk to 24 km² (including the lake interiors) mainly due to the expansion of settlement areas and proliferation of settlements (Rashid et al., 2017a). The lake was abundantly supporting sensitive aquatic macrophytes including *Eurayle ferox* (Lawrence, 1895; Mukerjee, 1921) and *Chara* sp. (Mukerjee, 1921), but as the pollution and eutrophication of the lake continued, the species were pressed to extinction from the lake (Kak, 2010). While it is believed that boatmen (locally known as *Ha'enz*) are the main culprits responsible for changing land use and land cover of the lake (Fazal and Amin, 2012), there are policy failures that have led to the majority of the lake area being highly deteriorated. The land use patterns and land cover of the lake are presently composed of open water (10.5 km²), aquatic vegetation (8.64 km²), floating gardens (2.89 km²), and settlements (2.02 km²) (Rashid et al., 2017a). The historical changes in the land system of the Dal Lake are shown in **Table 3**.

During the last 50 years, the rapid increase in houseboats, population pressure, encroachment, urbanization, pollution, and sewage has resulted in the decline of the quality of lake water (Amin et al., 2014). About 1,200 houseboats (Fazal and Amin, 2012) present in the lake are a major source of untreated sewage and pollution to the lake (Tanveer et al., 2017). The historical water quality changes in Dal Lake are reflected in **Table 4**.

Brari Nambal

Brari Nambal is a marshy lagoon situated between 34°05'12.88"N and 74° 48'50"E in Srinagar city. It is connected to the Dal Lake via a channel on the eastern side. Previously, there was an outlet channel known as *Nallah Mar/Mar* canal that used to provide navigability to Dal Lake to Anchar Lake via Khushalsar (Tantray and Singh, 2017). In addition, the Mar canal used to take the excess water from Brari Nambal to Khushalsar Lake. However, the channel was filled and converted into a motorable road during the 1970s (Wani et al., 2014), which resulted into the alteration of the hydrology (**Figure 3**). Brari Nambal has a narrow outlet on the western side and drains into river Jhelum through an underground channel. In 1971, Brari Nambal covered an area

TABLE 4 | Water quality changes in Dal ecosystem (Source: Trisal, 1977; Abubakr and Kundangar, 2009; Khanday et al., 2018).

Parameter	1974–1976	1985	1996–1997	2006–2007	2018
Dissolved oxygen (mg L ⁻¹)	10.25	8.7	8.6	6.8	7.07
Total alkalinity (mg L ⁻¹)	69.5	85.6	104	115	101.75
Nitrate nitrogen (μg L ⁻¹)	481	483	272	539	400
Ammoniacal nitrogen (μg L ⁻¹)	23.6	37.0	362	438	40
Ortho phosphate phosphorous (μg L ⁻¹)	65.5	80.5	135	93	40
Total phosphorus (μg L ⁻¹)	187.8	211.5	768	615	200
Total dissolved solids (mg L ⁻¹)	30.2	32.2	119.8	20	–

of 1 km² (water body—0.28 km² and Marshy area—0.72 km²) which reduced to 0.77 km² by 2002 (water body—0.21 km² and marsh—0.56 km²) (Fazal and Amin, 2011).

The population pressure, pollution, encroachments, and urbanization have led to great stress on the wetland, thereby deteriorating the water quality to the verge of extinction. Most of the sewage generated in the vicinity is treated at a sewage treatment plant constructed on the southern area of the wetland. The outflows from the sewage treatment plant have become a major source of pollution and nutrients to the wetland. The sewage treatment plant has failed in its operation as per prescribed norms; as a consequence, it discharges partially treated sewage, thereby turning the wetland into a gutter (Mukhtar et al., 2014).

Gilsar and Khushalsar Lakes

Gilsar and Khushalsar are twin lakes in highly deteriorated condition located toward the northwest of Srinagar city. The lakes receive waters from the Nigeen basin of Dal Lake via a water channel—Nallah Amir Khan (Nissa and Bhat, 2016). The total area of the lake is 1.06 km², and the average depth of the lake is

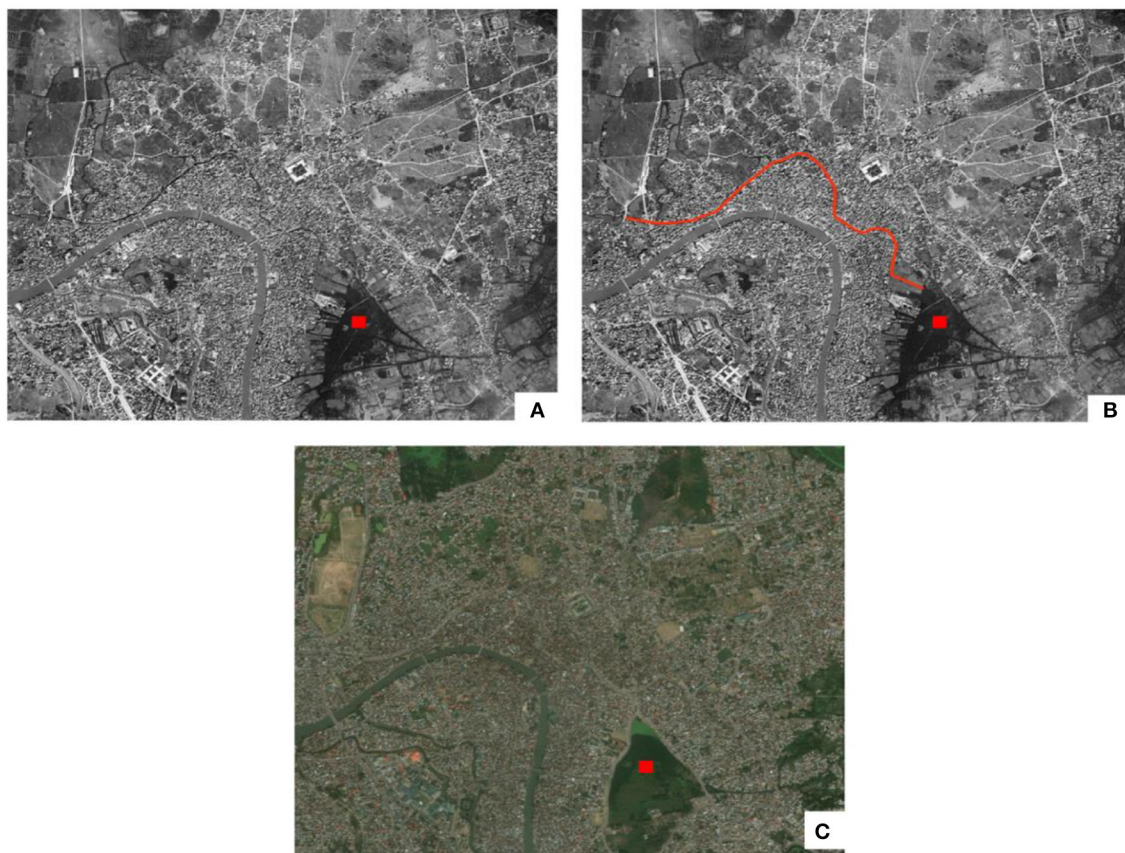


FIGURE 3 | Filling up of Mar Canal as seen from earth observation data **(A,B)** Declassified CORONA spy images showing Mar canal in 1965. Red Line in **(B)** indicates Mar canal. **(C)** Road built by filling up the Mar Canal. Red dot indicates Brari Nambal.

3.6 m. The lakes have been encroached upon at many places with illegal construction and landfilling (Chowdhury, 2017). These lakes receive sewage inputs estimated about 465 million liters per day (MLD) from the catchment containing ~ 2 metric tons (MT) of phosphorus and 1.71 MT of nitrogen (Kundangar, 2002) that has resulted into the proliferated growth of aquatic weeds and subsequent degradation of water quality.

Hokersar Wetland

Hokersar is the queen of the wetlands of Kashmir valley situated between $34^{\circ} 06' N$ latitude and $74^{\circ} 05' E$ longitude having an altitude of 1,580 m a.m.s.l. in the northern part of Doodhganga catchment, 10 km west of Srinagar city. The water supply of Hokersar wetland is maintained by the Doodhganga stream on the eastern side and by the Sukhnag stream on the western side. The depth of the wetland varies from a maximum of 2.5 m to a minimum of 0.7 m during spring and autumn, respectively. Hokersar is a game reserve and habitat for about 2 million species of migratory birds of Europe, Siberia, and Central Asia. The marshland supports various ecological and economic services, which include fisheries, food products, freshwater, and purification of water, and regulates global climate (Davis, 1993; Romshoo and Rashid, 2014). The wetland supports a broad

range of hydrological functions, for example, regulation of floods, recharge of groundwater, control stream flow (Joshi et al., 2002), and carbon sequestration (Romshoo and Rashid, 2014). In this context, the wetland was designated as a Ramsar site in November 2005. Due to increased human intervention and changing natural processes (Joshi et al., 2002), the area of the wetland has declined from 18.75 km^2 in 1969 to 13.00 km^2 in 2008 (Romshoo and Rashid, 2014; **Table 5**). This wetland has lost 5.75 km^2 of area during the last four decades (Romshoo and Rashid, 2014). During the last two to three decades, macrophytic species like *Acorus calamus*, *Euryale ferox*, and *Nelumbo nucifera* within the wetland had disappeared (Khan et al., 2004). The wetland is now choked by invasive species like *Azolla* spp., *Salvinia natans*, and *Menyanthes* spp. (Khan et al., 2004; Bano et al., 2018). The increased silt load from the catchment area due to deforestation of higher reaches is the possible cause for the disappearance of the species and depletion of water depth, which has been reported to have reduced from 1.12 m (Pandit, 1980) to 0.63 m (Rather and Pandit, 2002). It is also pertinent to mention that during the last few decades, the water quality of the wetland has deteriorated (Shah et al., 2019) severely (**Table 6**), mainly attributed to urbanization in the vicinity (Romshoo et al., 2011).

TABLE 5 | Area covered by different land use land cover types from 1992 to 2008 within Hokersar wetland (Source: Romshoo and Rashid, 2014).

Class name	Area 1992 (km ²)	Area 2001 (km ²)	Area 2005 (km ²)	Area 2008 (km ²)	Change from 1992 to 2008 (km ²)	% Change
Agriculture	4.26	3.69	3.23	4.95	0.69	3.69
Aquatic vegetation	2.5	3.48	4.56	4.46	1.96	10.73
Built up	0.01	0.05	0.12	0.11	0.1	0.55
Fallow	0.88	0.21	0.27	0.48	−0.4	−2.22
Marshy	7.74	8.06	7.27	5.62	−2.12	−11.86
Open water	0.85	0.43	0.31	0.36	−0.49	−2.72
Plantation	1.82	2.18	2.32	2.16	0.34	1.83
Road	0.03	0.03	0.03	0.03	0	0

TABLE 6 | Range value of various chemical parameters of Hokersar wetland (Source: Kaul et al., 1978; Kaul and Trisal, 1985; Shah et al., 2019).

Parameter	1978	2012–2013
Oxygen (mg L ^{−1})	3.2–12	2.4–10.1
pH	7.2–9.0	7.1–8.2
Alkalinity (mg L ^{−1})	85–256	91.3–254.7
Conductivity (μS cm ^{−1})	216–348	210–381
Cl (mg L ^{−1})	0.0–79.0	10.3–39.3
Dissolved inorganic phosphate phosphorus (μg L ^{−1})	19.0–47	-
Total phosphate phosphorus (μg L ^{−1})	11.2–306	134–390
NO ₃ -N (μg L ^{−1})	104–327	226.3–631.3
NH ₄ -N (μg L ^{−1})	3.0–10.0	16.7–242.3
SiO ₂ (mg L ^{−1})	3.0–9.0	-
Ca (mg L ^{−1})	24–61	44.2–107.2
Mg (mg L ^{−1})	11.0–18.0	6.2–28.1

BRIEF INVENTORY ON SERVICES PROVIDED BY WETLANDS

Having only a coverage of 0.6% of the Earth's surface, wetlands supply a tremendous proportion of ecosystem services such as recreational amenities, flood control, storm buffering, biodiversity, climate regulation, and socio-cultural values. They are important habitats for biodiversity contributing to primary productivity and home to many important migratory birds. Thus, wetlands have a diverse fauna with a relatively large number of endemic species. Goods and services derived from wetlands include livestock and cultivation, fisheries, fiber for construction and handicraft production, fuel wood, hunting for water fowl and other wildlife, aesthetic value of wetlands, storm buffering, flood water storage and stream flow regulation, water flow, sediment and nutrient cycling—water quality improvements, erosion control, carbon sequestration—climate change and mitigation, and cultural knowledge and traditions (Eftec, 2005). Wetlands are believed to have distinctive ecological features, which provide various goods and services to mankind. They constitute a natural resource of great economic, scientific, cultural, and recreational

TABLE 7 | Ecological and socio-economic importance of wetlands.

Services	Brief description
Regulating	
Air quality regulation	The wetland ecosystems have the ability to maintain air quality by extracting aerosols and chemical compounds from the atmosphere.
Climate regulation	Wetlands through their biologically mediated processes stabilize the micro-climate of the region, however at global scale; they moderate climate vagaries through the land cover and other processes.
Hydrological regimes	Wetlands regulate hydrological cycle and regulate water regime through ground water recharge, evapotranspiration, and by capturing and gradually releasing the water.
Pollution abatement and detoxification	Wetlands drastically reduce the nutrient input from through flowing surface and sub-surface run-off. The biotic and abiotic factors in the wetlands lead to the detoxification of the pollutants and xenic compounds carried into the ecosystem.
Erosion control	Wetland vegetation and biota reduce the erosion of soil through sediment binding and reducing current velocity.
Natural hazard mitigation	Wetlands help to lessen the negative impact of flooding by soaking up the water and reducing the speed at which flood water flows.
Biological regulation	Wetlands regulate population structure of the ecosystem through trophic relation.
Provisioning	
Food	Production of edible plants like <i>Nelumbo nucifera</i> and animals like fish (<i>Schizothorax</i> sp., <i>Cyprinus</i> sp.).
Fresh water	The biotic and abiotic processes taking place in the wetland ecosystem enhances water quality. Wetlands have vast ability to meet municipal water supply in the neighboring areas.
Fuel and fiber	Wetlands are home to a number of species or abiotic components with potential use for fuel or raw material.
Biochemical products and medicinal resources	Wetlands act as huge reservoirs of potentially beneficial chemical compounds having tremendous medicinal and cosmetic properties.
Genetic materials	Wetlands represent areas of high biological diversity and support gene pools of the most diverse assemblages of a wide variety of flora and fauna.
Ornamental species	Wetlands provide vital habitat for many species and other abiotic resources with Ornamental value.
Socio-economic	
Cultural heritage and identity	Wetlands symbolize the culturally significant landscapes by creating the sense of belongingness for certain features and species it beholds.
Tourism and recreational	Wetlands provide panoramic views of landscapes having humongous recreational potential for tourism.
Aesthetic	The aesthetics provided by the wetlands is based on greenness, tranquility, and diversity.

value. Wetland characteristics such as biodiversity, abiotic components, and ecological processes regulate a large number of functions that are first transformed into a list of services that can then be measured in appropriate units (biophysical or otherwise) and later used for economic valuation. Ecosystem functions represent the potential for benefits that may or may not be used directly by humans. Usually, the same function is linked to two or more ecosystem services. Some of the vital functions of the wetlands are listed in **Table 7**.

TABLE 8 | Threats perceived by wetlands of Srinagar city.

S. No.	Threats	Perceived impacts
1.	Floating gardens	Construction of floating gardens is a major threat to the lakes and wetlands of Srinagar city. This practice has resulted in conversion of large areas of open water into floating islands. The impact of these floating gardens is very grave, and unless this practice is stopped, the wetlands would be under a severe threat.
1.	Willow plantations	The creation of a network of willow plantations and populus trees within the wetlands (Anchar, Brari Nambal, Dal, Hokersar, Gilsar, and Khushalsar) is a serious threat to wetland ecology. These plantations have obstructed natural flow of drainage, causing deleterious morphological, hydrological, and ecological changes to wetlands.
2.	Houseboats	The direct discharge of untreated sewage from some 1,200 houseboats located in Dal lake is a growing threat to the lake ecosystem. This has resulted into deterioration of water quality, prolific growth of aquatic macrophytes, and pollution of the lake (Parvez and Bhat, 2014).
3.	Mechanical machines/harvestors	The mechanical dredgers and de-wedeers used in wetlands of Srinagar City (Dal lake, Brari Nambal) for removal of sediments and aquatic macrophytes has resulted into loss of species of fish, macro-invertebrates, zooplankton, and other biologically important organisms from the wetlands (Ali, 2014).
4.	Sewage treatment plants (STPs)	Pollutant loads from point sources (STPs) constructed along the banks of Dal lake and Brari Nambal has become the main source of pollution for these water bodies. The STPs have failed to operate as per prescribed norms resulting into increased nutrient loadings mostly nitrogen and phosphorus, organic matter, metals, pathogen, nutrients, and supplementary water pollutants to the wetlands (Mukhtar et al., 2014).
5.	Hospital effluents	The effluents discharged from SKIMS into Anchar lake has resulted into increased nutrient concentrations and toxic compounds into lake ecosystem that are very harmful for lake ecology and sometimes very toxic for fish and other aquatic biodiversity (Bashir et al., 2017; Gudoo et al., 2017).
6.	Red tide	The unprecedented occurrence and frequent seasonal recurrence of red tide in lakes and wetlands of Srinagar city constitutes a new environmental threat to the aesthetics and biological diversity of wetlands. The occurrence of red tide is due to ingress of untreated sewage from nearby residential areas to lakes and wetlands of Kashmir valley (Khan, 2000).
7.	Urban sprawl	Unplanned urbanization in the vicinity of wetlands in Srinagar is the biggest threat and an important cause for the reduction of wetland extent (Kuchay and Bhat, 2014; Romshoo and Rashid, 2014).
8.	Hydrological alterations	Hydrological changes including alterations in inflows and hydrological makeup by surface water extraction, water diversions, and stream channelization for a variety of human uses.
9.	Climate change in Himalayas	Recession of glaciers in Himalayas and changes in precipitation have a significant impact on wetlands and their associated species. Anticipated changes in regional climate could be one of the main drivers besides anthropogenic factors for loss of wetlands in Kashmir region given the depleting streamflow scenario as observed across Kashmir valley (Rashid et al., 2015; Zaz et al., 2019).
10.	Exotic species	Introduction of exotic species either deliberately or naturally into lakes and wetlands of Srinagar city has resulted into changes in wetland ecology, biodiversity, land uses, and water quality. For example, exotic fish species <i>Cyprinus carpio</i> introduced in Dal lake have eliminated the native <i>Schizothorax</i> sp. from Dal lake.
11.	Siltation and erosion	Siltation and erosion of sediments from the catchment areas is a major threat to the wetlands of the city. These result in loss in water spread area of wetlands.
12.	Roads	Constructions of roads within Dal and Khushalsar have not only fragmented these water bodies into sub-basins but also impacted the hydrology.

THREATS TO WETLANDS

Among the freshwater ecosystems, wetlands are the most widely used and are heavily exploited for sustainability and livelihood (Molur et al., 2011). The main threats to wetlands in Srinagar city are attributed to anthropogenic pressures that include urbanization (Farooq and Muslim, 2014), land use changes (Fazal and Amin, 2011), and large-scale encroachments (Wani and Khairkar, 2011; Kuchay and Bhat, 2014) in the catchment as well as in the wetlands itself (Rather et al., 2016). Besides, natural siltation associated with the anthropogenic siltation brought about by deforestation in the catchment areas has also been an important factor resulting in the loss of wetlands (Pandit and Qadri, 1990; Pandit, 1991; Shah et al., 2017; Amin and Romshoo, 2019). Another important driver of the loss of wetlands comes in the form of problems relating to drainage (Romshoo et al., 2017; Alam et al., 2018). The huge inflow of sewage from the catchment areas into the water bodies has resulted in excessive macrophytic growth (Dar et al., 2014). The main causes of wetland degradation in Srinagar are summarized in **Table 8**.

MANAGEMENT OF WETLANDS

In Srinagar, wetland ecosystems are continuously seen as isolated systems and hardly figure in any management plans. The principal responsibility for management of wetland ecosystems in Srinagar is with the Lakes and Waterways Development Authority (LAWDA), Srinagar. Although one of the wetlands in Srinagar—Hokersar—was declared as a Conservation Reserve by the Jammu and Kashmir Wildlife Protection Act (1978)¹ and selected as a Ramsar site under the Ramsar Convention on wetlands of international importance on 8 November, 2005, the wetland ecosystems are overlooked in management plans. A wetland management plan would be imperative for deriving sustained ecological and socio-economic services from these important freshwater ecosystems. The wetland management plan would adhere to various actions for protection, restoration, and manipulation of wetland ecosystems that provide values and functioning advocating to their sustainable usage (Walters, 1986).

¹http://www.jkdears.com/eers/pdf/J_K_Wildlife_Protection_Act.pdf

The various strategies involved in management process should aim at:

- Reducing the impact of current anthropogenic pressures and natural processes for long-term protection of wetlands.
- Prohibiting any kind of anthropogenic interference in wetland areas particularly where much of the natural functioning of wetland ecosystems have already been lost.
- Regulating inflows using water quality standards set by organizations for wetlands for their regular functioning while deriving monetary benefits in a sustainable way.
- Creating barrier or green zones for protection of wetland ecosystems, restraining detrimental human actions in the demarcated area of wetland ecosystems for restoring the wetlands.
- Addressing and treating point sources and non-point sources of pollution in the vicinity and catchments that would improve the trophic status of wetlands in peril.
- Setting up of robust treatment plants (STPs) within the wetlands and their immediate catchments that would improve wetland health.
- Involvement of local people, colleges, and universities for regular monitoring of the health of wetlands and establish a civil society–academia–policy interface that will help in the better understanding of these ecologically sensitive areas for formulating effective conservation and restoration efforts.
- Workshops and other programs with active participation of school and university children in the vicinity of wetland ecosystems on a regular basis so that hands-on education is imparted to the dwellers and inhabitants for protection of wetlands.

CONCLUSIONS

Wetlands are biologically most diverse and economically valuable ecosystems all over the world. They cover about 6% of the surface of the earth and provide important ecological and economic services. Srinagar city in the valley of Kashmir has several lakes and wetlands. These are important as socio-economic assets and function as absorption basins for flood waters. They help in the maintenance of biodiversity, purification, and recharge of groundwater. The wetlands of Srinagar city are degrading at an alarming rate mainly due to anthropogenic pressures and climate change. The major threats to wetlands in the city include pollution, siltation, encroachments, urbanization, and establishment of floating gardens. Robust management strategies must be adopted for conservation and protection of wetland ecosystems to ensure sustainable socio-economic and ecological benefits.

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FUTURE PERSPECTIVES

Wetlands all over the world function as important ecological assets and contribute largely to the well-being of the people. Studies have shown that the monetary value of wetland services far exceeds those provided by terrestrial ecosystems. Nowadays, there is a growing trend of utilizing constructed wetlands for treatment of wastewaters and industrial effluents. Due to encroachments and land use changes, the area of lakes and wetlands has drastically reduced during the last few decades. Therefore, the future of lakes and wetlands seems to be at stake, which would not only impact socio-economy but also increase the vulnerability of people to disasters. Despite the regulations and the presence of wetland management authorities, wetlands continue to degrade in the Kashmir region. Keeping in view their ecosystem services, wetlands need to be conserved for future generations. The wetland management authorities of Srinagar city need to look into the ecological degradation and the land conversion going on within and around the wetlands. The areas that require aggressive management approaches are restricting the expansion of floating gardens and limiting the growth of aquatic vegetation. It is these hotspots that are earth-filled and used for construction of houses. The siltation of wetlands in Srinagar is another important concern that should be looked into by the policy-makers so as to restore their original water holding capacities. This is important for reducing the vulnerability of population in Srinagar to floods. The effects of mismanagement of city wetlands will become visible only in a few decades and the wetland encroachers will bear consequences similar to what Srinagarites experienced during 2014 mega flood.

AUTHOR CONTRIBUTIONS

ShD, SB, and IR conceived, designed, and drafted this review, while SaD author helped in screening the relevant literature besides giving inputs on draft. All authors together at the end gave final shape to this manuscript.

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Soil Organic Matter as Catalyst of Crop Resource Capture

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The positive effect of soil organic matter (SOM) on crop yield has historically been attributed to the ability of SOM to supply crops with nitrogen and water. Whether management-induced increases in SOM meaningfully supplement water supply has received recent scrutiny, introducing uncertainty to the mechanisms by which SOM benefits crops. Here, we posit that to benefit crops SOM does not need to increase the supply of a growth-limiting resource; it only needs to facilitate root access to extant resource stocks. We highlight evidence for the ability of SOM to alleviate negative impacts of inadequate aeration (mainly waterlogging) and compaction on roots. Waterlogging, even if transient, can permanently downregulate root biosynthesis and call for expensive growth of new roots. Management practices that promote SOM reduce waterlogging by accelerating water infiltration and may promote aeration in non-saturated soils. Compaction as a restriction to root development manifests in drying soils, when mechanical impedance (MI) inflates photosynthate required to extend root tips, leading to short, thick, and shallow roots. SOM reduces MI in dry soils and is associated with root channels to subsoil, granting crops access to deep soil water. Both waterlogging and compaction necessitate additional belowground investment per unit resource uptake. In this framework, crop response to SOM depends on interactions of crop susceptibility to inadequate aeration or compaction, soil moisture, and “baseline” soil aeration and compaction status. By exploring the proposition that SOM catalyzes resource uptake by permitting root development, future research may constrain crop yield improvements expected from SOM management.

Keywords: aeration, available water capacity, compaction, crop yield, mechanical impedance, soil health and quality, soil organic matter, waterlogging

INTRODUCTION

Despite comprising a small proportion of the mass of agricultural soils, soil organic matter (SOM) is associated with improved soil structure (Feller and Beare, 1997; Six et al., 2000; Dexter et al., 2008; King et al., 2019). Multiple features of agricultural systems limit the extent by which management can alter SOM levels, but managing for even a modicum of increased SOM offers societal benefits, including climate change mitigation from the storage of carbon (C; Paustian et al., 2016; Minasny et al., 2017), a component of SOM, and reduced erosion (Barthès and Roose, 2002). Another, potential benefit of SOM is increasing crop yield (Pan et al., 2009; Oldfield et al., 2018), however, we have limited ability to explain inconsistent effects of management-induced increases in SOM on crops (Xin et al., 2016; Bradford et al., 2019; Wade et al., 2020) or to constrain potential crop benefits

from SOM in future climates, as crop stressors shift (IPCC, 2019). Progress on these fronts relies on a sound understanding of mechanisms through which SOM benefits crops in the first place.

Historically, improved crop yield from SOM has primarily been attributed to SOM's role in resource supply, either of water or nitrogen (Gregorich et al., 1994; Arshad and Martin, 2002; Lal, 2020). A large effect of SOM on available water capacity (AWC) was established without consideration of limits of management-induced SOM (Hudson, 1994). A recent synthesis, however, concluded that management-induced increases in SOM (10 g C/kg soil) effected on average only 1.16 mm additional AWC in the top 10 cm of soil (Minasny and McBratney, 2018). This is a limited contribution to crop transpiration, which can exceed 450 mm (Kimball et al., 2019, maize). If a 1.16 mm increase in SOM-derived AWC is multiplied by rain events during crop maturation, signifying the number of times AWC is "used," and including 10–20 cm soil, the augmentation of AWC by SOM is larger, but an effect on crop performance is likely context-dependent. While SOM is linked to AWC and management increases AWC in some situations, crop water supply does not appear well-justified as a universal mechanism linking management-induced SOM increases to crop yield.

Larger SOM pools are linked to higher production rates of plant-available nitrogen (N) via net N mineralization (Schimel, 1986; Booth et al., 2005), leading to the view that SOM benefits crops by increasing N supply. However, to determine that N supply from SOM limits crop growth, rates of plant N uptake should approach rates of N mineralization, indicating a potential for crop N demand that outpaces soil N supply. Results of this comparison depend on method, with net N mineralization deceasing (Brye et al., 2003; Loecke et al., 2012) but gross N mineralization exceeding (Osterholz et al., 2016) crop N demand. If gross N mineralization is indeed a better indicator of plant-available N than net N mineralization (Schimel and Bennett, 2004), or an underestimate given crop uptake of amino acids (Hill et al., 2011), then increasing SOM would simply be increasing an already sufficient N supply.

Here, we posit that SOM benefits crops not necessarily by increasing resource supply but by catalyzing crop resource capture (**Figure 1**). Roots, the locus of nutrient and water uptake, rely on contact with soil for nutrient (Wang et al., 2006), and water uptake (Javot and Maurel, 2002). Vigorous root development therefore multiplies the surface area of root-soil contact through which resource uptake occurs. We highlight below the consequences of inadequate aeration and compaction on roots and the ability of SOM-enhancing management practices to alleviate these stressors. While we acknowledge that relationships between SOM and soil structure are known (Karlen et al., 2001; Bünemann et al., 2018), we argue that their connections to related crop stressors—poor aeration and compaction—have received inadequate attention as mechanisms that link SOM to crops. We also acknowledge that compaction and inadequate aeration are often co-located, and we separate them to discuss how they manifest differently in the soil environment and as root stressors.

We refer to differences in SOM induced by agricultural practices, such as cover crops (Poeplau and Don, 2015), perennial forages (King and Blesh, 2018), manure application (Maillard and Angers, 2014), straw retention (Liu et al., 2014), or reduced tillage (in surface soil, Luo et al., 2010), except where noted. Although some of the mechanisms discussed may apply to naturally occurring variability in SOM, we defer their discussion for brevity.

AERATION

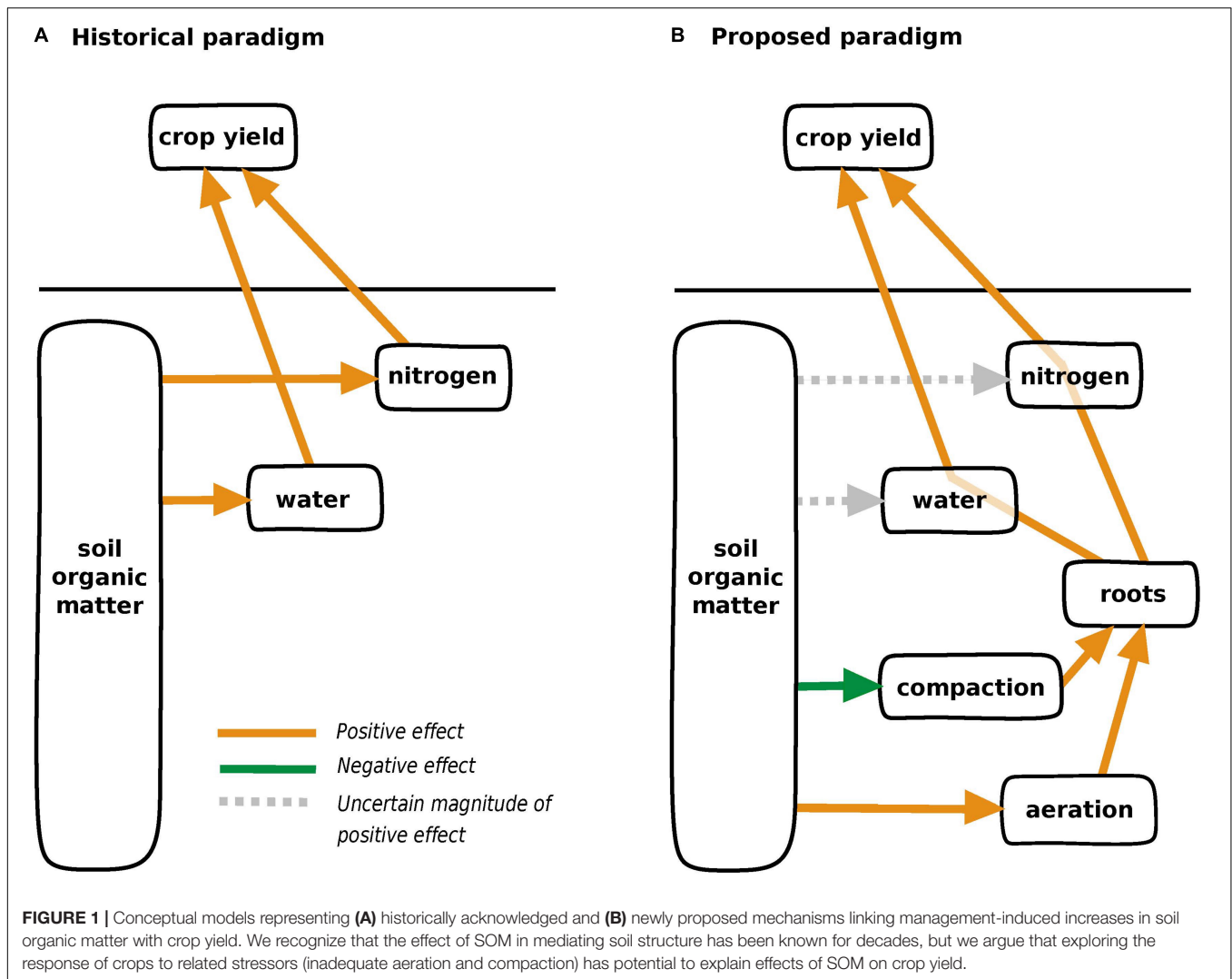
Inadequate Aeration in Waterlogged and Non-saturated Soils

As a substrate for respiration, O₂ and its transport potentially affect all functions of crop roots (Grable, 1966). To study the effects of O₂ deficiency in the field, researchers commonly impose an extreme constriction of aeration with waterlogging, the saturation of soil pores with water (Hodgson and Chan, 1982; Bange et al., 2004). Our consideration of inadequate aeration focuses accordingly on waterlogging, and we do not review impacts of inadequate aeration in non-saturated soils on crops, as these remain largely unexplored.

Waterlogging, Even if Transient, Can Indelibly Damage Roots

Waterlogging damages diverse crops worldwide (Velde and Van Der Tubiello, 2012; Shaw et al., 2013; Zhang et al., 2015; Li et al., 2019), and increases in waterlogging due to climate change are forecasted to exacerbate these damages (Rosenzweig et al., 2002). Waterlogging reduces soil aeration due to a 10⁴ fold slower rate of O₂ diffusion through water than through air (Grable, 1966). Within hours, soil O₂ can be depleted as root or soil organisms' respiration demands exceed atmospheric O₂ supply, with rate and extent of O₂ depletion depending on depth (Malik et al., 2001) and temperature (Trought and Drew, 1982). Over slightly longer time frames, waterlogged soils also accumulate byproducts of root or microbial metabolism, e.g., CO₂, Fe²⁺, and Mn²⁺, potential plant toxins (Shabala, 2011). Crop responses to waterlogging depend on crop species and cultivar (Huang, 1997; Boru, 2003; Ploschuk et al., 2018), as well as timing (Rhine et al., 2010; de San Celedonio et al., 2014; Ren et al., 2014) and duration (Malik et al., 2002; Rhine et al., 2010; Kuai et al., 2014; Ren et al., 2014; Arduini et al., 2019) of waterlogging, but a well-supported view holds that crop yield reductions from waterlogging are largely attributable to impaired function and inadequate recovery of roots (Malik et al., 2002; Herzog et al., 2016; Arduini et al., 2019).

In crops that are susceptible to waterlogging, stress response can be considered both during and after release from waterlogging. During waterlogging, O₂ deficiency induces an energy crisis in the root due to inefficient production of ATP (Gibbs and Greenway, 2003), which curtails energy-dependent nutrient uptake (Trought and Drew, 1980; Morard et al., 2000; Colmer and Greenway, 2011) and root growth (Palta et al., 2010; Arduini et al., 2019). After release from waterlogging,



root growth of certain root forms, i.e., seminal roots, can be permanently inhibited (Malik et al., 2002; Palta et al., 2010; Colmer and Greenway, 2011), attributable to cell death in apical meristems (Trought and Drew, 1980; Malik et al., 2002), beyond the reach of plant-transported O_2 (Colmer and Greenway, 2011). Crops must then rely on energetically-expensive production of new roots, i.e., adventitious roots (Palta et al., 2010; Steffens and Rasmussen, 2016), and/or increase nutrient uptake per unit root (Arduini et al., 2019). These adaptations do not necessarily allow crops to escape a waterlogging yield penalty, as reductions in root biomass (Grassini et al., 2007; de San Celedonio et al., 2017; Ploschuk et al., 2018) or root length density (Hayashi et al., 2013) are often linked to reductions in shoot biomass or yield.

Uncertainty remains about the threshold duration at which waterlogging damages crops. In some crops and growth stages, waterlogging as short as 3 days reduced yield (Malik et al., 2002; Ren et al., 2014), but the shortest waterlogging we found in field studies was 2 days (Rhine et al., 2010). Due to the risk of crop damage from even transient waterlogging, there is interest in management practices that reduce waterlogging risk

(Manik et al., 2019) and improve root aeration across the soil water spectrum (Rabot et al., 2018).

Soil Organic Matter: Means to Improve Root Aeration

Reducing Duration of Waterlogging

Management practices that promote SOM reduce risk and duration of waterlogging by increasing rate of water infiltration (Boyle et al., 1989; Adekalu et al., 2007; Abid and Lal, 2009; Blanco-Canqui et al., 2011), which increases the time soil can receive rain before ponding occurs (McGarry et al., 2000) and reduces time required to drain from saturation to field capacity (Wuest et al., 2005). Among the many measurable soil water variables, infiltration is the most commonly assessed, and we note the need to better establish relationships between infiltration, time to ponding, and drainage. It is also important to note, as reviewed by Blanco-Canqui and Ruis (2018) for no-till, that management practices that promote SOM can have a neutral effect on

water infiltration in some cases, despite positive effects in majority of cases.

Accelerated infiltration associated with SOM is attributable to several soil features. The redistribution of soil mass to larger aggregate size classes associated with SOM (King et al., 2019) helps to explain an increase in total (Pikul and Zuzel, 1994; Yang et al., 2011; Blanco-Canqui and Benjamin, 2013) or macroporosity ($>0.3\text{--}0.4$ mm, Deurer et al., 2009; Yagüe et al., 2016), although this effect is not detectable in all cases (Ruiz-Colmenero et al., 2013). SOM also stabilizes aggregates (Chenu et al., 2000; Annabi et al., 2011), minimizing their dissolution into smaller, and pore-clogging size fractions that seal the soil surface against water infiltration (Bissonnais and Arrouays, 1997; Lado et al., 2004). The most dramatic effects of SOM on infiltration can likely be traced to earthworms and/or termites and their creation of wide, continuous, vertically-oriented pores through which water flows preferentially (McGarry et al., 2000; Guo and Lin, 2018). More abundant – or more active (Pérès et al., 2010) – soil fauna may be due in part to reduced disturbance associated with some SOM-promoting practices, e.g., no-till. However, close relationships between SOM and earthworm abundance without the confounding effect of disturbance (Fonte et al., 2009; Guo et al., 2016) also indicate a role for SOM as faunal substrate supply.

Few studies have attempted to link SOM-induced reductions in waterlogging with crop yield. Gómez-paccard et al. (2015), however, find crop yields increased from reduced surface soil waterlogging associated with no-till. The ability of SOM-mediated reductions in waterlogging to benefit crops are most likely when (1) crop is sensitive to waterlogging and (2) rainfall intensity can be mediated by SOM on a timescale relevant to waterlogging stress (neither drizzle nor deluge); and (3) soil is otherwise poorly-drained (Rhine et al., 2010).

Promoting Aeration in Non-saturated Soils

If crops experience inadequate aeration in non-saturated soils, it is reasonable to expect that SOM would improve gas diffusivity given its effects on related parameters of soil structure (Neira et al., 2015, and below). Few studies investigate the isolated effect of SOM on gas diffusivity, however, Colombi et al. (2019) find a positive relationship between SOM and gas diffusivity at field capacity across a soil texture gradient. Future work should examine net effects of SOM on O_2 diffusivity and consumption in soils.

COMPACTION

Soil Compaction Constrains Root Development

Soil compaction reduces crop yields (Coelho et al., 2000; Ishaq et al., 2001a; Bayhan et al., 2002; Czyz, 2004; Whalley et al., 2008) and is quantified via either bulk density or mechanical impedance (MI; Ehlers et al., 1987; Bengough et al., 2011). MI estimates the force encountered by the elongation of a living root, and is consequential for crops because greater MI inflates the photosynthate required for root elongation

(Herrmann and Colombi, 2019). Although MI measurements ignore biopores used preferentially by roots (Stirzaker et al., 1996; White and Kirkegaard, 2010), MI is more descriptive than bulk density because it is sensitive to soil water. Drying soils present increasing MI (Vaz et al., 2011), and to isolate effects of water stress from compaction stress per se on crop development, researchers use experimental compaction.

Compaction studies indicate that reduced crop yield from compaction is due in large part to constraints on root development (Ishaq et al., 2001b; Czyz, 2004; Colombi and Keller, 2019). A root restricted by soil compaction is generally thicker than a root in non-compacted soil (Nadian et al., 1997), likely due to greater axial force needed to overcome compaction (Bengough, 2012). Reductions in total number of roots, rate of root elongation, total root length, or root biomass are also reported (Panayiotopoulos et al., 1994; Chan et al., 2006; Lipiec et al., 2012). Root length is generally more reduced than root dry mass (Panayiotopoulos et al., 1994), indicating the accumulation of belowground photosynthate without commensurate expansion of soil-contacting surface area available for nutrient and water uptake. Compaction is sometimes characterized by a hardpan around 20 cm depth, which leads to restricted root access to subsoil and concentrated root development in the topsoil (Czyz, 2004). This pattern of root development prevents crop access of deep soil water most implicated in crop drought resistance (Uga et al., 2013; Lynch, 2018).

A single threshold MI for crop sensitivity is unlikely to serve universally, and not only because cultivars (Houlbrooke et al., 1997) and crops (Rosolem et al., 2002) differ in MI tolerance. Threshold MI values also likely depend on definition by energy required to extend roots (Herrmann and Colombi, 2019) or by crop yield penalty. The MI required to reduce root growth efficiency is likely less than required to affect yields, and yield penalty due to restricted roots can be counteracted somewhat by fertilization (Robertson et al., 2009). Whatever the threshold, the detrimental effects of compaction on crops has generated attention toward means to reduce it.

Soil Organic Matter Reduces Compaction and Is Associated With Root Channels

Reduced Compaction: More Water Transpired Before Mechanical Impedance Limits Growth

Although SOM is often promoted for its ability to alleviate soil compaction and associated increases in MI (Hamza and Anderson, 2005), the generation of data confirming a negative SOM-MI relationship has been hampered by the convention of measuring MI in soils near field capacity (Duiker, 2002). Even in large datasets, no relationship between SOM and MI in soil near field capacity is found (Fine et al., 2017), likely because very wet soils ($\sim 1\text{--}10$ kPa) offer minimal MI regardless of SOM. It is as MI increases in drying soils (Vaz et al., 2011; Filho et al., 2014) that an effect of SOM becomes apparent (Stock and Downes, 2008; Gao et al., 2012).

We highlight two studies showing the effect of SOM in reducing MI as soils dry. Stock and Downes (2008) and

Gao et al. (2012) investigated soils differing only in SOM concentrations. With few exceptions, MI increased as soils approached permanent wilting point, but the increase in MI was not as much in higher SOM soils. In other words, SOM allows soil to become drier before reaching a potentially root-constraining MI. For instance, Stock and Downes (2008) find an MI of 1.5 MPa is reached in the 1% OM soil at $\sim$ 100 kPa, whereas the 3% OM soil reaches the same MI at about $\sim$ 200 kPa. For these soils, the difference in volumetric water content between -100 and -200 kPa is $\sim$ 0.02 m⁻³ H₂O m⁻³ soil, or $\sim$ 5 mm of water when considered over the top 25 cm. While Stock and Downes (2008) added organic amendments to glacial till to create fixed SOM percentages, their results resemble those of Gao et al. (2012), who compared fallow to grassland soils. The extent to which management-induced SOM benefits crops via reductions in compaction likely vary with context, particularly those relevant to MI thresholds (see section “Soil compaction constrains root development”).

Root Channels to Subsoil Water

Although not connected to the physical or biological properties of SOM, management practices that promote SOM may also alleviate the effects of compaction by facilitating crop root access to the subsoil. Deep-rooted cover crops or perennial crops create root channels to subsoil (McCallum et al., 2004), which are used by subsequent cash crops (Rasse and Smucker, 1998; Williams and Weil, 2004). Crops are most likely to benefit from these root channels if subsoil is compacted and if crops experience sufficient water stress for subsoil water stores to be relevant.

DISCUSSION AND OUTLOOK

In the historical conceptual model linking crop performance to SOM, SOM benefits crops primarily by supplying nitrogen and water. Here we propose SOM as a mediator of resource uptake via root growth. We note caveats to the proposed framework. The extent to which SOM affects nutrient and water supply still merits research, and not all mechanisms discussed are contingent on increased SOM. We focus on MI to characterize compaction, but SOM may alter soil structure in ways relevant to root growth that are not captured by MI. As a simple diagram, **Figure 1** does not depict that yield penalty in low SOM soils may be due to cost of constructing new or thicker roots. However, considering SOM as catalyzing resource uptake via root development can help explain recent reports of SOM effects on crops. Wade et al. (2020) report maize yield increases from management-induced SOM across a range of N fertilizer levels, consistent with the

concept that root N uptake—as well as soil N supply—can limit crop yield. Recognizing the importance of aeration for roots may also explain a parabolic crop response to a SOM gradient in a pot study (Oldfield et al., 2020), in which synthesized mixtures of minerals and organic horizons higher in SOM may have supported higher O₂ consumption by microbes without improved gas diffusivity expected in natural high-SOM soils (Colombi et al., 2019).

We propose the exploration of SOM as a catalyst for resource capture focuses on:

- *Characterizing the context-mediated effect of SOM on aeration and compaction* by describing the effect of SOM gradients on (a) aeration and risk and duration of waterlogging and (b) MI across a range of soil moisture contents.
- *Investigating crop response to SOM as a function of aeration and compaction* by identifying the thresholds of hypoxia affecting roots and yields. To relate SOM-mediated MI to root development, describing soil moisture status during crop maturation will be crucial.
- *Simulating future crop response to SOM*; currently, Basche et al. (2016) and Jarecki et al. (2018) are two of few examples to model the potential for management-induced SOM to stabilize crop yields under future climates.

We hope the lens proposed will help illuminate the effect of SOM on crops.

AUTHOR CONTRIBUTIONS

AK conceptualized and drafted the manuscript. GA, AG, and CW-R provided input to arrive at the final version.

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Biotic and Abiotic Mechanisms of Manganese (II) Oxidation in Lake Erie

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Manganese (Mn), an element sensitive to redox conditions in aquatic environments, plays a role in numerous biogeochemical cycles. Although the speciation of Mn in lakes largely tracks the availability of dissolved oxygen, direct oxidation of Mn by molecular oxygen occurs slowly and, instead, the majority of Mn oxidation occurs via biotic and abiotic mechanisms involving microbes, organic matter, light, reactive oxygen species, and mineral surfaces. While each of these mechanisms is either known or likely to occur in freshwater, the relative balance and interaction among these biogeochemical pathways in an intact plankton system remains uncertain. We investigated potential abiotic and biotic mechanisms contributing to Mn oxide formation in Lake Erie, which experiences seasonal hypoxia and accumulation of Mn during seasonal stratification. Overall, Mn oxidation rates were much higher in the shallow and highly productive western basin (up to $1.5 \mu\text{moles L}^{-1} \text{d}^{-1}$) compared to the deeper and less productive central basin, where we observed very little Mn oxidation over 7 days. Our experiments suggest that abiotic mechanisms involving mineral surfaces played a larger role than biotic mechanisms, particularly in the light where Mn oxidation was highest. Reactive oxygen species exhibited antagonistic roles: hydrogen peroxide acted as a net reductant for manganese oxides and completely masked oxidation of Mn by the superoxide free radical. These findings show that multiple mechanisms may exert control over the fate of Mn and suggest that Mn released from sediments during hypoxia can potentially remain dissolved in the water for an extended period of time. Since the severity of Lake Erie hypoxia has increased in recent years, and we are becoming increasingly aware of the health effects of Mn in drinking water sources, these findings should help inform efforts to predict when and where Mn will accumulate in the lake water.

Keywords: manganese cycling, Mn(II), reactive oxygen species, Lake Erie, manganese oxidation

INTRODUCTION

Lake Erie, one of the Laurentian Great Lakes, plays a valuable role to the ecology, economy, and population of the Great Lakes region and supplies drinking water to over 11 million people. It is unique among the Laurentian Great Lakes as it is the shallowest lake and its three distinct basins (eastern, central, and western) vary in depth, productivity, susceptibility to thermal stratification, and terrestrial inputs (Beletsky et al., 2012; Scavia et al., 2014; Cory et al., 2016; Prater et al., 2017). The western basin is shallow and most affected by riverine inputs of nutrients, which sustain high

primary production and recurrent cyanobacterial blooms (Bertani et al., 2016; Scavia et al., 2016). The eastern basin is deepest and least productive. The central basin is intermediate in depth and productivity, but is characterized by widespread seasonal hypoxia in bottom waters (Scavia et al., 2014; Zhou et al., 2015). One of the consequences of hypoxia for water quality is the release of dissolved manganese (Mn) from the sediments into the water, which occurs not only in Lake Erie (Burns and Nriagu, 1976; Lum and Leslie, 1983), but other lakes and reservoirs (Davison, 1981; Munger et al., 2019). Hydrodynamic upwellings in the central basin have the potential to transport hypoxic water toward shore (Rowe et al., 2019), which can result in elevated Mn concentrations entering intake pipes for public water utilities (Ruberg et al., 2008). Mn is a secondary contaminant in drinking water owing to aesthetic effects (United States Environmental Protection Agency, 1996) and it is now known that micromolar concentrations of Mn in drinking water can cause neurologic impairments, particularly in children (Bouchard et al., 2011; Khan et al., 2012). Elevated Mn in seasonally anoxic bottom waters of Lake Erie has been documented previously, and with increased spatial extent of hypoxia in recent years (Zhou et al., 2013), there is a need to understand the biogeochemical cycling and fate of Mn in Lake Erie.

Mn in aquatic environments exhibits three oxidation states, with each one impacting different ecological and biogeochemical processes. Mn(II) is the most reduced form of Mn and it is a soluble antioxidant within aquatic ecosystems (Wuttig et al., 2013) and inside bacterial cells (Daly et al., 2004). Mn(IV) is insoluble and forms numerous different oxide minerals (reviewed in Post, 1999; Tebo et al., 2004). Mn(III) is an unstable intermediate that forms ligand complexes with dissolved organic matter and metal oxides (Oldham et al., 2017) and serves as both an oxidant and reductant (Yakushev et al., 2009; Madison et al., 2013; Oldham et al., 2019). Mn(VII) is soluble and a powerful oxidant, but to date, is not known to be an important Mn species in aquatic environments (Sunda et al., 1983; Hansel and Learman, 2015; Hansel, 2017) and would likely react rapidly with organic matter. Mn(III,IV) oxides are important in the environment as they have a high affinity to sorb other heavy metals (e.g., lead and copper), bind nutrients like phosphorus (Yao and Millero, 1996; Post, 1999), and also can oxidize complex organic molecules (Tebo et al., 2004; Spiro et al., 2009). Mn (III, IV) oxides are also important as they can serve as terminal electron acceptors for microbes (Nealson and Saffarini, 1994; Tebo et al., 2005) and facilitate anaerobic degradation of organic matter.

The distribution and fate of Mn in aquatic systems depends on various factors, the predominant one being oxidative and reductive processes. In particular, oxidation of Mn(II) to Mn(IV) leads to the formation of insoluble (oxy)hydroxide minerals that tend to accumulate in sediments (Tipping et al., 1984; Post, 1999). Thus, the oxidation of Mn is key to understanding persistence of dissolved Mn(II) in the water column of lakes (Davison, 1981), rivers (Anderson et al., 2011), caves (Carmichael et al., 2013), and the ocean (Sunda and Huntsman, 1988, 1990). Homogeneous oxidation of Mn(II) by molecular oxygen is thermodynamically favorable at the pH of most lakes, but kinetically limited in the absence of a biotic or abiotic catalyst and will occur slowly

(Diem and Stumm, 1984). However, abiotic oxidation of Mn(II) by molecular oxygen can be catalyzed via complexation with organics compounds (Nico et al., 2002; Duckworth and Sposito, 2005) or by Mn oxide mineral surfaces (Diem and Stumm, 1984; Davies and Morgan, 1989; Junta and Hochella, 1994). Biotic Mn(II) oxidation can be catalyzed enzymatically by a taxonomically diverse group of bacteria and fungi (Boogerd and De Vrind, 1987; Miyata et al., 2006; Dick et al., 2008; Anderson et al., 2009; Geszvain et al., 2011; Butterfield et al., 2013). Biotic oxidation of Mn(II) is important both in contemporary (Sunda and Huntsman, 1990; Tebo, 1991) and geological timescales (Ossa Ossa et al., 2018) and occurs directly via enzymes such as multicopper oxidases or heme peroxidases (Dick et al., 2008; Anderson et al., 2009; Butterfield et al., 2013; Nakama et al., 2014), indirectly due to high pH micro-environments produced by photosynthesis (Chaput et al., 2019), and indirectly through the biological production of the superoxide free radical (O_2^-) (Learman et al., 2011a; Hansel et al., 2012). In addition to producing superoxide, many microbes are known to produce hydrogen peroxide as a metabolism byproduct (Dixon et al., 2013; Marsico et al., 2015), which could serve to reduce Mn oxides and potentially compete with Mn oxidation caused by superoxide (Sunda and Huntsman, 1994; Learman et al., 2013). Other abiotic processes include photo-reduction of Mn oxides (Sherman, 2005) and interactions with organic matter ligands (Oldham et al., 2017; Oldham et al., 2019).

Much of our understanding of the mechanisms of Mn oxidation comes from marine and estuarine systems, but far less is known about these mechanisms in freshwater ecosystems. A longstanding conceptual model in freshwater has been that the distribution and speciation of Mn within the water column is a simple balance between Mn reduction by microbes, which is thought to occur only in anoxic sediments or water, and abiotic Mn mineralization, which occurs rapidly in oxygenated water and results in sedimentation of Mn oxy(hydr)oxides (Davison, 1981, 1993). This conceptual model is based on vertical patterns of Mn, redox gradients, and dissolved oxygen in lakes and reservoirs subject to seasonal or permanent stratification (Mortimer, 1971; Burns and Nriagu, 1976; Hongve, 1997; Munger et al., 2017). This understanding of Mn cycling has been useful in applied lake management – artificial aeration of bottom water in reservoirs effectively slows Mn release and results in decreased concentrations of Mn in the water (Zaw and Chiswell, 1999; Gantzer et al., 2009; Gerling et al., 2014; Munger et al., 2016). Studies in freshwater have shown that Mn is oxidized more rapidly than Fe(II) (Hsiung and Tissue, 1994; Stumm and Morgan, 1996) and also that rates of Mn oxidation measured in lake water are higher than expected from homogeneous oxidation by molecular oxygen (Delfino and Lee, 1968; Diem and Stumm, 1984; Hsiung and Tissue, 1994; Zaw and Chiswell, 1999). This disparity in rates has led to the hypothesis that Mn oxidation in marine (Tebo, 1991) and freshwater ecosystems is a primarily mediated by biological processes (Chapnick et al., 1982; Tipping, 1984; Hsiung and Tissue, 1994; Aguilar and Nealson, 1998).

While all of the mechanisms described above have been demonstrated individually, the relative contribution of different biotic and abiotic mechanisms to Mn oxidation in freshwater

TABLE 1 | List of hypotheses, representative chemical reactions, and outcomes.

Hypotheses	Possible Mn redox reaction(s)	Expectation for treatment effects	Supported?
H1: Dissolved oxygen is not a major oxidant ^a	$Mn(II) + O_2 \rightarrow Mn(III/IV)$	Filtered.Dark = 0	Yes
H2: Oxidation by mineral surfaces ^{a,b,c}	$Mn(II) + mineral\ particles \rightarrow Mn(III/IV)$	Killed.Dark > Filtered.Dark or Killed.Light > Filtered.Light	Yes
H3: Oxidation by heterotrophic microbes ^{d,e,f,g,h,i,j,k,l}	$Mn(II) + microbes \rightarrow Mn(III/IV)$	Killed.Dark < Control.Dark	Yes
H4: Oxidation by photosynthetic microbes ^{m,n}	$Mn(II) + microbes + light \rightarrow Mn(III/IV)$	(Control.Light-Killed.Light) > (Control.Dark-Killed.Dark)	No
H5: Oxidation by superoxide (O_2^-) ^{d,o}	$Mn(II) + O_2^- + 2H^+ \rightarrow Mn(III) + H_2O_2$	SOD.Dark < Control.Dark or SOD.Light < Control.Light	No
H6: Reduction by hydrogen peroxide (H_2O_2) ^{o,p}	$Mn(III/IV) + \frac{1}{2}H_2O_2 \rightarrow Mn(II) + \frac{1}{2}O_2 + H^+$	Catalase.Light > Control.Light or Catalase.Dark > Control.Dark	Yes
H7: Competing superoxide radical and hydrogen peroxide ^{o,p}	$Mn(II) + O_2^- + 2H^+ \rightleftharpoons Mn(III/IV) + H_2O_2$	SODCatalase.Light > SOD.Light or SODCatalase.Dark > SOD.Dark	Yes

References for the hypotheses are as follows: ^a(Diem and Stumm, 1984), ^b(Davies and Morgan, 1989), ^c(Junta and Hochella, 1994), ^d(Learman et al., 2011a), ^e(Tebo et al., 2005), ^f(Nealson et al., 1988), ^g(Boogerd and De Vrind, 1987), ^h(Miyata et al., 2006), ⁱ(Dick et al., 2008), ^j(Anderson et al., 2009), ^k(Gesztvain et al., 2011), ^l(Butterfield et al., 2013), ^m(Richardson et al., 1988), ⁿ(Chaput et al., 2019), ^o(Learman et al., 2013), ^p(Andeer et al., 2015).

systems is poorly understood (but see Nealson et al., 1988; Tebo, 1991). Further, these different mechanisms have not been examined simultaneously in experiments with an intact plankton system which includes phototrophs, heterotrophs, mineral particles, and dissolved organic matter (DOM). Due to the relevance of Mn cycling to water quality in lakes, such as Lake Erie, there is a need to examine the relative impact of different proposed mechanisms in a real biological system.

A greater understanding of the fate of Mn in Lake Erie would not only provide new insights into the cycling of this metal in freshwater ecosystems, it would also provide data for water managers as higher levels of Mn in water intakes are becoming more frequent. Here, we present the results of an experiment designed to test whether these proposed mechanisms occur in an intact freshwater plankton system. We used water from Lake Erie and applied experimental manipulations to examine the roles of: particles (using filtration); biological particles (using formaldehyde to kill microbes); photosynthetic metabolism (using light vs. dark); superoxide (using the enzyme superoxide dismutase to scavenge superoxide); and hydrogen peroxide (using the enzyme catalase to remove hydrogen peroxide). As our experiments were performed in an intact plankton system, where multiple mechanisms are occurring simultaneously, we use **Table 1** to outline the hypothesized mechanisms for Mn oxidation, the representative reactions, and our expectations in terms of Mn oxide concentrations and Mn oxide production rates.

MATERIALS AND METHODS

Sample Stations and Collection

We collected water from two biogeochemically contrasting areas of Lake Erie: the western basin, which is characterized by high biological production, sediment resuspension, and generally oxic conditions and the central basin, which is deeper and has lower biological production and undergoes seasonal hypoxia in the

TABLE 2 | Ambient conditions at each sampling location.

Station Date		Water Temperature (°C)	Chlorophyll-a (μg/L)	pH	Dissolved Oxygen (mg L ⁻¹)
CB2	June 4, 2019	13.6	1.36	8.24	10.64
ER43	June 27, 2019	16.0	3.12	ND	10.26
WE2	June 17, 2019	18.9	2.76	8.23	9.46
WE2	July 08, 2019	25.3	28.35	8.87	6.10

ND denotes parameters that were not measured in a sample. Archived methods are available in the NOAA NCEI archive Accession 0187718. We measured water temperature and dissolved oxygen using either a CTD profiler (SeaBird Scientific) or portable sonde (EXO Yellow Springs Instruments). ND denotes data not determined.

hypolimnion. We sampled on June 17 and July 8, 2019 in the western basin aboard the National Oceanic and Atmospheric Administration (NOAA) vessel R4108 at NOAA Great Lakes Environmental Research Laboratory station WE2 (41.762°N, 83.33°W). In the central basin, we sampled from two different stations located within 15 km (**Figure 1**). On June 4, 2019, we sampled station NOAA station CB2 (41.7588°N, 82.1352°W) aboard the NOAA vessel R5503. On June 27, 2019, we sampled at EPA station ER43 (41.7883°N, 81.9450°W) aboard the R/V Lake Guardian. Hereafter, we will refer to these locations collectively as the western and central basins. All water samples were collected in Niskin bottles from a depth of 1–2 m below the surface. Samples were stored in the dark at the same ambient temperature as the lake (**Table 2**). Although we collected water from the surface mixed layer for these experiments, it is important to note that bottom water was not hypoxic at the time of collection (i.e., > 2 mg L⁻¹ dissolved oxygen). We initiated the experiments within 24 h of collecting the samples.

Experimental Design

Each experiment followed the same experimental design, with six primary treatments in complete factorial with incubation

in either the light or dark. All treatments received 5 $\mu\text{moles L}^{-1}$ Mn as MnCl_2 . The control treatment consisted of whole lake water. For the filtered treatment, we removed particles larger than 0.2 μm using a Whatman GD/X filter. For the killed treatment, we added formaldehyde to a final concentration of 0.1% (w/v). Preliminary experiments showed that higher concentrations of formaldehyde caused reduction of Mn(VII) oxides and interfered with the leucoberberlin blue method. We did not observe significant increases in absorbance at 624 nm in any of the experimental units treated with formaldehyde. The enzyme superoxide dismutase (SOD) has been used previously to inhibit the activity of superoxide as an oxidant of Mn(II) (Learman et al., 2011a, 2013; Andeer et al., 2015). In the SOD treatment, we added 12.5 KU of superoxide dismutase (from bovine erythrocytes, Sigma-Aldrich item 574594) to unfiltered lake water. This concentration was equivalent to 5% of the amount used by Learman et al. (2011a) and reflects our expectation that concentrations of superoxide would be lower in lake water than in a dense laboratory culture. For the catalase treatment, we added 4 KU of catalase (from bovine liver, Sigma-Aldrich item C40) to unfiltered lake water in order to scavenge hydrogen peroxide. This concentration was also scaled to 5% of the amount used by Learman et al. (2011a) in cell culture, similar to the SOD treatment. Because catalase is unstable, we added 4 KU 1 h prior to each of the timepoints on the first day of the experiments and thereafter at 1 h prior to sampling. For the SOD/catalase treatment, we applied both the SOD and catalase amendments to unfiltered lake water, including periodic additions of catalase at the same timing and concentration as in the catalase only treatment.

Each treatment was incubated under two different light levels: complete darkness (wrapped in aluminum foil) or high light (300 $\mu\text{moles m}^{-2} \text{ s}^{-1}$ photosynthetically active radiation, PAR). We selected this intensity because it corresponds to the mean daytime PAR intensity experienced in the surface 2 m of Lake Erie (Weiskerger et al., 2018). Continuous illumination was provided by an array of light emitting diodes (Waveform Lighting) that illuminated all experimental units at the same intensity ($\pm 5\%$) and broad PAR spectrum from 400 to 800 nm. The LEDs were upward-facing beneath a glass platform on which the experimental units were arranged. Experimental units had a volume of 100 mL and were contained in 125 mL borosilicate glass Erlenmeyer flasks that had previously been soaked in 3.7% hydrochloric acid and rinsed with type II water prior to use. We performed each experiment in an environmental chamber that maintained the units at the same temperature as the lake water (Percival Scientific). Flasks were randomly arranged in the environmental chamber and incubated over the course of 7 days.

Sampling Procedures

We measured the production of Mn oxides over the course of each experiment using the dye leucoberberlin blue (LBB), which produces an intense blue color and absorbance peak at 624 nm when reacted with Mn oxides (Krumbein and Altmann, 1973; Lee and Tebo, 1994; Learman et al., 2011a; Oldham et al., 2017). We made two modifications to this method to suit our

experiment. First, we decreased the concentration of LBB (Sigma-Aldrich 432199) in order to minimize background absorbance from unreacted dye. Second, we corrected for the background absorbance in the sample and buffer. In our modified method, we mixed 2 mL of sample with 0.67 mL of either 136 mM acetic acid buffer (in water) or acetic acid buffer with 294 $\mu\text{moles L}^{-1}$ LBB. Samples were incubated in the dark for 15 min to allow for reaction with the reagent and buffer. Then, absorbance was measured at 624 nm using a Perkin Elmer Lambda 40 spectrophotometer and a 1 cm pathlength. For each sample, we quantified the difference in absorbance between the sample with buffer only and the sample with buffer and LBB. We made standard curves using 0–2 $\mu\text{moles L}^{-1}$ potassium permanganate. Since each molecule of permanganate oxidizes five molecules of LBB, whereas one molecule of MnO_2 would oxidize two molecules of LBB, 2 $\mu\text{moles L}^{-1}$ as permanganate should produce the same absorbance as 5 $\mu\text{moles L}^{-1}$ as manganese (IV) dioxide. Because both Mn(III) and Mn(IV) oxides are known to react with LBB, and a given sample may contain a mix of Mn(III) and Mn(IV) oxides (Oldham et al., 2017), we chose to convert the absorbance produced by reaction with LBB to Mn(IV) equivalents.

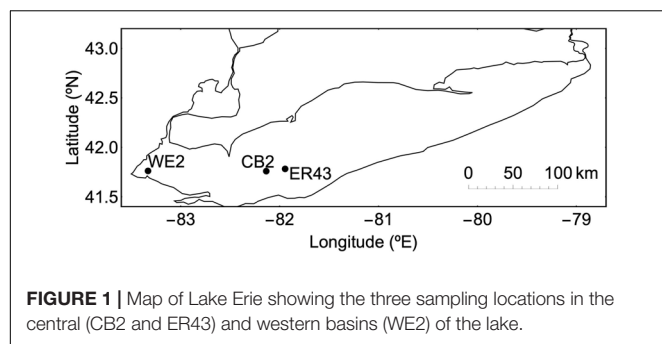
We sampled each experimental unit at the start of the experiment and twice more during the first day of the experiment. Flasks were consequently sampled once per day for the remaining 6 days of the experiment. Prior to sampling, the flasks were manually mixed for 3 s to homogenize the plankton. At the final sampling in each experiment, we measured Mn oxide concentration in both the raw and filtered ($<0.2 \mu\text{m}$) water from each treatment.

Statistical Analyses

The treatments exhibited peak Mn oxide concentrations at different times during the experiments (**Supplementary Material**), thus we summarized the measurements from each experimental unit in terms of the maximum Mn oxide concentration and maximum Mn oxide production rate. We analyzed the maximum Mn oxide concentrations in each experiment using a two-way analysis of variance (ANOVA) with fixed effects of treatment and light. We quantified the maximum rate of Mn oxide formation observed during each time series from the increase in Mn oxide equivalent concentration vs. time. For each flask, we applied a moving window of four consecutive timepoints and used linear regression to estimate the rate across each window. We report the highest rate from each flask as the maximum Mn oxide production rate. Changing the window size from four timepoints to three timepoints caused little change in terms of the mean rate estimates, but did affect the variance. We evaluated pairwise differences among treatments using Tukey's method. All statistical analyses were performed in R version 3.4.2 using the function "lm" in the "stats" package. The R code and complete dataset are provided as **Supplementary Material**.

Electron Microscopy

We performed transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDS) on Mn oxide particles produced in both lake water from Lake Erie and in the



catalase treatment of our experiment. The lake water was sampled from the anoxic hypolimnion at station CB2 (central basin) on September 19, 2019, aerated, and stored at 4°C for 7 days prior to centrifugation at 1000 g for 20 min to form a pellet. The catalase treatment samples (sampled on July 8, 2019 in the western basin) were stored at −20°C and then were thawed, sonicated, and centrifuged at 14,000 g for 15 min. The pellets from the catalase treatment and the Lake Erie water were resuspended in 2 mL of ultrapure water, sonicated, and the centrifuged at 14,000 g for 15 min. The water was decanted and then the pellet was resuspended in 0.2 mL of ultrapure water and sonicated, and then the concentrated samples from each condition was loaded onto a copper TEM grid with a lacy carbon coating (Ted Pella) and then placed in a desiccator to allow water to evaporate. Imaging was performed at Central Michigan University on a Hitachi HT7700 TEM and EDS data was collected on a Thermo Scientific NSS Pathfinder to confirm the presence of Mn.

RESULTS AND DISCUSSION

Homogenous Oxidation by Dissolved Oxygen

We hypothesized that homogenous oxidation by dissolved oxygen would not result in appreciable formation of Mn oxides (H1, **Table 1**). Our results support this hypothesis: **Figure 2** shows that in the absence of particles, Mn oxide formation was undetectable in both the western and central basins (<2% of added Mn), even after 1 week in well-oxygenated dark incubations. This result shows that reduced Mn is not rapidly oxidized by dissolved oxygen under the conditions present in Lake Erie and that homogenous oxidation is not a primary means of Mn oxide formation and removal from the surface mixed layer. This finding is consistent with previous studies reporting that it can take several years for Mn(II) to be oxidized by oxygen in the absence of microbes or mineral particles (Diem and Stumm, 1984; Tipping, 1984). Although homogenous oxidation by dissolved oxygen is not responsible for appreciable Mn oxidation in Lake Erie, we did find support for several of the other proposed mechanisms of Mn oxidation.

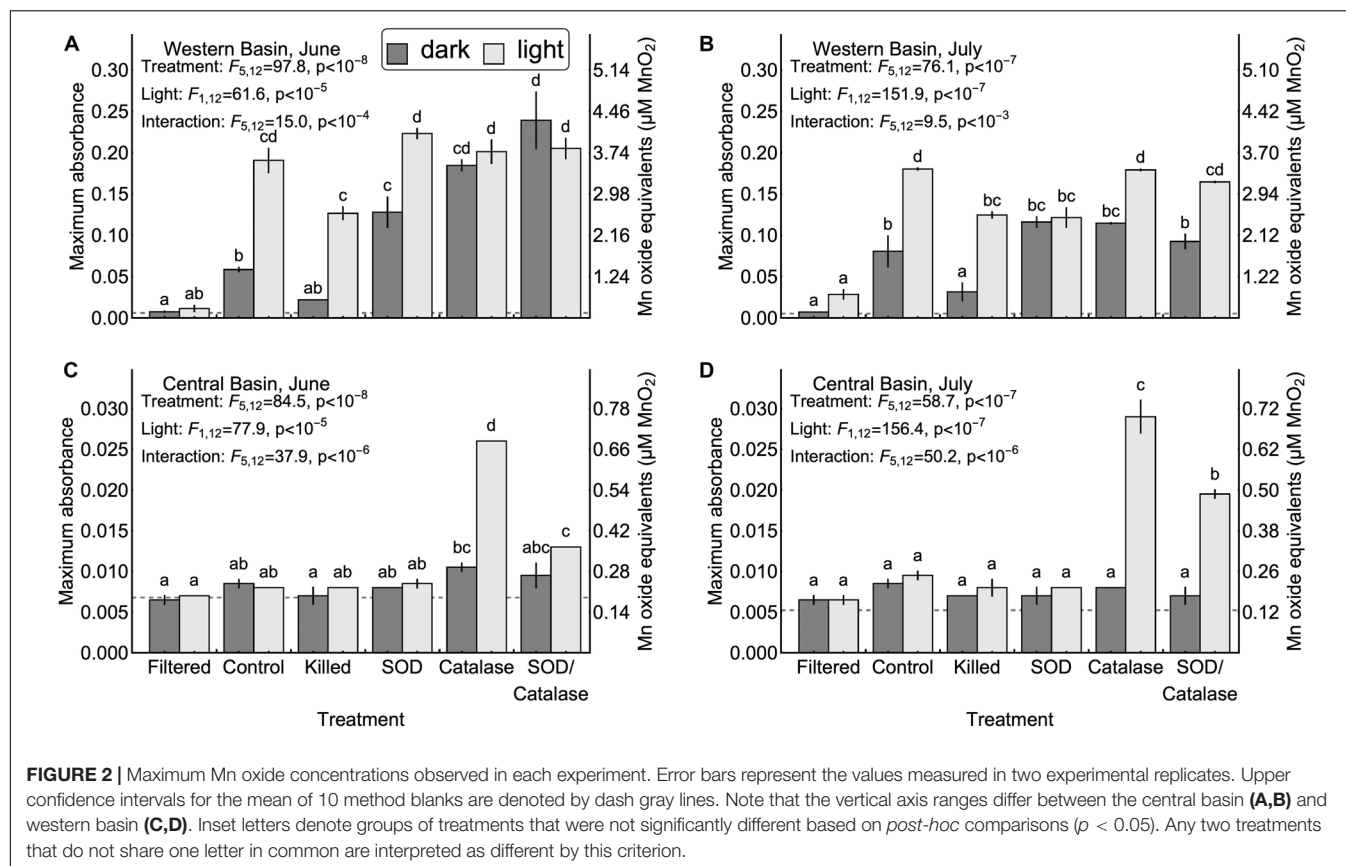
Abiotic Mn Oxidation

Next, we tested the hypothesis that mineral surfaces can facilitate the oxidation of Mn(II) (H2, **Table 1**). We did not observe

measurable production of Mn oxides in the central basin. In the western basin, the killed experimental treatment (containing only mineral particles and dead biological particles) had higher concentrations of Mn oxides than the filtered treatment (no particles), although this difference was only significant in the light (**Figure 2**). Similarly, the killed treatment applied to the western basin showed significantly higher rates of Mn oxide production compared to the filtered treatment (except June dark, **Figure 3**). Higher production of Mn oxides in the presence of particles (minerals, detritus, and killed cells) is consistent with Hypothesis 2 and suggests that minerals are catalyzing oxidation reactions (**Table 1**). When those particles were removed by filtration we did not observe any production of Mn oxides. Although oxidation by molecular oxygen is kinetically limited at pH below 10, Mn oxides and other minerals can catalyze the oxidation of Mn(II) by molecular oxygen (Davies and Morgan, 1989; Tebo et al., 2005; Learman et al., 2011b; Hansel and Learman, 2015). Suspended sediments in Lake Erie contain both Mn and Fe oxides (Burns and Nriagu, 1976; Mudroch, 1984) and the predominant inputs of suspended sediments to the surface mixed layer is through river inputs and wind-driven resuspension in the shallow western basin (Binding et al., 2012; Shuchman et al., 2013). Concentrations of non-volatile suspended solids were higher in the western basin experiments (33.7 mg L^{−1} in June 5.25 mg L^{−1} in July) compared to the central basin (<1 mg L^{−1}), which potentially explains why we did not observe production of Mn oxides from the central basin under the killed treatment. This finding is also consistent with other studies in marine systems showing that Mn(II) oxidation rates are faster in nearshore waters where Mn oxide particles are present (Tebo and Emerson, 1985; Sunda and Huntsman, 1987; Tebo, 1991). Moreover, previous experiments have shown that rates of Mn oxidation catalyzed by mineral surfaces are faster in the light than in the dark (Nico et al., 2002; Learman et al., 2011b, 2013). Although there is no consensus explanation for this phenomenon, it could be attributable to production of ROS through reactions involving DOM or mineral particles (Learman et al., 2011b). Nonetheless, the effects of visible light on abiotic oxidation by mineral particles is consistent with the effects that we observed in the western basin (**Figure 3**). While particle-associated reactions made up the majority of the abiotic oxidation in the light, we did see limited production of Mn oxides in the filtered treatment (western basin, July), which suggests that photochemical reactions with dissolved organic matter may play a small role in Mn oxide formation in Lake Erie (Painter et al., 2001; Hansel, 2017), or that nanoparticles smaller than 0.2 microns help catalyze oxidation of Mn (Madden and Hochella, 2005).

Biological Mn Oxidation

Our third hypothesis (H3, **Table 1**), that heterotrophic biological activity causes Mn oxidation, was supported by significant differences in Mn oxide concentrations (**Figure 2B**, July $p < 0.05$) and Mn oxide production rates (**Figure 3A**, June $p < 0.05$) between the killed + dark and control + dark treatments applied to the western basin. We did not observe detectable rates of Mn oxidation in the control treatments from the central basin. These findings show that biological processes played a critical role in



Mn oxidation, which is consistent with both experimental (Tebo et al., 2005) and observational studies (Chapnick et al., 1982; Tipping et al., 1984; Hsiung and Tissue, 1994).

We also hypothesized that photosynthetic biological activity causes Mn oxidation (H4, **Table 1**). In our experiments from the western basin, the control treatments produced significantly higher Mn oxide concentration in the light than the dark, which initially suggests that photosynthetic organisms might play a role in Mn oxide formation (**Figure 2A**). If this were true, then the addition of formaldehyde should obliterate much of the positive effect of light on Mn oxide production. However, the absolute decrease in Mn oxide concentration (or Mn oxide formation rate) with addition of formaldehyde was similar between the light and dark treatments (**Figure 2A**), suggesting that the biocide's effect may have been to reduce oxidation by non-photosynthetic biological processes only (e.g., heterotrophic microorganisms). This suggests that photosynthetic activity does not represent the sole mechanism of Mn oxidation in Lake Erie and the majority of biological Mn oxidation may be due to heterotrophic microorganisms. An alternative explanation for this pattern may be that photosynthetic microbes inside colonies or other aggregates were not killed by the addition of formaldehyde in the experiments. Although we did not observe detectable increases in absorbance (without LBB) in the killed treatments, we cannot rule out some remaining contribution from metabolism. Further studies with different biocide treatments (Tebo, 1991) and the addition of mineral

particles could help to determine the importance of light for mineral surface interactions.

Role of Reactive Oxygen Species

We hypothesized that superoxide is an important oxidant of Mn (II) in Lake Erie (H5, **Table 1**). We tested this hypothesis by adding superoxide dismutase to remove the effect of the free radical. We observed no effect of SOD on Mn oxidation in the central basin. Addition of SOD led to inconsistent effects on Mn oxide concentration and Mn oxide production rates in the western basin (**Figures 2, 3**). In particular, the July experiment from the western basin did show a decrease in Mn oxide concentration with SOD (in the light only). As a result, our experiments provide little support for superoxide as a primary mechanism of Mn oxidation. This finding was surprising given previous laboratory experiments showing that biologically produced superoxide is a potent oxidant of Mn(II) and Mn(III) (Learman et al., 2011a; Hansel et al., 2012). While addition of SOD did not significantly decrease production of Mn oxides in any of the experiments, the effect of formaldehyde addition shows that some of the Mn oxide production was driven by biological processes. This suggests that other biological mechanisms, besides superoxide, could play an important role. Such biological mechanisms could include Mn specific oxidases (Miyata et al., 2006; Dick et al., 2008; Anderson et al., 2009; Butterfield et al., 2013; Nakama et al., 2014) or exudation of DOM (Duckworth and Sposito, 2005;

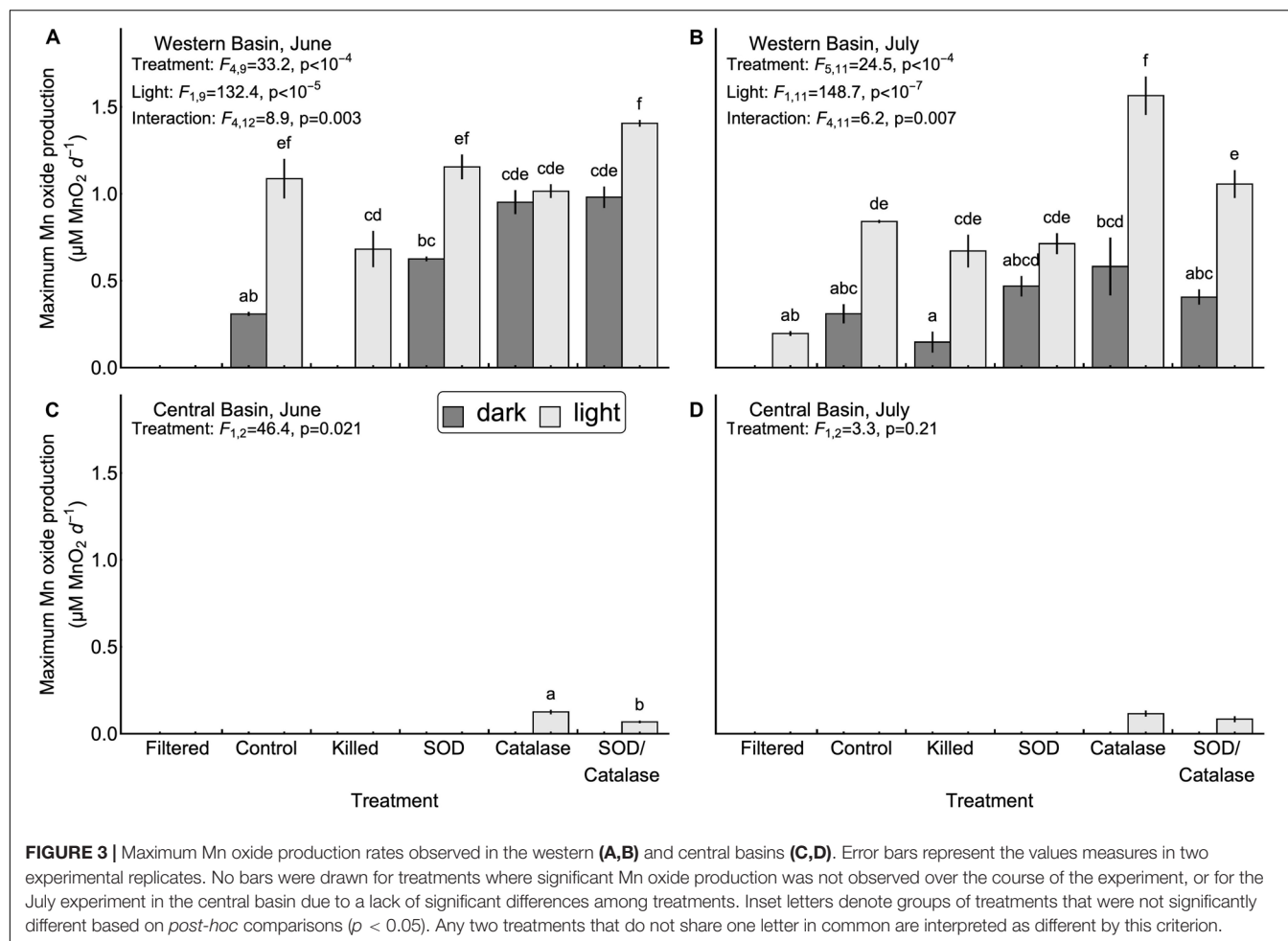


FIGURE 3 | Maximum Mn oxide production rates observed in the western (A,B) and central basins (C,D). Error bars represent the values measures in two experimental replicates. No bars were drawn for treatments where significant Mn oxide production was not observed over the course of the experiment, or for the July experiment in the central basin due to a lack of significant differences among treatments. Inset letters denote groups of treatments that were not significantly different based on *post-hoc* comparisons ($p < 0.05$). Any two treatments that do not share one letter in common are interpreted as different by this criterion.

Hansel, 2017), both of which can catalyze the oxidation of Mn(II) by dissolved oxygen.

Although hydrogen peroxide is not an oxidant of Mn(II) under the conditions in Lake Erie, we hypothesized that hydrogen peroxide has an inhibitory effect of Mn oxide accumulation and production rates by acting as a reductant for Mn(III, IV) oxides (H6, Table 1). We tested this hypothesis by adding catalase to remove the effect of hydrogen peroxide as a reductant. Our experiments in the central basin showed significantly higher Mn oxide concentrations and Mn oxide production rates in the catalase treatment compared to the control, although only in the light (Figures 2, 3). In the western basin there was no difference in maximum Mn oxide concentration between the control and catalase treatments, through there were significant increases in Mn oxide production rate in June (dark treatment) and July (light treatment). However, photosynthetic activity is also known to produce hydrogen peroxide (Painter et al., 2001) and may explain why the inhibitory effect of hydrogen peroxide was greater in the light than in the dark.

Our final hypothesis was that superoxide and hydrogen peroxide acts as competing redox reactions with Mn (H7, Table 1). Compared to the SOD only treatment, addition

of SOD/catalase in the western basin significantly increased Mn oxide concentration (June, dark) but had no significant effect on maximum Mn oxide production rate. In the central basin the addition of SOD/catalase led to higher Mn oxide concentrations and Mn oxide production rates compared to the SOD only treatment. In the June experiment the SOD/catalase treatment lowered Mn oxide production rates by 45% compared to catalase only treatment. These results suggest that superoxide was responsible for approximately half of the gross Mn oxidation under those conditions, but the net effect is not detectable due to the effect of hydrogen peroxide. Consistent with Hypothesis 7, our experiments show that both hydrogen peroxide acting as a reductant of Mn oxides can potentially negate the effect of superoxide acting as an oxidant, which has been documented in other studies (Archibald and Fridovich, 1982; Nico et al., 2002; Hansard et al., 2011; Learman et al., 2011a, 2013; Hansel et al., 2012). In several of our experiments the SOD/catalase treatment exhibited higher Mn oxide concentrations and Mn oxide formation rates than the control treatment (Figures 2, 3). If superoxide and hydrogen peroxide were the only mechanisms at play, we would expect the SOD/catalase treatment to produce no more Mn oxides than the control. In contrast, our results

suggest that not all of the Mn oxidation was attributable to superoxide and that the other mechanisms of oxidation are normally overwhelmed by the effect of hydrogen peroxide acting as a reductant.

These results suggest that reactive oxygen species could lead to rapid cycling among different redox states within the surface mixed layer, similar to what has been observed in marine and estuarine systems (Sunda and Huntsman, 1988, 1990). The presence of both oxidizing and reducing mechanisms under the conditions in the surface mixed layer has two important implications. First, the presence of reducing mechanisms in the surface mixed layer could serve to slow advection of Mn from the surface mixed layer, similar to what has been proposed in marine systems (Sunda and Huntsman, 1988, 1990). Second, because Mn(III, IV) oxides are among the strongest oxidants in aquatic systems (Yakushev et al., 2009; Oldham et al., 2019), cycling of Mn oxides in the surface mixed layer could have important consequences for organic matter (Allard et al., 2017).

Light-Mediated Effects on Mn Oxide Production

We found that light treatment had a significant and overall positive effect on Mn oxide concentration in all four experiments (Figure 2). This is consistent with other recent work showing that light can enhance Mn oxide production via both photosynthetic microbes (Richardson et al., 1988; Chaput et al., 2019) and/or abiotic mechanisms (Nico et al., 2002; Learman et al., 2011b, 2013). As discussed above, our experiment does not suggest that photosynthesis was solely responsible for the positive effect of light on Mn oxide formation. Instead, our results are consistent with an abiotic photochemical process catalyzed by mineral particles. This phenomenon has been reported previously (Learman et al., 2011b, 2013), but remains poorly understood. We did not find evidence for direct photoreduction of Mn oxides or photo-mediated reduction of Mn oxides by DOM, both of which are known to occur (Sunda and Huntsman, 1988, 1990; Bertino and Zepp, 1991). It is possible that photoreduction did occur but was slower than oxidation, which would have masked this reaction.

Despite these dramatic effects of light on Mn oxidation, our experiment did not completely mimic the natural intensity and spectrum of light in the surface mixed layer of the lake, which could impact interpretation of the experiment in two distinct ways. First, although the lights that we used for our experiment provide the complete PAR spectrum at an intensity representative of daytime PAR conditions in the surface 2 m of Lake Erie, it is possible that some photosynthetic microbes were under-saturated or, more likely, over-saturated under these conditions. If phytoplankton were undersaturated for light, it is possible that our estimates of light-mediated Mn oxidation underestimate the true net effect of light that may occur at the surface of the lake. Second, although the light intensities were high throughout the visible spectrum, our light source did not extend into the ultraviolet portion of the spectrum. Light-mediated reduction of Mn, particularly by ultraviolet

wavelengths, is known to occur directly through photo-reduction of Mn(III, IV) (Sunda and Huntsman, 1988, 1990) and indirectly through reaction with DOM or mineral oxides (Sunda et al., 1983; Bertino and Zepp, 1991).

Lake Erie is similar to other freshwater lakes in that UV light is more rapidly attenuated with depth than visible wavelengths or PAR (Morris et al., 1995), and wavelengths corresponding to UV-B and lower do not penetrate beyond the first few meters at the surface. Smith et al. (1999) measured extinction coefficients for PAR, UV-A, and UV-B in the western and central basins of Lake Erie. Based on their median extinction coefficients for each basin, PAR is attenuated to 1% of its surface intensity by 5.3 and 6.8 m (western and central basins, respectively), UV-A by 2.0 and 2.7 m, and UV-B by 1.1 and 1.5 m below the surface. Because photo-reduction of Mn oxides is known to occur even in the absence of UV wavelengths (Sunda et al., 1983), our estimates of net Mn oxidation do not reflect rapid cycling of Mn redox states that may occur in the lake as a result of photo-reduction (Sunda and Huntsman, 1994). Moreover, photo-reduction of Mn oxides in Lake Erie could allow the persistence of dissolved Mn even under oxic conditions and regions of the lake where hypoxia does not typically occur (e.g., the western basin). Determining the effect of photo reduction on Mn cycling and distribution in the lake will ultimately require further experiments that explicitly test the effects of UV at intensities expected in the water of Lake Erie.

Relative Contribution of Biotic and Abiotic Mechanisms

Previous work in freshwater has suggested that the majority of Mn(II) oxidation is attributable to biological processes (Chapnick et al., 1982; Tipping, 1984; Aguilar and Nealson, 1998). While we did find evidence for biological oxidation of Mn (Hypothesis 3), our experiments suggest that abiotic mechanisms involving mineral surfaces also play an important role. In the western basin experiments, biological activity was responsible for 64% of the Mn oxides produced in the dark and 32% of the Mn oxides produced in the light, with the remainder being attributable to mineral surface interactions [(control – killed)/(control – filtered)]. It is important to note, however, that these proportions of biotic and abiotic contribution are based on net Mn oxide formation. Because the effect of hydrogen peroxide acting as a reductant of Mn oxides was evident in all four experiments, this means that gross Mn oxide formation rates were potentially much higher and thus the contribution of biological processes to gross Mn oxidation could be different.

Effect of Lake Basin on Mn Oxidation

Mn oxide concentrations and Mn oxide production rates from the western basin were much higher than those measured in the central basin. Maximum Mn oxide concentration after 7 days in the control + light treatment was 0.13 $\mu\text{moles L}^{-1}$ in the central basin but 3.4 $\mu\text{moles L}^{-1}$ in the western basin. Rates of Mn oxidation in the central basin were undetectable over 7 days, except for the light treatments including catalase (Figures 2A,B). Our results suggest that under the fastest rates in the western basin, about 90% of the added Mn(II) was converted

to Mn(IV) in a week, but previous work has shown that the oxide minerals produced are a mix of Mn(II,III,IV) and thus the 90% value is likely an underestimate of the total removal of reduced Mn (Tipping, 1984). The dramatic differences in Mn oxidation between the western and central basins could reflect differences in suspended sediment and biological productivity between these two areas of the lake (Shuchman et al., 2013). Temperature can also have an effect on rates of Mn oxidation (Hem, 1981; Tipping, 1984), but the difference in temperature between the central (14–16°C) and western basins (19–25°C) is inadequate to explain the lack of measurable Mn oxidation in the central basin.

Products of Mn Oxidation

The Mn oxide minerals produced in our experiments were analyzed for morphology via transmission electron microscopy (TEM). TEM images collected from both natural and experimental samples show particles that contain Mn (confirmed with Energy-dispersive X-ray spectroscopy, EDS, **Supplementary Figure S1**) with fiber like morphology (**Figure 4**). Samples without Mn(II) amendments or relatively high concentration of Mn(III,VI) (measured with LBB) did not contain particles with this morphology. The morphology documented here is similar to biogenic phylomanganate mineral birnessite, which has been seen with previous laboratory studies (Villalobos et al., 2003; Tebo et al., 2004; Learman et al., 2011b). Further, birnessite is known for its autocatalytic properties (Coughlin and Matsui, 1976; Bargar et al., 2005; Learman et al., 2011b), thus provides possible support for mineral surface oxidation of Mn(II) (H₂) in Lake Erie. The characteristic form of this mineral suggests that it should be readily removed by filtration. At the end of each experiment, we measured the Mn oxide concentration in the unfiltered and filtered (<0.2 µm) fractions from each treatment. In every case, filtration of the water resulted in undetectable concentration of Mn oxides. For those experimental units with detectable Mn oxide production in whole water ($n = 55$), the median removal by filtration was 99.3%.

Dynamics of Mn Cycling in Lake Erie

Taken together, this study shows that the cycling of Mn in the surface mixed layer is impacted by both biotic and abiotic mechanisms and the importance of these reactions varies substantially between contrasting basins of the lake. Our study suggests that Mn(II) in Lake Erie will not be oxidized by dissolved oxygen unless this reaction is catalyzed by a biotic or abiotic catalyst, particularly mineral surfaces and heterotrophic plankton. Light also has a strong effect on oxidation, and this is not solely due to the production of ROS or photosynthetic organisms. Our study showed that both superoxide and hydrogen peroxide impact cycling of Mn in Lake Erie, but their combined net effect was highly variable and warrants further study. Lastly, the difference in Mn oxidation rate between the western and eastern basin helps underscore the importance of biological processes and suspended sediments as controls on the fate of Mn once it is released during hypoxia in the central basin. Additional studies examining the effects of UV exposure, *in situ* metabolism, and hydrodynamic mixing are required to determine the relative impact of these mechanisms in the lake.

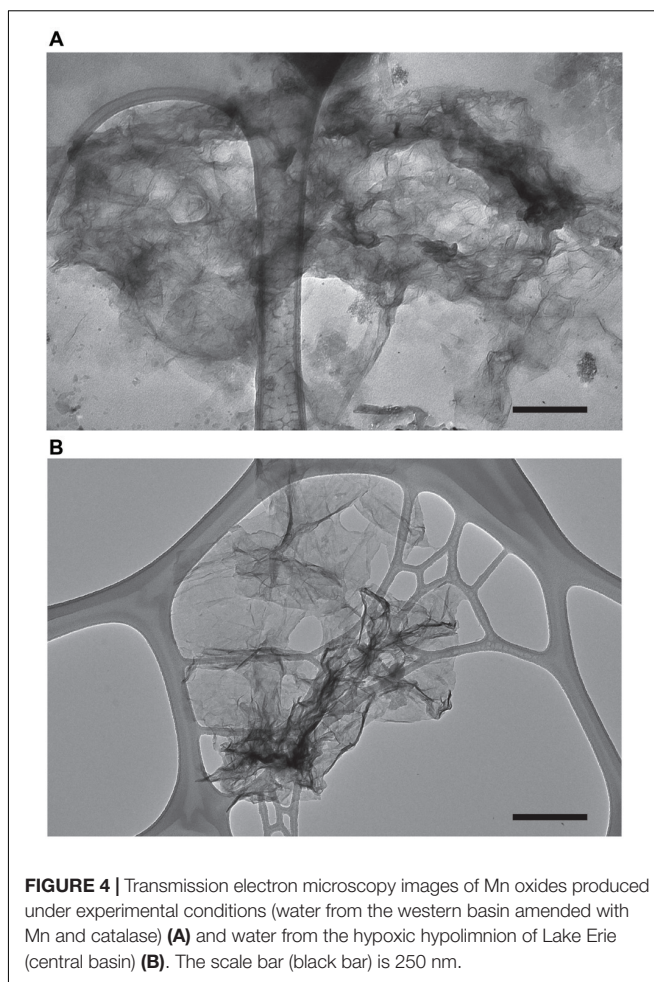


FIGURE 4 | Transmission electron microscopy images of Mn oxides produced under experimental conditions (water from the western basin amended with Mn and catalase) (**A**) and water from the hypoxic hypolimnion of Lake Erie (central basin) (**B**). The scale bar (black bar) is 250 nm.

Implications for Lake Erie Water Quality

Seasonal hypoxia is largely a phenomenon of the central basin, which is deep enough to stably stratify during the summer but typically has a thin hypolimnion, which becomes anoxic by mid-summer due to sediment oxygen demand (Rowe et al., 2019). Recently, it has been documented that Mn(II) is being released from the sediments in the central basin during these hypoxic events (Ruberg et al., 2008). Thus, the region of the lake experiencing accumulation of Mn(II) in the water (central basin) is also the region with slowest rates of removal (via natural oxidation). The low rates of Mn oxidation in the central basin are important because they mean that when Mn-laden hypoxic water mixes with the surface layer (via diffusion or upwelling), Mn(II) can persist for weeks or longer. While there are likely seasonal patterns in Mn oxidation rate in the central basin, our experiments suggest that co-location of Mn(II) release due to hypoxia and low Mn oxidation rates make the central basin particularly susceptible to accumulation of Mn during stratification.

While a lake-wide survey or budget will be required to quantify patterns of Mn accumulation in relation to hypoxia and removal by oxidation, our results have at least three implications for drinking water intakes. First, our experiment shows that

biogeochemical processes leading to net oxidation of Mn are an important control on its fate in Lake Erie. When Mn is eventually oxidized to form birnessite, it will either sink to the sediments or be readily removed by filtration during water treatment. While the problem of elevated dissolved Mn in Lake Erie is ultimately attributable to release of Mn from sediments under hypoxia ($\sim 50 \mu\text{moles m}^{-2} \text{d}^{-1}$, Adams et al., 1982), the low rates of Mn oxidation even under oxic conditions may serve to exacerbate this problem. Second, the low rates of Mn oxidation under oxic conditions present in the surface mixed layer of the central basin mean that dissolved oxygen concentration is an imperfect proxy for whether dissolved Mn(II) will be present. Based on our experiments, a more reliable prediction for potential Mn oxidation and removal from the water would consider suspended sediments, plankton biomass, and light. Third, although homogenous oxidation by dissolved oxygen is not among the mechanisms supported by our experiments, several of those other mechanisms do require dissolved oxygen as a reactant. Indeed, other studies have shown that Mn oxidation rates are sensitive to dissolved oxygen concentration (Tebo and Emerson, 1985; Tebo, 1991; Hsiung and Tissue, 1994). The dependence of these mechanisms on dissolved oxygen concentration means that oxidation rates in the hypoxic (i.e., $<2 \text{ mg L}^{-1}$ dissolved oxygen) areas of central basin are likely even lower than we measured. As a result, the accumulation of Mn(II) in the hypolimnion may potentially begin even before the onset of anoxia (Munger et al., 2019). These findings underscore the importance of understanding the mechanisms of Mn oxidation in order to predict the fate of Mn in freshwater systems.

DATA AVAILABILITY STATEMENT

The complete dataset is provided in the **Supplementary Material**.

AUTHOR CONTRIBUTIONS

JZ executed the experiments. DL performed the electron microscopy. CG wrote the computer code

and drafted the figures. All authors contributed to the experimental design, analysis of data, and writing the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2020.00057/full#supplementary-material>

FIGURE S1 | EDS spectra collected on fiber like material produced under experimental conditions using water from the western basin amended with Mn and catalase (left) and water from the hypoxic hypolimnion of Lake Erie's central basin (right). High Cu peaks are due to the copper grids used to prepare the samples for TEM.

TABLE S1 | Summary data for each experimental unit.

TABLE S2 | The complete experimental dataset.

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A Decade of Air Quality in Bogotá: A Descriptive Analysis

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In this work we apply a rigorous and reproducible data analytics process for validation and analysis of the historical data from Bogotá (Colombia) air quality monitoring network since 1998. The reasons for addressing this research study stem from the lack of a consistent approach for cleaning, validating and reporting air quality data. By analyzing the whole dataset, we are aiming at providing citizens and the city authorities with a clear view of the current situation of air quality and of its historical evolution. Without any loss of generality, we focus our analysis on both respirable and fine particulate matter (PM₁₀ and PM_{2.5}) concentrations, which in Bogotá and worldwide are source of concern for their negative impacts on human health. We develop a reproducible and flexible data cleaning methodology for particulate matter concentration data reported by the local authorities, which allows customizing and applying configurable validation rules. Then, we present statistical descriptive analyses by providing intuitive data visualizations, characterizing historical and spatial change of air pollutant levels. Results raise concerns for the high percentage of invalid data, as well as the high levels of PM_{2.5} and PM₁₀ ambient concentrations as observed in the valid portion of the available data, which frequently exceed national and international air quality standards. The data exhibit encouraging signs of air quality improvement, particularly for PM₁₀. However, the analyses indicate that significant differences exist across Bogotá, and particularly in the south-west zone of the city annual concentrations of particulate matter are up to three or four times the WHO recommendations. We are confident on the methodology and results from our analysis are useful both for local environmental authority and the general public to help in obtaining consistent conclusions from the available data.

Keywords: air quality, particulate matter, data validation, visual analytics, urban health

INTRODUCTION

Urban air pollution is a major environmental problem due to its negative effects on human health and quality of life (Chow et al., 2004; Gurjar et al., 2008; Romero-Lankao et al., 2013; Baklanov et al., 2016). In many Colombian cities, respirable particulate matter (PM₁₀) and fine particulate matter (PM_{2.5}) are the two most critical air pollutants. The plot in **Figure 1** shows the 2018 official data from IDEAM, the Colombian Institute for Hydrological, Meteorological and Environmental Studies, for the yearly average concentrations of particulate matter for several Colombian cities

equipped with air quality monitoring networks that measure both PM₁₀ and PM_{2.5} ambient concentration (Instituto de Hidrología, Meteorología y Estudios Ambientales [IDEAM], 2020). **Figure 1** depicts a concerning situation where most cities exceed the threshold values recommended by WHO for the average yearly values, 25 µg/m³ for PM₁₀, and 10 µg/m³ for PM_{2.5} (World Health Organization [WHO], 2018), which are marked as black contours. Notice that the average yearly PM₁₀ concentration of Yumbo is actually not displayed in the chart, as its extreme value (83 µg/m³) would impair the visualization. Also notice that multiple values are reported for two cities (Barranquilla and Bogotá) that are equipped with multiple monitoring stations. It is important to notice that a few cities exceed the national threshold set for the annual average concentration of PM₁₀ of 50 µg/m³ (Ministerio de Ambiente y Desarrollo Sostenible de Colombia [MADS], 2017).

In Bogotá, Colombia (one of Latin American largest cities), PM₁₀ and PM_{2.5} ambient concentrations are often well above national air quality standards (Franco, 2012; Instituto de Hidrología, Meteorología y Estudios Ambientales [IDEAM], 2016). Even more alarming, during the last decade, annual PM₁₀ and PM_{2.5} concentrations were more than twice higher than World Health Organization reference values. Recent studies showed that Bogotá ranks among the first cities in the world for the duration of travel times (INRIX, 2018), and that the exposure of citizens traveling across the city with both public and private transportation means can be hazardous for health (Franco et al., 2016; Morales et al., 2019).

This is a matter of concern since Colombia's national government stated that in Bogotá about 10% of the total deaths are attributable to urban air pollution, representing costs equivalent to 2.5% of the city's gross domestic product (National Planning Department [DNP], 2017). Moreover, local public health authorities have identified respiratory illnesses as the main cause of morbidity and mortality for children under 5 years of age (Hernández-Flórez et al., 2013).

Much of the effort in terms of air pollution management in Bogotá has been focused on an accurate diagnosis of the problem, mainly strengthening the city air quality-monitoring network and leading to a better understanding of the relevance and impact of the emission sources (Pachón, 2018; Mendez-Espinosa et al., 2019; Morales et al., 2019; Ramírez et al., 2020).

Bogotá air quality network (RMCAB) started its operation in 1998 under the administration of the District Secretariat of Environment, and today it comprises 14 automatic monitoring stations (13 fixed stations and a mobile one) geographically located within Bogotá's urban area (see **Figure 2**). Each station measures ambient air pollutant concentrations and meteorological conditions on an hourly basis. RMCAB monitors and sensors use standard methods tested by the United States Environmental Protection Agency (EPA), which are periodically calibrated and receive preventive and corrective maintenance. Specifically, PM₁₀ and PM_{2.5} instruments use the Beta attenuation monitoring technique to continuously measure the concentrations of these compounds (Secretaría Distrital de Ambiente [SDA], 2019). The data collected by the measuring devices is sent to a central database server managed by the

District Secretariat of Environment. Open-access is provided to the data, which can be downloaded in the form of text files from the page of the RMCAB monitoring network (Secretaría Distrital de Ambiente [SDA], 2020).

Data from the RCMAB has been crucial for characterizing the problem, leading to the identification of particle-related pollutants as the most critical in Bogotá. Also, it has been relevant when formulating public policies in the city, such as the Ten-Year Air Pollution Abatement Plan (Secretaría Distrital de Ambiente [SDA], 2011). However, much is yet to be done to improve the use and dissemination of the air quality data in the city. While the collected data is open, the public access provided for its visualization and analysis is limited, still focused on the needs of the data owner rather than on those of other data users, including research institutions and the general public. Certainly, one barrier that hinders exploiting historical air quality data is the difficulty in reliably extracting information out of it in a statistically significant way. This data analytics process requires significant technical knowledge not generally available. We believe this is an opportunity given the crucial role of data when formulating pollution control strategies and for improving urban air quality management.

Understanding such data potential, we worked together with the environmental authorities in a collaborative project that defines a structured and replicable methodology for air quality data acquisition, cleaning, validation, analysis and visualization, using big-data and analytics open access instruments. We present in this paper a first glimpse of what this collected data says about air quality in Bogotá, including current condition and historical tendencies.

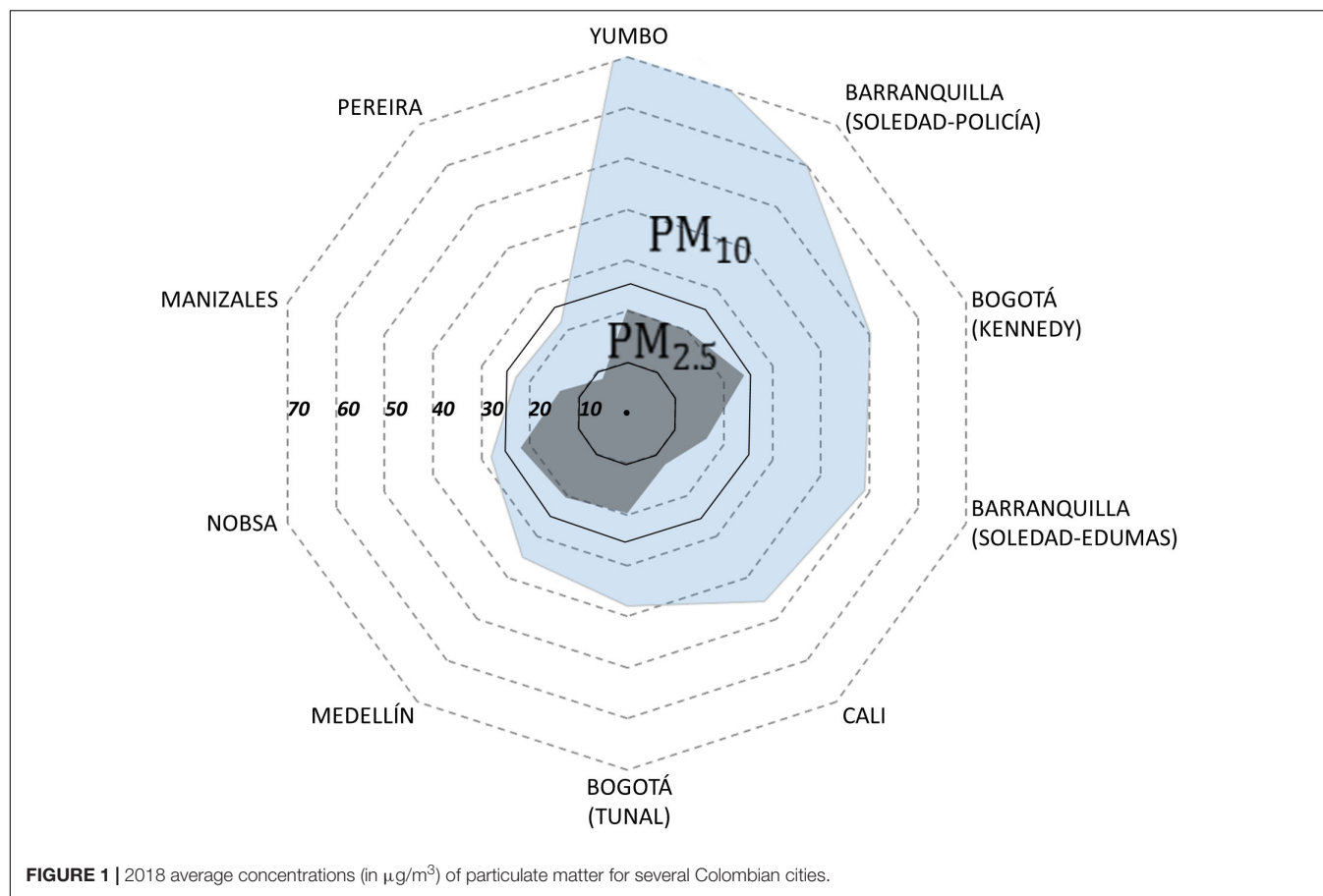
MATERIALS AND METHODS

Validation of Air Quality Data

We analyzed PM₁₀ and PM_{2.5} concentrations data collected by Bogotá Air Quality Monitoring Network. Local environmental authority provided us with the crude datasets for each of the existing 13 fixed stations, containing hourly observations for the 1998–2018 period. The mobile monitoring station data is not included in this analysis since it has been dedicated to the evaluation of air pollution in different heavy traffic roads (at several locations). Also, data from Fontibon and Bolivia monitoring stations are not part of the analysis, as the first station has been in process of site evaluation in the recent years and the latter only reports meteorological data.

We used the R[®] statistical package (R Core Team, 2018) to consolidate a single tidy dataset. With the aim of defining a simple and repeatable process, we developed a rule-based data validation. Two distinct types of rules were considered for validation:

- Generally applicable rules, which do not refer to the nature of the measured variable, for instance those used to exclude from posterior analysis non-numerical data and reported measurements that are out of the detection range of the monitoring equipment. Also, rules that



smooth the data series by removing single points that are extreme peaks/valleys, i.e., data values that drastically differ from the two immediately adjacent measurements. Since monitoring stations are purposefully located far from major emitting sources, such sudden variations are likely due to erroneous measurements.

- Contaminant-specific rules, which do take into account the physical properties of the variable being measured. They are used for instance to invalidate negative values reported for particulate matter measurements, since they are of the nature of concentration and impossible to be negative. Moreover, whenever simultaneous measurements of PM_{10} and $\text{PM}_{2.5}$ are available at a monitoring station, a rule is applied that verifies whether the $\text{PM}_{10} > \text{PM}_{2.5}$ relationship is maintained. Since $\text{PM}_{2.5}$ (particles whose diameter is not larger than $2.5 \mu\text{m}$) is a component of PM_{10} (particles whose diameter is not larger than $10 \mu\text{m}$), valid measurements must satisfy such inequality. In case it is not, this specific rule invalidates both measurements.

Finally, according to the practices of local authorities, we included a rule that removes isolated data points, based on a configurable minimal threshold set on the availability of data in a moving 24-h time window. When applied to the dataset, each rule sets a validation flag for each measurement, without actually removing the invalid values. The specific set of rules to

use can thus be flexibly chosen. Each analysis can in principle use a distinct set of validation rules for and new rules can be added to the data validation process in a modular way.

Descriptive Analysis of Air Quality Data

Descriptive analyses of air quality data allow understanding the behavior of pollutant concentrations and provide indications about their trends over time and location. Also, they allow to characterize the very same data availability, defining the scope and statistical significance of the analysis results that can be obtained from such set of data. To portray data availability, we worked on the design of very intuitive visualizations, which can be used to obtain a clear picture of the impact that the validation rules have on the data series. Our objective here was to provide information that is useful for understanding the type of statistical analyses that can be performed with the valid data, as well as to offer a way to analyze the patterns that may exist in the invalid data distribution.

We then determined distributions that adequately fit the valid data on particulate matter, which allows constructing useful models of contaminant behavior. We used the *fitdistrplus* R[®] package (Delignette-Muller and Dutang, 2015) to identify suitable fitting distributions for both PM_{10} and $\text{PM}_{2.5}$ data sets. With this package, we verified the position of the data samples in the Cullen and Frey graph (Cullen and Frey, 1999), which

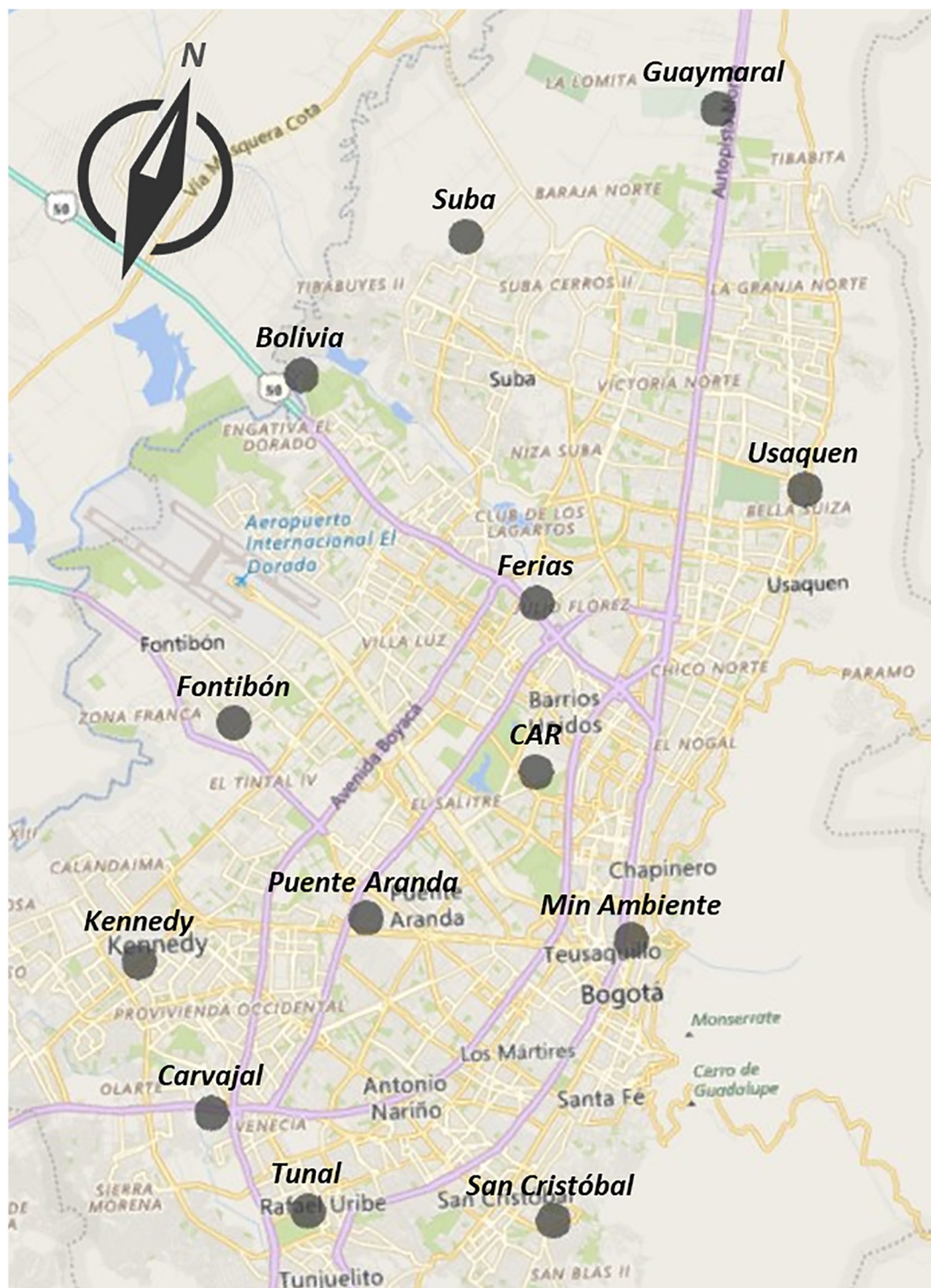


FIGURE 2 | Map of Bogotá urban area with the geographical location of the air quality monitoring stations.

based on the skewness and kurtosis of the data determines feasible distributions to be fitted. Then, we used numerical indicators such as Loglikelihood, Corrected Akaike's Information

Criterion (AICc) and Bayesian Information Criterion (BIC) to determine estimates for the error incurred when using a certain distribution as a model. According to the location of

the data samples in the mentioned Cullen and Frey graph, we computed these indicators for the Lognormal, Weibull and Gamma distributions.

Descriptive analyses of the data reported by the different air quality monitoring stations at different time granularities were performed. PM_{10} and $PM_{2.5}$ concentrations distribution and variation through hours, days of the week, and years were analyzed. Box plots and confidence intervals (using the Bonferroni method, with a confidence level of 95%) for the average values were produced and mean concentrations were compared to national standards and WHO guidelines.

Valid Data Availability

An important point that we would like to make is that we focused our analysis on achieving an understanding of the $PM_{2.5}$ and PM_{10} distribution and tendencies, without dealing with causality factors such as emission sources or factors that influence pollution distribution such as meteorology, or changes in the inventory of sources, quality of fuels, restrictions and regulations. We chose to limit the scope of our work to providing a descriptive analysis of the whole RMCAB dataset, a task that has not been tackled before. We approach our exploration of data through well-stated validation rules, which ensure that our results are reproducible. Finally, we focus on providing intuitive visualizations of the historical air quality of the city. Even though these visualizations are not explicative, they are easily understandable and offer a glimpse into a complex phenomenon that is of significance to the academia, government and the general public.

RESULTS

Valid Data Availability

The data reported by the Bogotá air quality monitoring station includes a set of air pollutants and weather related measurements. Data available for PM_{10} and $PM_{2.5}$ in the period 1998–2018 (hourly measurements) was downloaded from the web site of the Bogotá Secretariat of Environment and consolidated in a dataset of around 140 Mb. Then, the rule-based validation process was applied to the dataset.

Figure 3 visualizes the result of the rule-based validation process. It shows the percentage of data that passed all validation rules and those that were invalidated per tidying rule, for the $PM_{2.5}$ measurements (left panel) and for the PM_{10} measurements (right panel). A very high percentage of invalid data is remarkable. For PM_{10} , only five monitoring stations have at least 50% of valid data, with just Carvajal and Suba stations having a valid data proportion over 70%. For $PM_{2.5}$, the percentage of valid data per station is even smaller, also because in the very first years of the monitoring network not all stations were equipped with $PM_{2.5}$ measurement devices.

Most data were invalidated due to the string removal rule (orange portion of the bars). According to the local environmental authority, such string data is inserted in the dataset as a result of instrument failures, preventive maintenance activities, power supply failures, incorporation of

new monitoring equipment and communication failure due to port damage. It is therefore worthwhile remarking that most of the data unavailability is due to equipment outages rather than unreliable measurements.

For both pollutants, monitoring stations do not exhibit an appreciable correlation of the percentage of data in the different categories generated by the validation process. Also, no correlation is evident between the amount of valid data for $PM_{2.5}$ and PM_{10} at the same station, which suggests that data unavailability is primarily determined by causes that independently affect each contaminant monitoring unit. The subset of data records that passed all the validation tests accounted for around 60 Mb out of the 140 Mb of the original dataset.

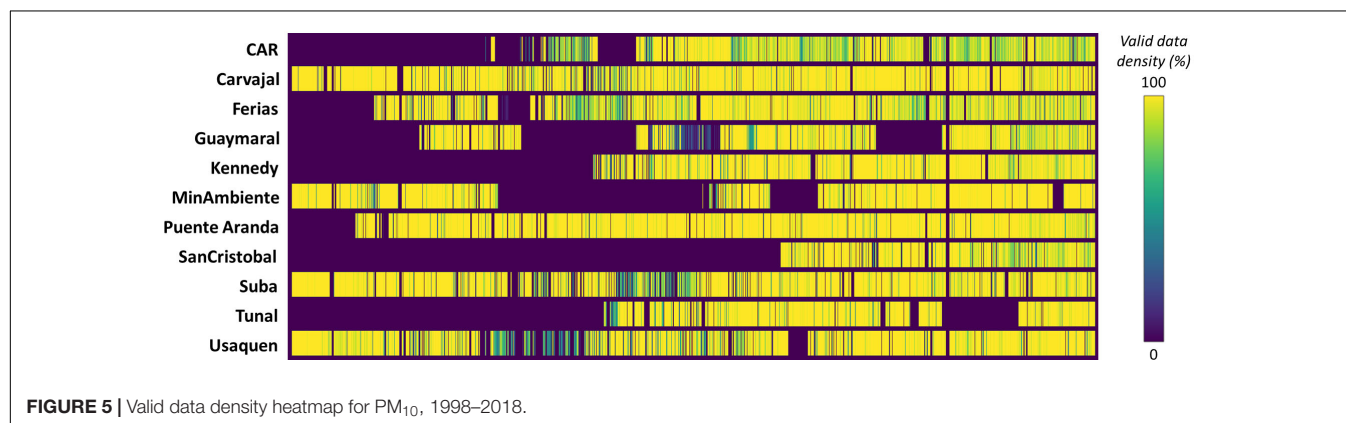
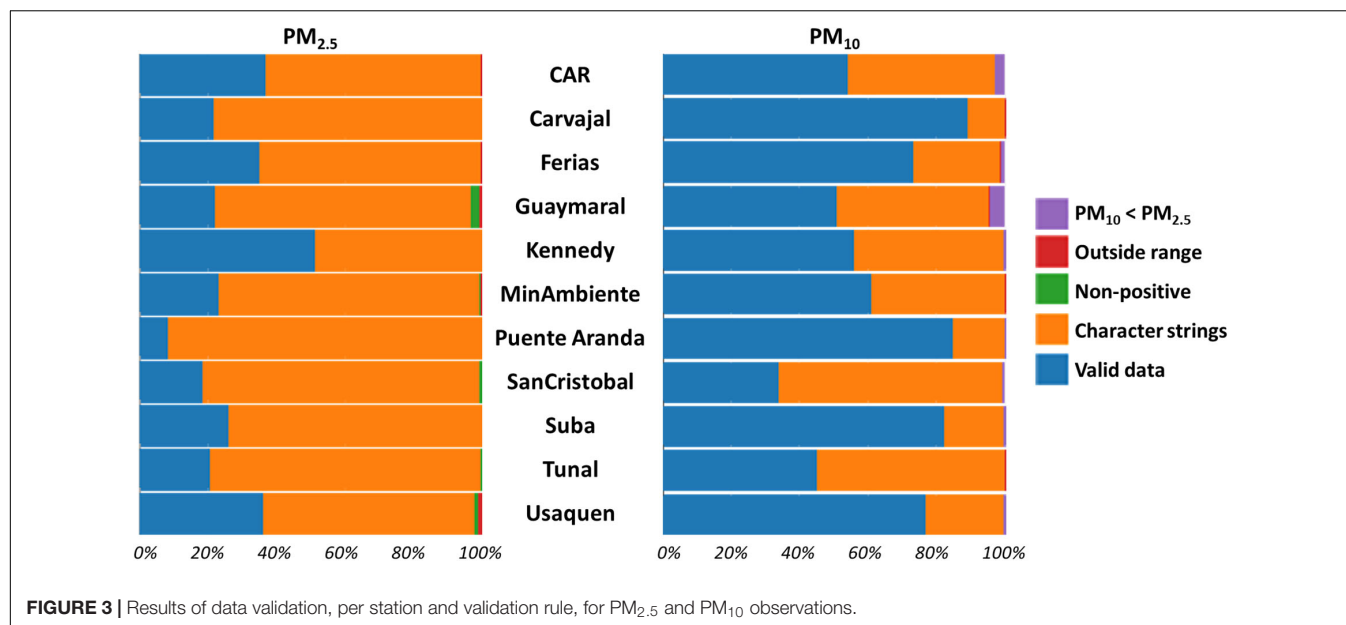
Figure 4 shows a heatmap of the daily valid $PM_{2.5}$ data density for the whole period of analysis (1998–2018). The heatmap reports the density of the valid data with a colored scale according to which a yellow data point indicates the full availability of hourly valid data – 24 valid data points – for a day, while purple denotes no available data. For $PM_{2.5}$ before 2008 only three stations have reported valid data, and after that valid data was only reported intermittently, while data availability improves significantly from 2009 onwards. Also, for 9 months in year 2013, among all stations only Kennedy reported valid data for $PM_{2.5}$.

As shown in **Figure 5**, valid data availability is higher for PM_{10} than for $PM_{2.5}$. Several stations, such as Carvajal, Suba, Ferias and Puente Aranda have been consistently reporting valid data within the period of analysis. These visualizations allow determining for which periods it is feasible to generate statistically aggregated indicators for the whole city, and in which ones the available data only allows local or zoned air quality characterization. According to these valid data density results, further analysis in this paper will only take into consideration the 10-year period beginning January the 1st of 2009 and ending December the 31st of 2018.

Statistical Data Treatment

Figure 6 shows the distribution of $PM_{2.5}$ and PM_{10} concentrations, with the upper tails in the figure truncated at one fourth of the maximum observed value. The observed $PM_{2.5}$ measured concentrations ranged in the interval [1.5, 416] $\mu\text{g}/\text{m}^3$, with an average of 20.05 $\mu\text{g}/\text{m}^3$ and a coefficient of variation (standard deviation/mean) of 0.75, while PM_{10} ranged in the interval [1.6, 998] $\mu\text{g}/\text{m}^3$, with an average of 55.64 $\mu\text{g}/\text{m}^3$ and a coefficient of variation of 0.72. **Figure 6** also reports WHO annual reference value for particulate matter, i.e., 10 $\mu\text{g}/\text{m}^3$ for $PM_{2.5}$ and 25 $\mu\text{g}/\text{m}^3$ for PM_{10} (World Health Organization [WHO], 2018), and Colombian national standards, i.e., 20 $\mu\text{g}/\text{m}^3$ for $PM_{2.5}$ and 50 $\mu\text{g}/\text{m}^3$ for PM_{10} (Ministerio de Ambiente y Desarrollo Sostenible de Colombia [MADS], 2017). The average values measured for both pollutants are twice above WHO guidelines and for PM_{10} exceed the threshold value set by the national regulation.

We plotted the data points in the Cullen and Frey graph to determine suitable distributions for valid data fitting (**Figure 7**). The orange points are obtained by 500 bootstrapped samples from the original data and allow to see the uncertainty associated



with the possible fitting distributions. According to the skewness and kurtosis for the historical data both pollutants can be fitted with a Weibull, Gamma or Lognormal distribution. It should be reiterated that data is obviously auto-correlated, but a time series analysis is not part of this work and only a preliminary descriptive analysis is being conducted.

We also compared the data against the three best fitting distributions of each type, finding that for both $PM_{2.5}$ and PM_{10} datasets the Gamma distribution was the best choice, as determined by the Loglikelihood, AIC and BIC scores (see **Table 1**). The best fitting Gamma distribution for the $PM_{2.5}$ dataset has shape $k = 1.839$ and rate $\beta = 0.091$, while the best

fitting Gamma distribution for PM_{10} has shape $k = 2.217$ and rate $\beta = 0.026$.

Analysis of Air Quality in Bogotá

We start our analysis by exploring the valid data measured at each monitoring station. **Figure 8** provides a boxplot of the data (without any aggregation) for the historical concentrations of $PM_{2.5}$ (left chart) and PM_{10} (right chart) per station. The

boxplots' center value is the median of the data, while quantiles 75 and 25% are the limits of the box, and the upper/lower whiskers report respectively the largest and smallest measurements within 1.5 times the inter-quartile range. All hourly valid data in the chosen time window (2009–2018) has been considered for this analysis. The chart in **Figure 8** describes a situation in which significant differences exist among geographical areas in the city. Monitoring stations located in the southwestern part of Bogotá,

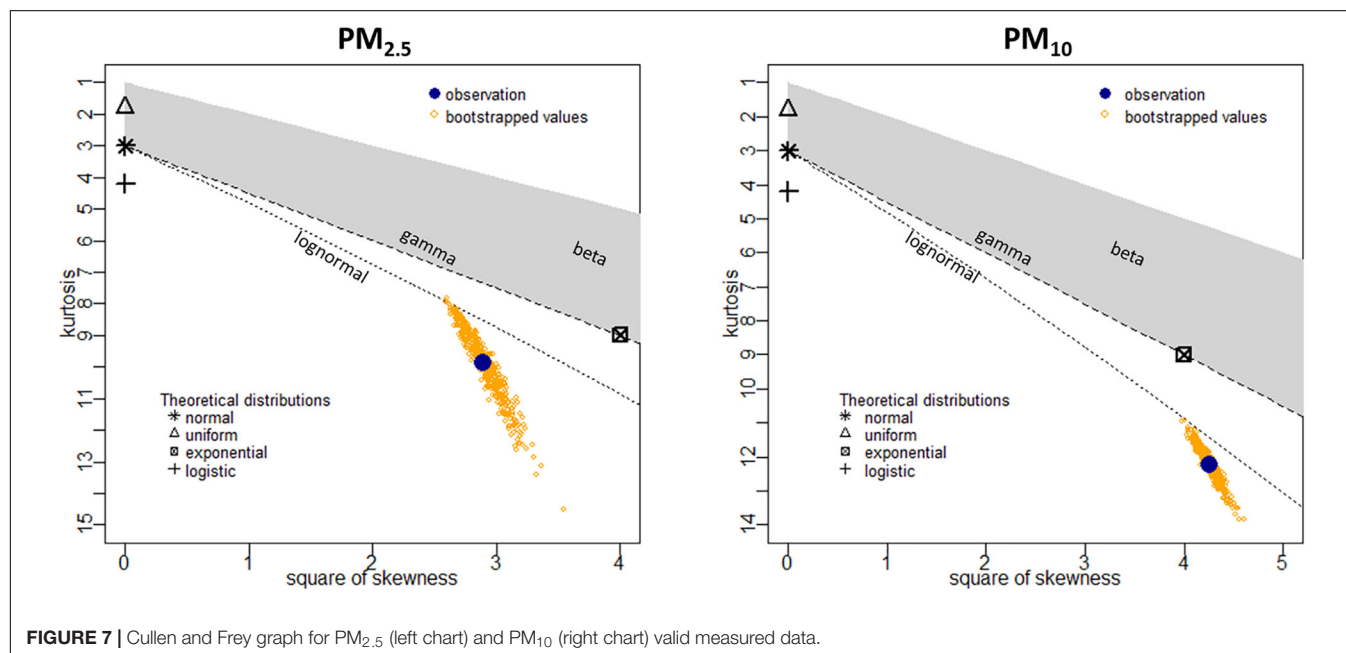
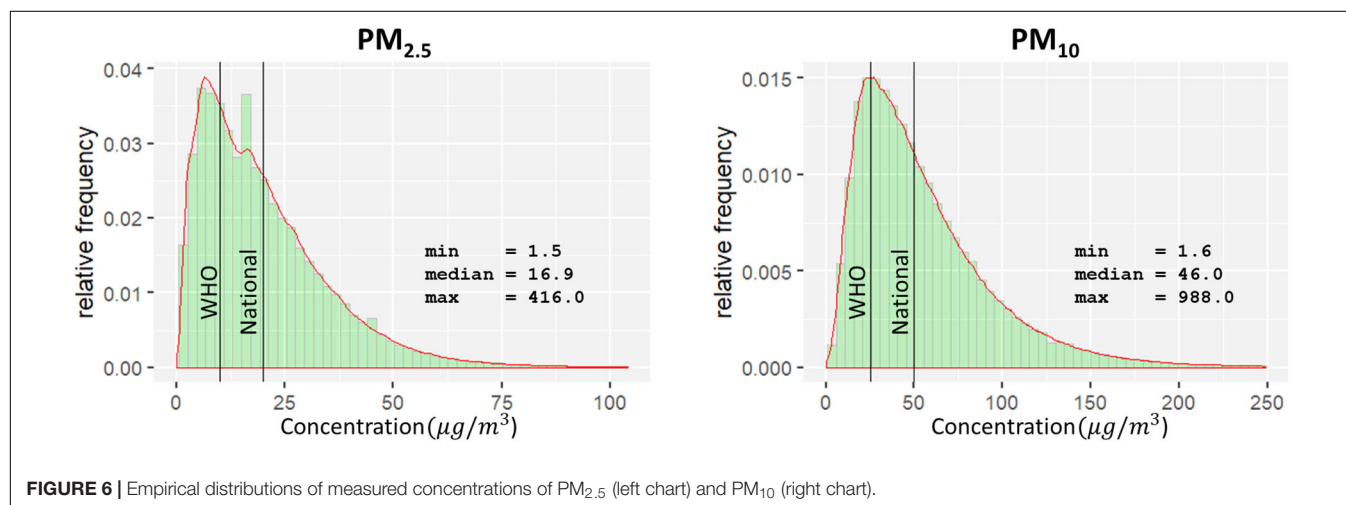


TABLE 1 | Loglikelihood, AIC and BIC indicators for $PM_{2.5}$ and PM_{10} data fitting.

	$PM_{2.5}$			PM_{10}		
	Lognormal	Weibull	Gamma	Lognormal	Weibull	Gamma
Loglikelihood	1917386	1910667	1906015	6002062	6033753	6001738
AIC	3834775	3821338	3812034	12004129	12067508	12003480
BIC	3834798	3821360	3812056	12004153	12067532	12003504

such as Carvajal and Kennedy, consistently reported higher concentrations of pollutants.

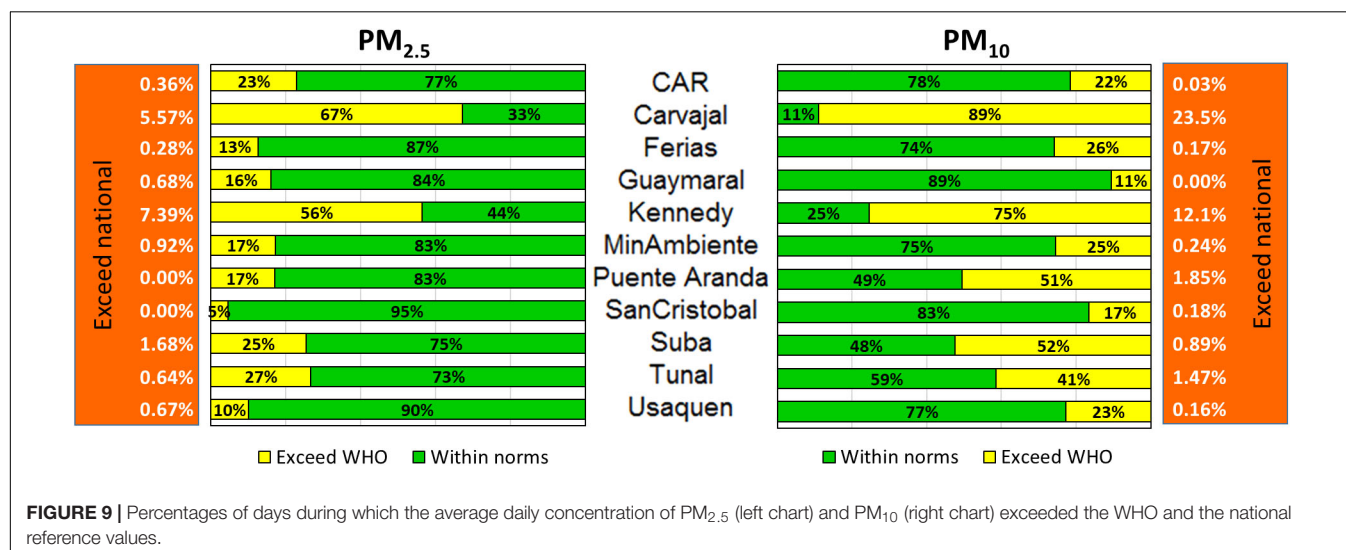
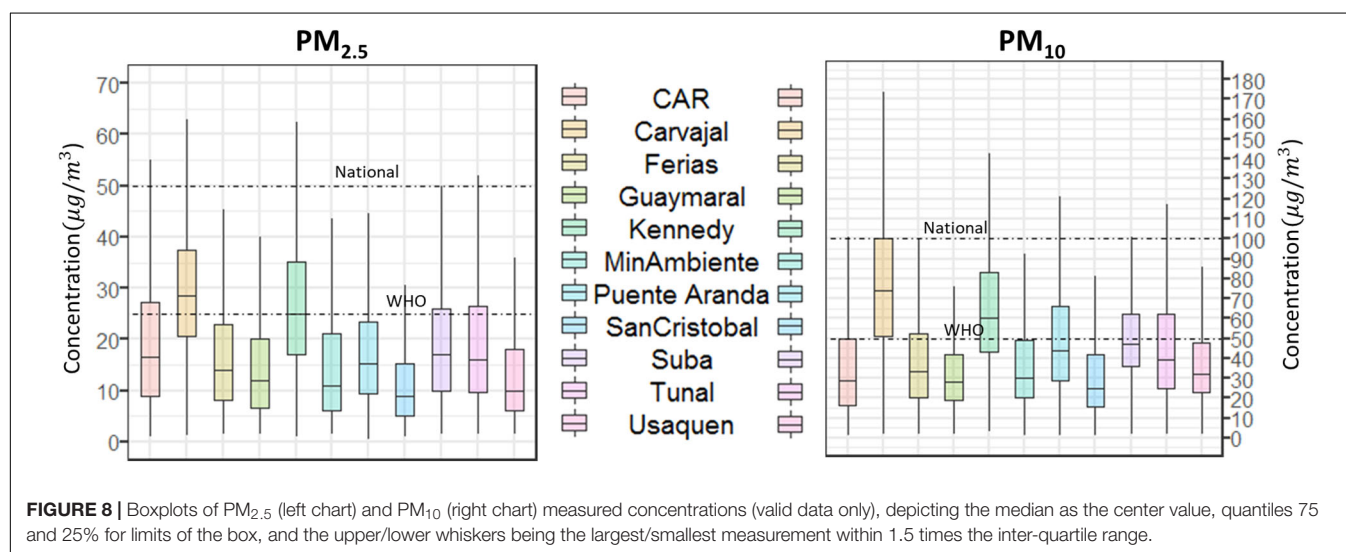
We report in **Figure 8** the average daily reference values from WHO for $PM_{2.5}$ ($25 \mu\text{g}/\text{m}^3$) and PM_{10} ($50 \mu\text{g}/\text{m}^3$), as well as the threshold for the average daily concentrations set as per the Colombian national regulations ($50 \mu\text{g}/\text{m}^3$ for $PM_{2.5}$ and $100 \mu\text{g}/\text{m}^3$ for PM_{10}).

To better appreciate the differences among measurements at distinct stations, we show in **Figure 9** the percentage of days, across the whole 2009–2018 period, for which the average daily pollutant concentration measured exceeded the WHO recommendation. The magnitude of these exceedances is shown by the yellow bars, for $PM_{2.5}$ (left chart) and PM_{10} (right chart). The percentages inside the red boxes are for the days in which the average concentration of pollutants exceeded the national threshold values.

From **Figure 9** we can observe that Carvajal reported 89% of the measurement days with average PM_{10} concentration

values above WHO standards, and Kennedy reported 75% days above WHO guidelines. Moreover, only two monitoring stations (Guaymaral, located in at the northern border of the city, and San Cristobal, located on the cliffs at the south) registered a percentage of PM_{10} exceedance days below 20%. In a significant part of the exceedance days of Carvajal and Kennedy, the national threshold for PM_{10} is exceeded as well, by 23.5 and 12.1%, respectively.

This is also the case for $PM_{2.5}$ for which Carvajal and Kennedy stations reported more than 50% of the days above WHO reference values and more than 5% above national thresholds, while in stations located in other areas of the city (San Cristobal, Usaquen) almost 100% of the concentration data for this pollutant were below the national threshold values. The reasons for the observed differences can be rooted not only in the meteorological conditions, but in the differential presence of emission sources such as industry and heavy traffic operating by diesel which are much more popular in some areas than in others.



To help identifying the presence of drifts in the measured concentration of pollutants, **Figure 10** shows multiannual monthly average concentrations charts, for the period of analysis (2009–2018) for $PM_{2.5}$ and PM_{10} . In the charts, the orange shadowed area is defined by the 95% confidence intervals for the monthly averages. The continuous line is a smoothing of the data obtained by local regression. One first thing that appears to be a constant for both pollutants through the period of analysis is a seasonal trend. January, February and March are the most polluted months of the year, while June, July and August are the months with lower ambient concentrations. Several studies have demonstrated an influence of meteorological conditions on the air quality of the city. There is evidence showing that the first months of the year are associated to more stable atmospheric conditions while mid-year months are characterized by more intense advections, as well as that distinct microclimate conditions exist in the same urban area, leading to detectable differences in air quality

(Secretaria Distrital de Ambiente [SDA], 2019). This is one of the main reasons for which recent guidelines recommend reporting air quality at the station and not at the city level.

Historical PM_{10} concentrations exhibit a steady downward drift (continuous dark line in right panel in **Figure 10**). For $PM_{2.5}$ the tendency is less evident. The peak in measurements reported around 2012–2013 corresponds to a period of limited data availability. As visualized in the miss-map for $PM_{2.5}$ reported in **Figure 4**, for most of 2013 only the Kennedy monitoring station was providing valid data. As shown in our previous analyses, Kennedy is one of the monitoring stations that consistently reports very high concentration of pollutants.

Since the charts in **Figure 10** suggest that the time-series of pollutants may not be stationary, in the subsequent analyses we shall consider explicitly the time dimension of observations.

Figure 11 presents the daily clustered values for $PM_{2.5}$ and PM_{10} . We show the average daily values and their 95% confidence interval (colored strip) for each of the years of analysis. For both

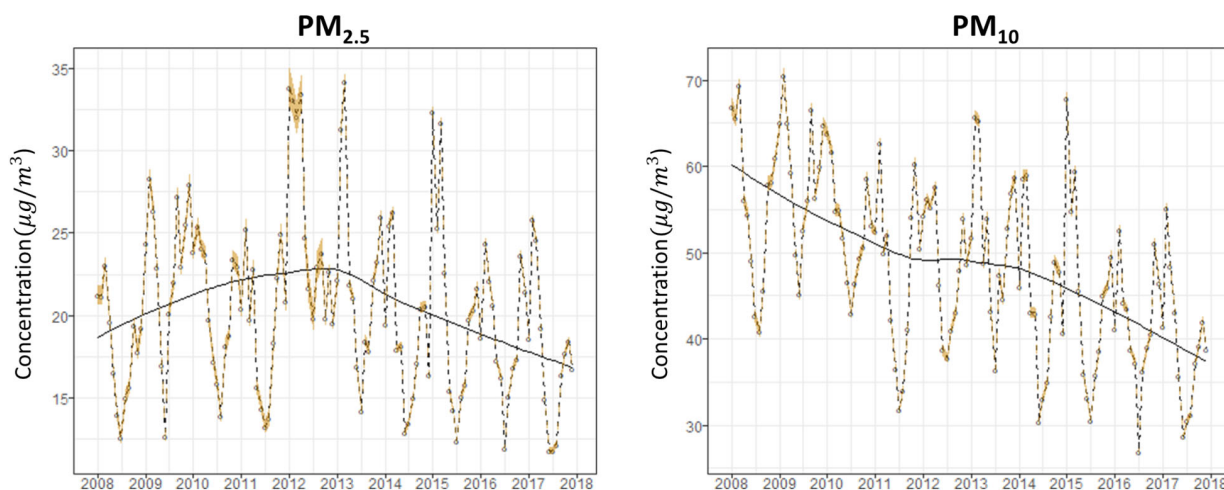


FIGURE 10 | Variations in $PM_{2.5}$ (left chart) and PM_{10} (right chart) monthly average concentrations (valid data only).

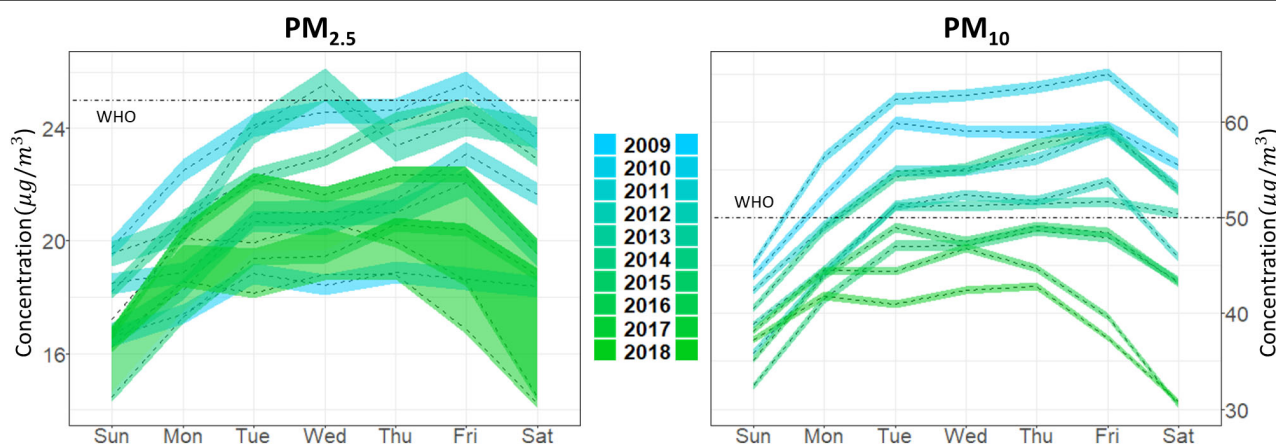
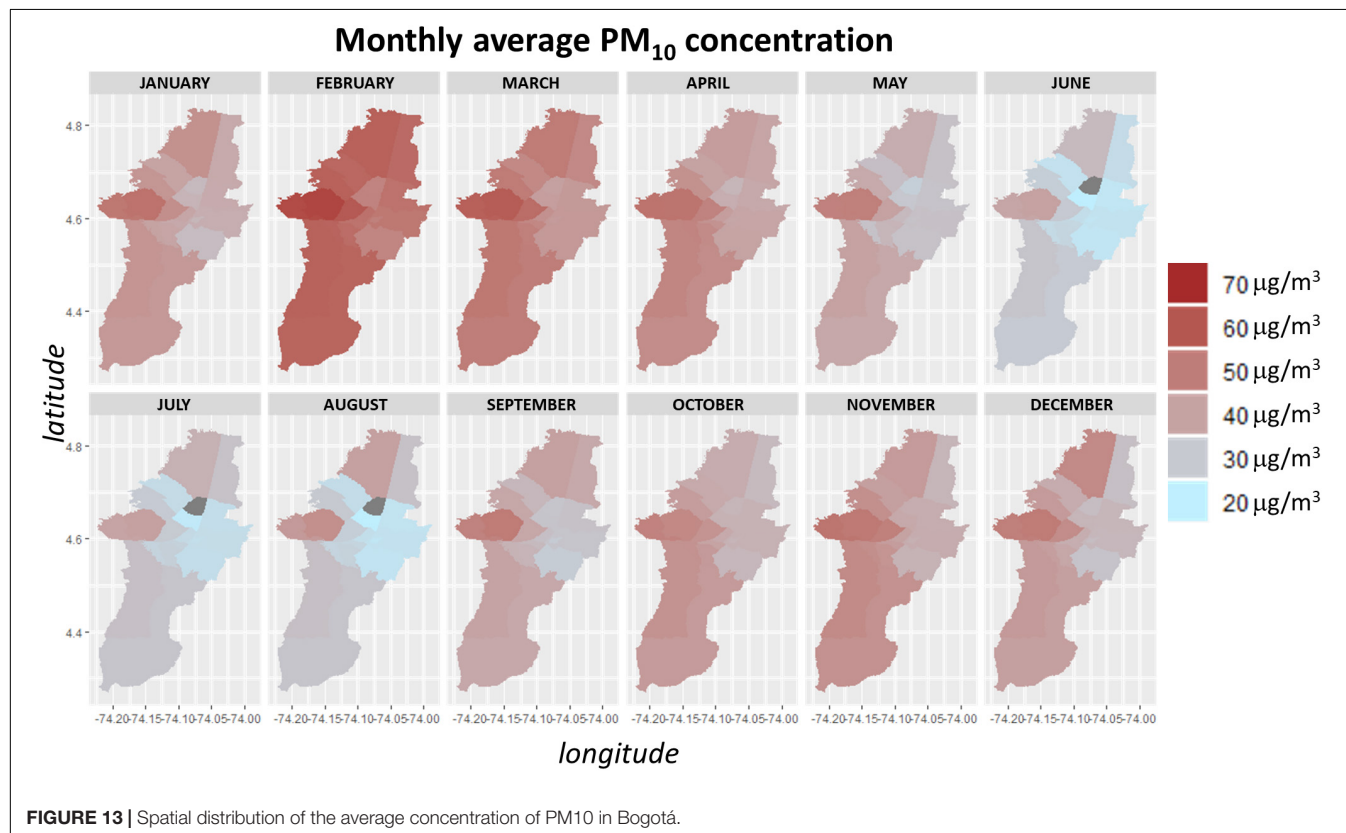
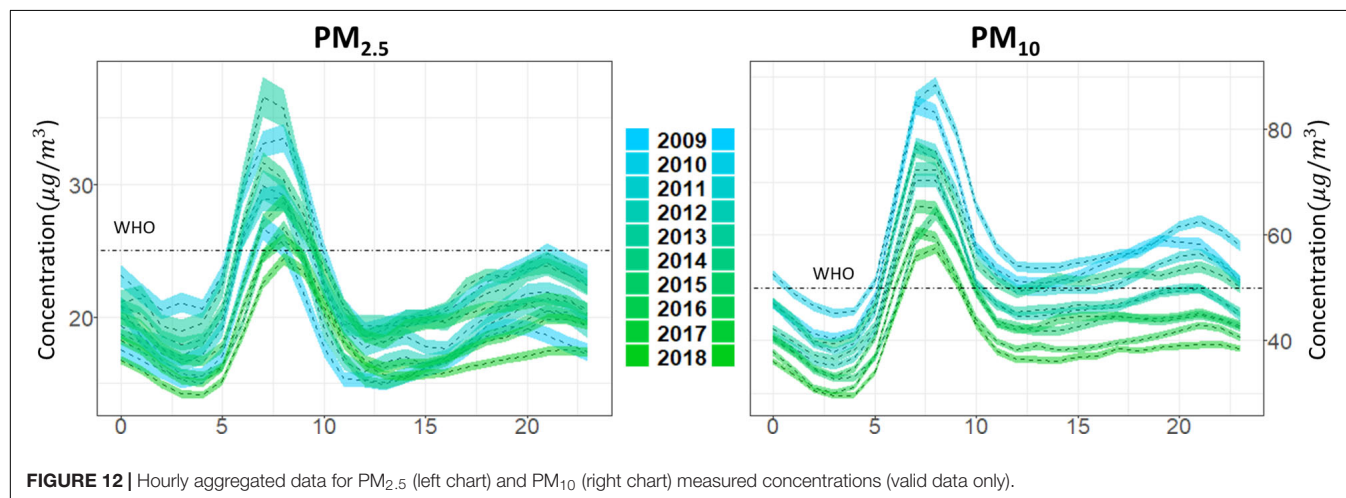


FIGURE 11 | Daily aggregated data for $PM_{2.5}$ (left chart) and PM_{10} (right chart) measured concentrations (valid data only).

PM_{2.5} and PM₁₀ there is a consistent increasing drift throughout the week, Sunday being the least polluted day and Friday the most polluted. Even though it is beyond the scope of this article to identify causes or explain the reasons of the observed air pollution levels, it is easy to speculate on mobile sources being the main cause of the pollutant accumulation over the week, as there is a significant statistical difference between Saturdays and Sundays (when heavy traffic is reduced) and weekdays. Also, lower concentrations on Mondays can be partially explained by the fact that most of the national holidays take place on this day

of the week (about 15 bank holidays throughout the year). While for PM_{2.5} average daily concentration (left chart in **Figure 11**) there is not an evident tendency to improvement, for PM₁₀ (right chart), we observe a steady encouraging decreasing drift in the measured average daily values over the years. While in 2009 all days of the week except for Sundays were exceeding the 50 $\mu\text{g}/\text{m}^3$ WHO reference value, since 2015 all the average daily values are consistently below such threshold.

Figure 12 shows PM_{2.5} and PM₁₀ data at the hourly aggregation level. The concentrations of both pollutants peak in



the time interval between 6:00 a.m. and 9:00 a.m. A secondary, lower peak of concentration arises in the late afternoon until night hours. Such bimodal behavior may be related with the traffic patterns in the city, with high vehicular activity in the morning rush hour and then intense but more dispersed traffic flows throughout the rest of the day. During peak hours, the concentrations of $PM_{2.5}$ and PM_{10} are above WHO 24-h reference values, meaning that Bogotá citizens are exposed to unhealthy air quality conditions during that part of the day. It is however, encouraging to observe that, at least for PM_{10} concentrations (right chart in **Figure 12**), an improving pattern of change is appreciable, with the average hourly concentration consistently descending over time in the whole hourly range. The analysis in **Figure 12** is suggesting that the different traffic restriction strategies that have been put in place in Bogotá have not affected $PM_{2.5}$ and PM_{10} daily patterns over time.

DISCUSSION, CONCLUSION AND FUTURE WORK

Bogotá's historical air quality data was validated and analyzed with the intention of representing tendencies of the most critical pollutants (PM_{10} and $PM_{2.5}$) over time. We consider this work (presented as a short communication) to serve the purpose of establishing a structured and well-grounded procedure for data cleaning, and of conducting a descriptive analysis using modern analytics tools. Moreover, we present a clean picture of what data says about air quality in the city over the past decade. The results of this work are useful both for local environmental authorities and the general public, since it defines repeatable steps for data preparation and effective visualization of analysis results, which help in obtaining consistent conclusions from the available data.

We would like to highlight three main issues that data say about air quality in Bogotá. First, historically there is a high percentage of invalid data (using the proposed tidying procedure). This is a matter of concern, since data quality is crucial when considering which periods to analyze, as well as what variables and stations to include in analysis. Second, $PM_{2.5}$ and PM_{10} ambient concentrations in Bogotá frequently exceed national air quality standards, meaning citizens are exposed to air pollution levels considered harmful for people's health. In particular, the south-west part of the city, where concentrations of particulate matter are frequently much higher than the values indicated in WHO guidelines.

Moreover, no single mean concentration for the whole city should be used to conclude the air quality conditions in Bogotá, and a spatially differentiated analysis should always be done. From the data collected at the monitoring stations we estimated

the average PM_{10} concentration, for each month of the year, at each one of the 19 administrative subdivisions (*localidades*, in Spanish) of the main urban area of Bogotá. As it can be easily appreciated from the panel chart in **Figure 13**, significant differences exist among those different areas of the city within the same month. A distance weighting formula has been used to determine the average pollution level in each area from the valid network measurements.

Third, even though mobile sources in the city have doubled in the last decade, PM_{10} concentrations show a consistent downward drift, and $PM_{2.5}$ concentrations do not appear to have worsened. This may suggest that control strategies, such as improvements in diesel sulfur content, and technological upgrades of industries have had beneficial impacts on particulate matter emissions and a subsequent effect on air quality in the city.

We understand urban air quality as a result of the interaction of different factors (i.e., meteorological conditions, emissions). Our future work will consider the integration of such factors in the analysis. Also, we are working on the development of an air quality data open access visualization tool for both the public and decision makers. We believe the type of analyses hereby presented and the continuity of such academic work in conjunction with environmental and public health authorities can lead to a better understanding of the problem, and to improve the extent of implementation of air pollution control actions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

All the authors equally contributed to the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Potential of Sentinel-2 for Crop Production Estimation in a Smallholder Agroforestry Landscape, Burkina Faso

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Crop production statistics at the field scale are scarce in African countries, limiting potential research on yield gaps as well as monitoring related to food security. This paper examines the potential of using Sentinel-2 time series data to derive spatially explicit estimates of crop production in an agroforestry parkland in central Burkina Faso. This type of landscape is characterized by agricultural fields where cereals (millet and sorghum) and legumes (cowpea) are intercropped under a relatively dense tree canopy. We measured total above ground biomass (AGB) and grain yield in 22 field plots at the end of two growing seasons (2017 and 2018) that differed in rainfall timing and amount. Linear regression models were developed using the *in situ* crop production estimates and temporal metrics derived from Sentinel-2 time series. We studied several important aspects of satellite-based crop production estimation, including (i) choice of vegetation indices, (ii) effectiveness of different time periods for image acquisition and temporal metrics, (iii) consistency of the method between years, and (iv) influence of intercropping and trees on accuracy of the estimates. Our results show that Sentinel-2 data were able to explain between 41 and 80% of the variation in the *in situ* crop production measurements, with relative root mean square error for AGB estimates ranging between 31 and 63% in 2017 and 2018, respectively, depending on temporal metric used as estimator. Neither intercropping of cereals and legumes nor tree canopy cover appeared to influence the relationship between the satellite-derived estimators and crop production. However, inter-annual rainfall variations in 2017 and 2018 resulted in different ratios of AGB to grain yield, and additionally, the most effective temporal metric for estimating crop production differed between years. Overall, this study demonstrates

that Sentinel-2 data can be an important resource for upscaling field measurements of crop production in this agroforestry system in Burkina Faso. The results may be applicable in other areas with similar agricultural systems and increase the availability of crop production statistics.

Keywords: remote sensing, parkland, Sudano-Sahel, intercropping, yield mapping, crop statistics, food security, monitoring

INTRODUCTION

Smallholder agricultural systems are the dominant livelihood strategy in most of sub-Saharan Africa (Morton, 2007), with a very high proportion of food and cash crop production coming from farms that are generally smaller than 2 ha (Gollin, 2014; Lowder et al., 2016). The importance of these systems in the light of the projected population growth and subsequent food production requirements on the continent cannot be overstated. At present, low actual crop yields relative to potential yields limit the possibilities to achieve food security and reduce poverty (World Bank, 2008). Such yield gaps and their causes need to be understood in order to design effective measures to increase crop production (Tittonell and Giller, 2013).

A basic requirement for conducting research on yield gaps is the availability of field level crop production estimates. Such data make it possible to understand causes of yield gaps and to evaluate the effectiveness of interventions and changes in land use techniques, as well as impacts resulting from changes in agricultural policy. However, agricultural statistics collected by field surveys in Africa are mainly reported on national level and generally considered to be unreliable (Carletto et al., 2015b; Burke and Lobell, 2017).

Satellite remote sensing represents an alternative method for deriving quantitative estimates of crop production and grain yield with a number of potential benefits, including the ability to cover large areas, repeated observations and low costs (Lobell, 2013; Chivasa et al., 2017). Crop production estimation has been a key research topic for several decades (Atzberger, 2013; Rembold et al., 2013). The main efforts have been directed toward intensive commercial systems where the fields are generally large, crops are homogeneous, and reliable agricultural statistics are accessible to calibrate and validate remote sensing based estimates (Lobell, 2013; Chivasa et al., 2017). Yields of these agricultural systems can be easily estimated even if the remotely sensed imagery has relatively coarse spatial resolution.

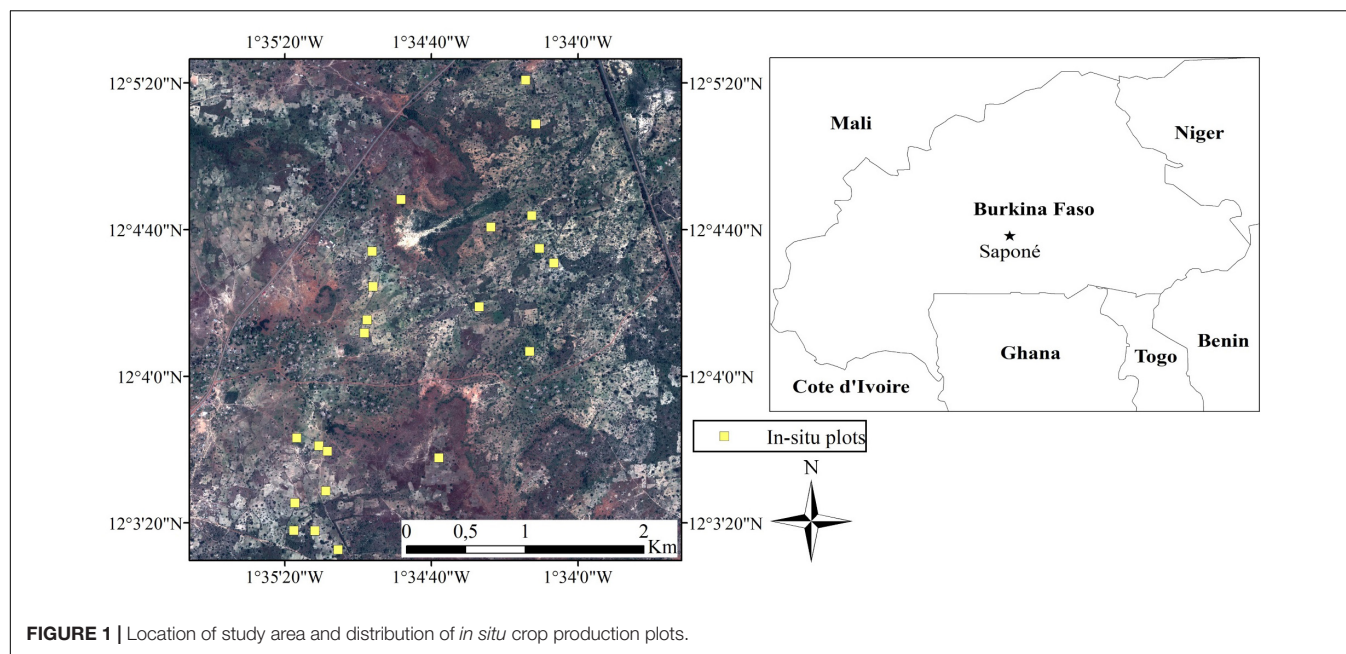
Such preconditions do not apply in the heterogeneous African smallholder systems where the agricultural fields are typically very small, irregular in shape and discontinuous in space and in crops cultivated (Burke and Lobell, 2017). Estimates based on household surveys from four African countries suggest that more than 50% of the fields are below 0.4 ha in size and 25% are smaller than 0.2 ha (Carletto et al., 2015a). In addition, a large proportion of the fields are intercropped, with high within-field heterogeneity in productivity (Bayala et al., 2014, 2015) and *in situ* measurements of crop production are generally unavailable (Tittonell et al., 2007; Bayala et al., 2014). A further complicating condition is that many of the smallholder farmers,

in particular in West Africa, practice agroforestry where trees and shrubs are integrated in the fields and thereby pose an additional challenge for remote sensing-based estimates of crop production (Vancutsem et al., 2013; Sweeney et al., 2015; Bégué et al., 2018).

Consequently, coarse-to-medium spatial resolution systems, such as MODIS and Landsat, provide limited possibilities for accurate crop production estimation in these heterogeneous agricultural landscapes because of the inability to distinguish small individual fields. Under such conditions, these satellite systems integrate the spectral measurement over an area that goes beyond the field boundaries, which means that other landscape components influence the signal and thereby results in mixed pixels. An important technological development was achieved when the Sentinel-2 satellite systems became operational (2A in 2015 and 2B in 2017). Sentinel-2 combines key spectral wavelengths at 10–20 m spatial resolution with a relatively short revisit-period (5 days at Equator) and an open-access data policy. These improvements have opened up new possibilities for more frequent high-resolution observations of agricultural fields, which is a precondition for satellite-based crop production estimation in African smallholder systems (Duncan et al., 2015).

Recent research in both eastern and western Africa has demonstrated the feasibility of satellite-based crop production estimation in smallholder systems. Burke and Lobell (2017) used 1 m Terra Bella Skysat imagery to estimate maize production during two growing seasons in Kenya and found promising agreement between satellite and *in situ* crop production estimates at individual field level. These authors concluded that the low availability of cloud-free imagery was an important limitation for improving accuracy in the estimates. Jin et al. (2017, 2019) expanded on the research in Kenya by also assessing the utility of satellite imagery from RapidEye and Sentinel-2. They found that the inclusion of red-edge wavelengths was useful for crop production estimation and reported considerable improvements in accuracy when aggregating field scale estimates to district level. Also using Sentinel-2 imagery, Lambert et al. (2018) developed crop type specific regression models to estimate production of millet, sorghum, maize and cotton in Mali. They showed that peak growing-season values of vegetation index (VI) and leaf area index (LAI) were the best estimators for the different crops.

The aim of this study is to assess the utility of Sentinel-2 data to estimate crop production at individual field scale in a smallholder farming system in central Burkina Faso. The landscape type in focus is referred to as agroforestry parklands in which the fields include a significant tree component and represents an important subsistence base in West Africa (Bayala et al., 2014). We investigated several critical aspects of satellite-based crop production estimation in this farming system,



including (i) choice of vegetation indices (VI), (ii) effectiveness of different periods for image acquisition and temporal metrics, (iii) consistency of the method between years, and (iv) influence of intercropping and trees on accuracy of the estimates. We also assessed the relationship between crop above ground biomass (AGB) and grain yield based on the reasoning that AGB has a stronger influence on the remote sensing data and is therefore easier to estimate.

MATERIALS AND METHODS

Study Area

The study area is an agroforestry parkland system situated in the rural commune of Saponé (12°04'48"N, 1°34'00"W) located 30 km south from Ouagadougou in central Burkina Faso (Figure 1).

This is a typical Sudano-Sahelian smallholder landscape where the fields are rainfed and farmers primarily grow pearl millet (*Pennisetum glaucum* (L.) R. Br.), sorghum (*Sorghum bicolor* (L.) Moench) and legumes, such as cowpea (*Vigna unguiculata*), for household consumption. Parklands are the main production system throughout this region. Farmers apply various forms of organic amendment to the fields, including manure, crop residues, woody litter and compost, but rarely mineral fertilizers (Koussoubé and Nauges, 2016). Doses of applied manure vary from 0.8 to 15 t ha⁻¹, with an average dose of 5.5 ± 0.72 t ha⁻¹ (Sanou, 2010). Crop residues also represent an important source of fodder for the small-scale livestock production, which is a common activity throughout this region.

The local terrain is relatively flat with small variations in elevation (293–363 m above sea level). The soils are shallow (on average ca. 60 cm), sandy loamy reosols (FAO classification) with very low nutrient content in terms of nitrogen, phosphorus and organic material (Bazié et al., 2012). In addition, the soils are weakly acidic with low cation exchange capacity (Bayala et al., 2002). Mean tree canopy cover is 15% and mainly consists of traditional agroforestry species, in particular *Vitellaria paradoxa*, *Parkia biglobosa*, *Lannea microcarpa*, and *Mangifera indica* (Karlson et al., 2015).

Average annual rainfall is around 800 mm with high inter-annual variability, which strongly influences crop development and production. Total rainfall was 850.7 mm in 2017 and 789.1 in 2018, with relatively large inter-annual differences in the monthly distribution (Figure 2). Annual mean potential evapotranspiration (PET_{Penman}) is 1900 ± 210 (Bazié et al., 2018). The rainy season generally extends between May and October, with high variability between seasons, and the main proportion of rain is usually falling between July and September. The cropping season starts between May and July, depending on crop type and the onset of the rainy season, whereas harvesting generally

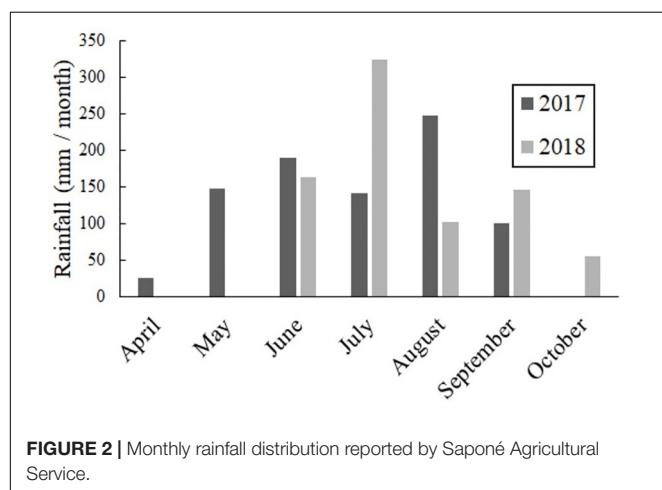


FIGURE 2 | Monthly rainfall distribution reported by Saponé Agricultural Service.

takes place at the end of October to November. However, unreliable rainfall at the start and end of the rainy season can lead to large windows for both sowing (May 15–July 15) and harvesting (October 15–December 15). This is even more variable nowadays due to high climate variability, as evidenced by farmers sometimes continuing to sow in August, as well as rains continuing to the beginning of November.

Crop Production Data

We measured total AGB and grain yield in 22 plots (20 × 20 m) during the harvest period (late October to early November) in 2017 and 2018 (Figures 1, 3). The plot locations included agricultural fields with different tree canopy cover and crop combinations, where agreements with the local landowners were organized. Tree canopy cover within and around the fields ranged between 1 and 34% (see section “Analysing the Influence of Tree Cover and Intercropping on Estimation Accuracy”). Each plot was geo-referenced using a Garmin Oregon GPS device with a locational error <3 m. Three different crops were cultivated in the sampled fields, including sorghum, pearl millet and cowpea. The plots included both intercropped and single crop fields. For each plot and crop, we cut, dried and weighed the different plant components. The millet and sorghum plant components included ears, grain and straw, and for cowpea the components included pods, grain and residues. Total AGB represents all dried plant components, including the grain.

Satellite Data

Sentinel-2 A/B data from 2017 to 2018 growing seasons (June–October) were processed to Level 2A using version 1.8.3 of the Sentinel-2 for Agriculture system (Bontemps et al., 2015; Defourny et al., 2019). Following these protocols, atmospheric correction, detection of clouds and shadows and retrieval of aerosol optical thickness were done using the MACCS algorithm (Hagolle et al., 2010, 2015). From the two growing season time series, images with cloud cover below 30% were selected for further processing, resulting in six 100 × 100 km granules (30PXU) from 2017 (9/6, 29/6, 29/7, 7/9, 7/10, 22/10) and seven from 2018 (14/6, 14/7, 24/7, 17/9, 27/9, 7/10, 22/10). The image acquisition aimed to select dates from similar points in time for each of the 2 years. MACCS output enabled masking of clouds and shadows in the time series images. Sentinel-2 acquires red-edge and shortwave infrared bands at 20 m resolution. These

bands were resampled to 10 m using bilinear interpolation to allow integration with the visible and near-infrared bands.

In addition, we acquired a cloud free Pléiades image (0.5 m spatial resolution) in October 2017 and used it to delineate tree crowns around the field plots. This ancillary dataset enabled an assessment of the potential influence of parkland trees on the Sentinel-2-based crop production estimation.

Vegetation Indices

We used various spectral VI derived from the Sentinel-2 data as estimators of crop production. Table 1 presents candidate VI previously used for estimating crop production in similar agricultural systems. These were identified in recent studies focusing on satellite based estimation of crop production and yield at high spatial resolution in smallholder systems located in comparable agro-ecological zones with similar crops, including Burkina Faso (Forkuor et al., 2018), Mali (Lambert et al., 2018), and Kenya (Jin et al., 2017). Our review indicated that VI with a strong relationship to crop production often included red-edge wavelengths or were designed to be sensitive to canopy chlorophyll content. We also included traditional VI, such as NDVI and EVI, for reference.

Temporal Metrics for Crop Production Estimation

The image dates used to derive VI, as well as the method used to integrate a time series of vegetation index values to derive temporal metrics, such as peak or mean values, are critical issues that strongly affect the accuracy of crop production estimation (Chivasa et al., 2017). First, clouds limit the possibility to acquire gap free observations during the growing season, especially in tropical areas, and thereby generally reduces the possibility to acquire dense time series (Roy et al., 2010). Secondly, the phenological phase characterized by the observations is critical for establishing strong relationships between VI and crop production (Duncan et al., 2015). The optimal timing of image acquisition or method for deriving temporal metrics can differ between years, geographical regions and crops, and also depends on the planting period and climatic factors, in particular rainfall (Rasmussen, 1992; Maselli et al., 2000). We evaluated both individual dates and temporal metrics (Table 2) as estimators of crop production. The temporal metrics were derived from the

TABLE 1 | Vegetation indices (VI) selected for crop production estimation in Saponé, Burkina Faso.

Vegetation index (VI)	Equation	References
Normalized Difference Vegetation Index (NDVI)	$\frac{(B8 - B4)}{(B8 + B4)}$	Rouse et al., 1974
Enhanced Vegetation Index (EVI)	$\frac{2.5 \times (B8 - B4)}{(B8 + 6 \times B4 + 7.5 \times B2 + 1)}$	Huete et al., 1997
Red Edge 1 NDVI	$\frac{(B8 - B5)}{(B8 + B5)}$	Forkuor et al., 2018
Red Edge 2 NDVI	$\frac{(B6 - B4)}{(B6 + B4)}$	Forkuor et al., 2018
Triangular Vegetation Index (TVI)	$0.5 (120 (B4 - B3) - 200 (B4 - B3))$	Broge and Leblanc, 2001
MERIS Terrestrial Chlorophyll Index (MTCI)	$\frac{(B8 - B5)}{(B5 - B4)}$	Dash and Curran, 2004

The equation references the Sentinel-2 band numbers.

full growing season (GS; June–October), as well as the end of the growing season (EGS) defined here as September to October.

Crop Production Estimation

In order to identify the most effective estimators for crop production, we compared average and maximum Pearson correlation coefficients ($p < 0.05$) between *in situ* measurements of crop production and the candidate VI. The comparison was done for both the temporal metrics and individual acquisition dates. In the final selection of VI to use as estimators in the crop production models, we gave preference to those that provided high correlation coefficients for both 2017 and 2018. The reason for this was to test whether a crop production model developed in 1 year (2017) was applicable for estimation of crop production in the following year (2018).

Crop production estimation models were calibrated using the most effective estimators identified in the previous step as independent variables in simple Ordinary Least Squares regression. Due to the relatively small sample size ($n = 22$), we used 10-fold cross validation for model calibration and for computing model accuracy measures (Hastie et al., 2008), including coefficient of determination (R^2), root mean square error (RMSE), relative RMSE (relRMSE), and standard deviation (SD).

Analysing the Influence of Tree Cover and Intercropping on Estimation Accuracy

Using the 2017 Pléiades image as reference, we manually delineated individual tree crowns in 60 m × 60 m areas centred over the crop harvest plots and tree canopy cover (%) was calculated within each 3600 m² area. From the *in situ* crop production data, we also computed the proportional contribution of cowpea to total AGB, which enabled an assessment of the influence of intercropping on the crop production estimation. Cowpea is a low growing legume and represents the primary crop to combine with cereals (millet and sorghum). We hypothesized that a higher proportion of cowpea in the fields would increase the error in the estimates since this crop is partly obscured from the view of the satellite sensor by the taller cereal canopy. Separate linear regression models were computed to analyse the relationships between residuals from the crop production estimation models and the field level proportion of cowpea AGB, as well as percent tree cover.

TABLE 2 | Temporal metrics used for crop production estimation based on full growing season (GS) and end of growing season (EGS) VI time series.

Temporal metric	Time period
GS mean VI	June–October
GS median VI	June–October
GS peak VI	June–October
EGS mean VI	September–October
EGS sum VI	September–October

RESULTS

Crop Production Measurements

Crop production measured *in situ* differed substantially between the 2 years, in particular for AGB, which was higher in 2018, whereas inter-annual differences in mean grain yield was comparatively small (Table 3). On the other hand, maximum and minimum values, and consequently the range of AGB and grain yield showed large differences between the 2 years with considerably higher values in 2018. The higher AGB in 2018, when total rainfall was lower compared to 2017, can be explained by variations in the interannual rainfall distribution. Relatively high rainfall in July (2018) can provide more favorable conditions for plant growth, whereas heavy rains in August (2017) can damage the more developed plants through effects of sustained flooding of the soil.

There was strong linear relationship between AGB and grain yield in both years, with an R^2 of 0.88 and 0.86 in 2017 and 2018, respectively (Figure 4). However, the slope of the regression line differed between the years with a lower AGB to grain yield ratio in 2018 compared to 2017. This suggests that AGB is a very strong indicator of grain yield despite the high prevalence of intercropping of cereals and legumes, which differ markedly in their structure. The results also show that the relationship can be subject to considerable inter-annual differences. We choose to focus the remaining analysis of satellite-based crop production estimation on total AGB instead of grain yield for two reasons. Firstly, AGB is what influences the signal in the Sentinel-2 data and any attempt to estimate grain yield is dependent on the strength of this relationship. Secondly, in addition to the grain yield, all other crop material constitutes an important source of fodder for livestock, which represents a key component of the area's livelihood (Bayala et al., 2014).

Identification of Crop Production Estimators

The strength of relationships between the candidate VI and AGB varied slightly with no clear consistency between the years, with the exception of MTCI that did not provide a significant correlation in either 2017 or 2018 (Table 4). When considering both 2017 and 2018, RE NDVI 2 proved to be the most stable VI and was therefore selected as the VI to develop regression models for crop production estimation. The temporal metrics with the strongest relationship to AGB also differed between the years, with GS peak VI consistently ranking highest in 2017 and GS median VI in 2018.

In addition to the temporal metrics, we also investigated the potential of the most effective VI (i.e., RE NDVI 2) from individual dates as estimators of AGB. Figure 5 shows a relatively similar temporal pattern for both years, where RE NDVI 2 from early to mid-September appears to provide the strongest correlation to AGB. The difference in the September image acquisition dates between 2017 and 2018 needs to be considered when comparing the maximum correlation coefficients.

TABLE 3 | Descriptive statistics of *in situ* crop production measurements in Saponé, Burkina Faso.

Year	Crop combination	AGB ton ha ⁻¹			Grain yield ton ha ⁻¹		
		Min	Max	Mean	Min	Max	Mean
2017	Sorghum (<i>n</i> = 4)	0.74	2.09	1.18	0.17	0.57	0.33
	Millet (<i>n</i> = 6)	0.06	1.79	0.66	0.01	0.38	0.15
	Sorghum – cowpea (<i>n</i> = 7)	0.10	2.19	1.10	0.05	0.89	0.36
	Millet – cowpea (<i>n</i> = 1)	0.73	0.73	0.73	0.15	0.15	0.15
	Millet-sorghum – cowpea (<i>n</i> = 4)	0.56	1.85	0.99	0.16	0.87	0.41
	Total	0.06	2.19	0.96	0.01	0.89	0.30
2018	Sorghum (<i>n</i> = 8)	0.29	4.67	1.92	0.04	1.12	0.37
	Millet (<i>n</i> = 5)	0.53	4.89	2.1	0.08	0.82	0.35
	Sorghum – cowpea (<i>n</i> = 3)	0.29	2.51	1.13	0.05	0.73	0.31
	Millet – cowpea (<i>n</i> = 5)	0.65	1.38	0.98	0.14	0.26	0.22
	Millet-sorghum – cowpea (<i>n</i> = 1)	1.73	1.73	1.73	0.36	0.36	0.36
	Total	0.29	4.89	1.62	0.04	1.12	0.32


FIGURE 3 | Two examples of field plot locations. Early growing season (left) and before harvest (right).

Estimating Crop AGB Production

The regression equations and the validation metrics for the AGB estimations based on temporal metrics and individual dates are presented in **Table 5**. The strength of the linear regression models between *in situ* AGB and VI estimates is generally high, with R^2 ranging between 0.41 and 0.80. However, there are clear

TABLE 4 | Correlation coefficients between vegetation index (VI) temporal metrics and aboveground biomass (AGB) in Saponé, Burkina Faso.

Vegetation index (VI)	2017		2018	
	Average	Max	Average	Max
EVI	0.63 (4)	0.69 – Peak	0.68 (5)	0.73 Median
NDVI	0.68 (4)	0.77 – Peak	0.65 (5)	0.75 Median
RENDVI 1	0.70 (1)	0.70 – Peak	0.53 (4)	0.58 Median
RENDVI 2	0.68 (6)	0.76 – Peak	0.73 (5)	0.83 Median
TVI	0.62 (4)	0.77 – Peak	0.57 (5)	0.60 Peak
MTCI	–	–	–	–

Numbers in parentheses indicate the number of temporal metrics with a significant ($p \leq 0.05$) relationship to AGB that were used to compute the mean value.

differences in accuracy between the 2 years when using VIs from individual dates as estimators. Specifically, the regression model based on RENDVI 2 from September 7 (2017) resulted in the most accurate estimates overall, with an R^2 of 0.80 and relRMSE of 32.2%. This contrasts with the lowest accuracy for AGB estimates that were derived using RENDVI 2 from September 17 in 2018, both in terms of R^2 (0.41) and relRMSE (63%). Using temporal metrics (GS peak and median) derived from the full growing season time series as estimators resulted in relatively high accuracy for both 2017 and 2018 with small inter-annual differences.

Above ground biomass estimates in 2017 were within the range of the *in situ* measurements and the estimation models did not show any signs of bias (**Figure 6**). In 2018, the AGB estimates were mostly between 0.5 and 3 ton ha⁻¹ and generally smaller as compared to the *in situ* measurements. The 2018 temporal metric (median) showed less tendency for bias and clearly outperformed the individual date (September 17, 2018) in terms of accuracy.

Inter-Annual Consistency

As an additional experiment, we applied the 2017 peak RENDVI 2 regression model to estimate AGB in the

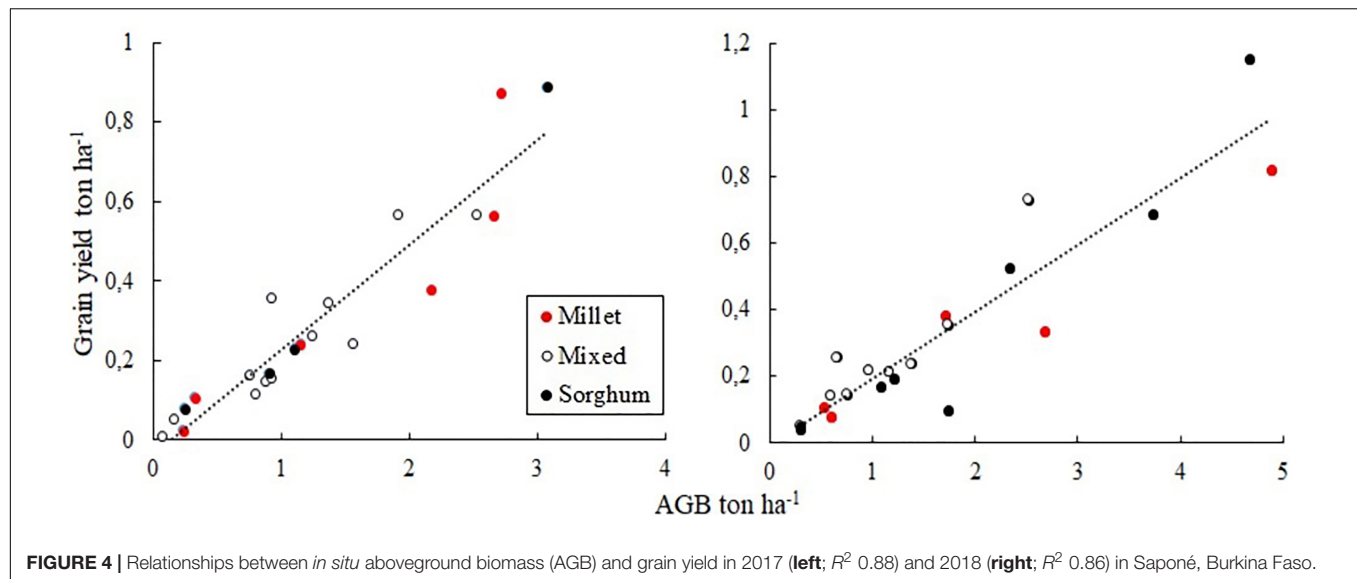


FIGURE 4 | Relationships between *in situ* aboveground biomass (AGB) and grain yield in 2017 (left; R^2 0.88) and 2018 (right; R^2 0.86) in Saponé, Burkina Faso.

TABLE 5 | Linear regression models used for aboveground biomass (AGB) estimation and validation metrics for 2017 and 2018 for the Saponé site in Burkina Faso.

Year	Regression model	R^2	RMSE (ton ha ⁻¹)	Rel RMSE (%)	SD (ton ha ⁻¹)
2017	AGB (ton ha ⁻¹) = $-4.0 + 12.7 \times \text{peak RENDVI } 2$	0.62	0.54	56.2	0.68
	AGB (ton ha ⁻¹) = $-2.7 + 10.4 \times \text{Sep 7 RENDVI } 2$	0.80	0.31	32.2	0.76
2018	AGB (ton ha ⁻¹) = $-8.3 + 31.9 \times \text{median RENDVI } 2$	0.68	0.77	47.5	0.93
	AGB (ton ha ⁻¹) = $-2.1 + 9.6 \times \text{Sep 17 RENDVI } 2$	0.41	1.02	63.0	0.80

Remotely sensed estimators are highlighted in italics.

following year using the 2018 Sentinel-2 time series as input data. The 2017 peak RENDVI 2 model was able to produce relatively accurate AGB estimates in 2018 in terms of R^2 (0.66), RMSE (0.81 ton ha⁻¹) and relRMSE (50%; **Figure 7**). However, the AGB ranges and mean values are different between the years, and AGB in 2018 was generally underestimated when using the model developed in 2017. The

estimates for plots with only millet in particular resulted in large errors, whereas AGB in plots with mixed crops were accurately estimated.

Influence of Tree Cover and Intercropping on Estimation Accuracy

We found no significant correlations between residuals and percent tree cover inside and around the fields (**Figure 8**), nor between regression residuals and the proportional contribution of cowpea to the total AGB. Consequently, these factors do not appear to significantly influence the satellite-based estimation of AGB in this study.

DISCUSSION

The ability to produce accurate and spatially explicit estimates of crop production at sub-field level from freely available satellite data would be a major development for the monitoring and analysis of smallholder farming systems in Africa. This study demonstrates that this may be feasible even in the highly heterogeneous and complex agroforestry parklands that extend over large parts of the Sudano-Sahel in West Africa. The following section discusses the relevance of our results for the application of the method to estimate crop production at regional or national level.

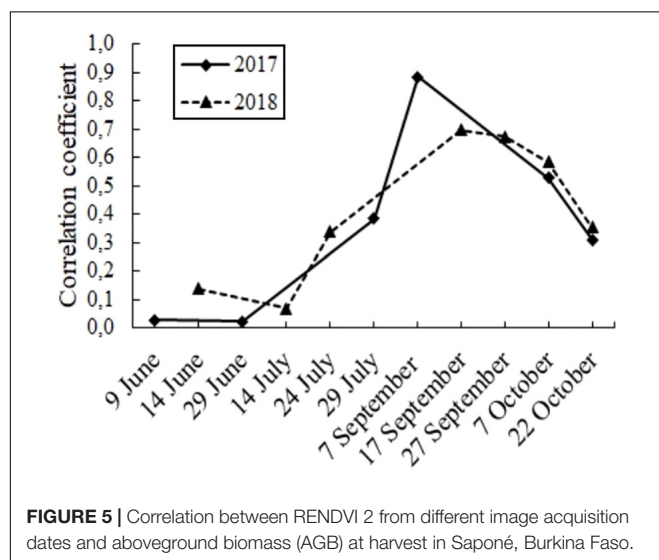


FIGURE 5 | Correlation between RENDVI 2 from different image acquisition dates and aboveground biomass (AGB) at harvest in Saponé, Burkina Faso.

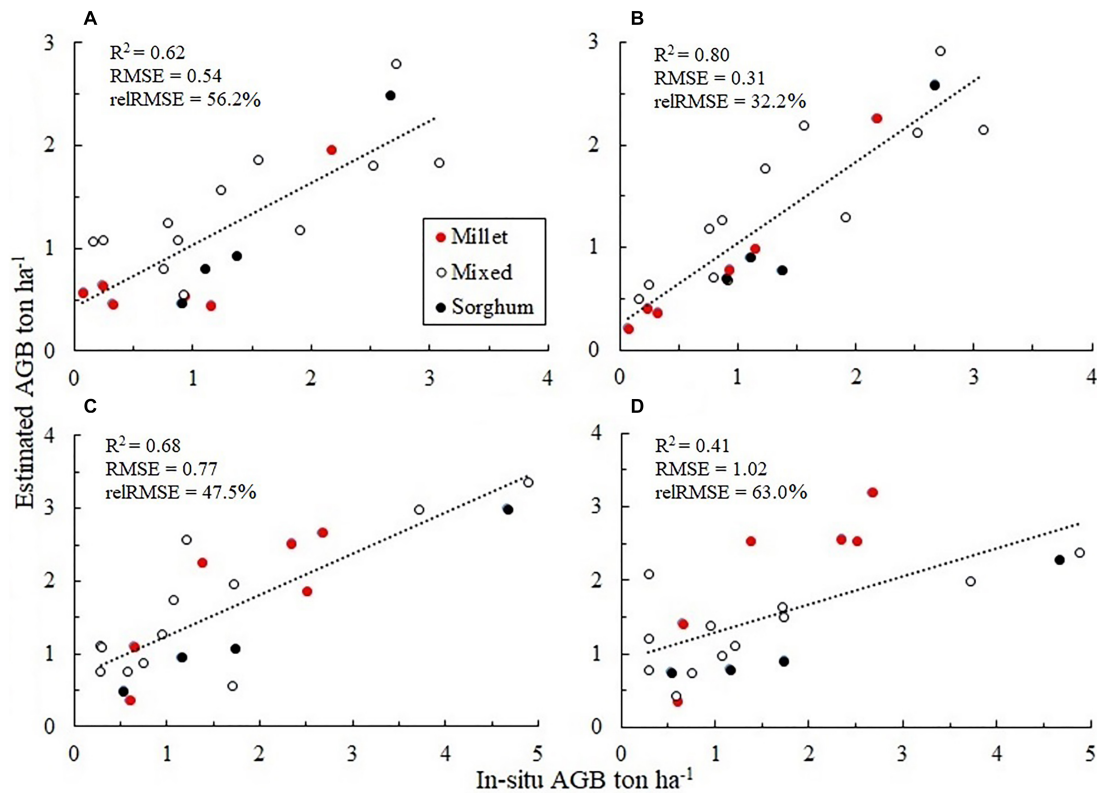


FIGURE 6 | Relationship between in situ and estimations of aboveground biomass (AGB) based on peak VI 2017 (A), 7 September 7, 2017 (B), median VI 2018 (C), and September 17, 2018 (D) for Saponé site in Burkina Faso.

Comparison to Previous Crop Production Estimation Based on Satellite Imagery

Depending on the estimator used and the year considered, our method was able to explain between 41 and 80% of the variation in the *in situ* crop production measurements, with RMSE ranging between 0.31–0.54 and 0.77–1.02 ton ha^{-1} AGB in 2017 and

2018, respectively. Previous research on the use of medium to high spatial resolution satellite imagery for crop production estimation in Africa is limited compared to other continents. In addition, comparing results from this study to previous research is complicated due to differences in the crops in focus, the spatial scale used for estimating crop production and measures used to report accuracy. While the commonly reported RMSE

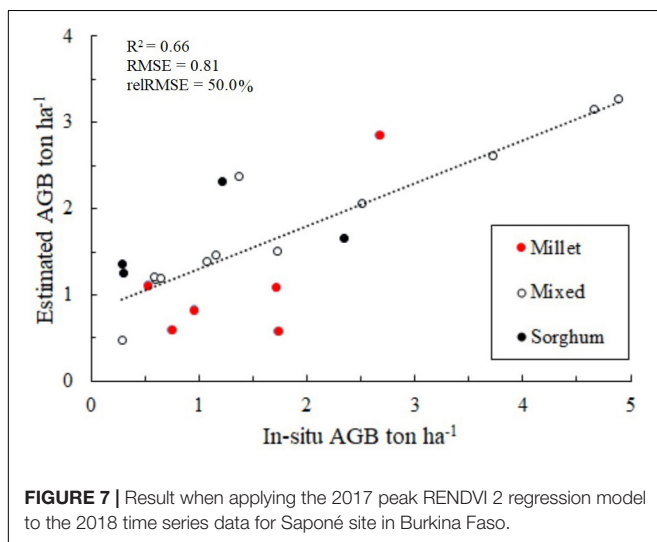


FIGURE 7 | Result when applying the 2017 peak RENDVI 2 regression model to the 2018 time series data for Saponé site in Burkina Faso.

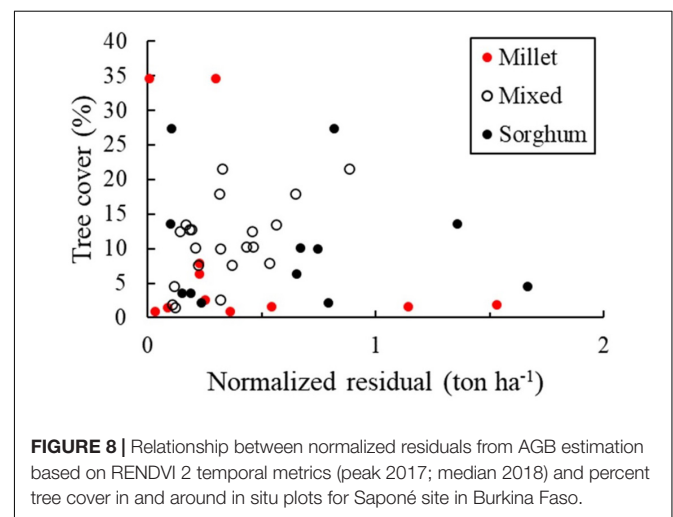


FIGURE 8 | Relationship between normalized residuals from AGB estimation based on RENDVI 2 temporal metrics (peak 2017; median 2018) and percent tree cover in and around in situ plots for Saponé site in Burkina Faso.

provides a quantitative measure of accuracy, its interpretation is dependent on the local crop production conditions, in particular the production per spatial unit.

Earlier efforts that focused on similar crops (i.e., millet and sorghum) primarily used coarse resolution AVHRR imagery for estimating crop production at district level (Rasmussen, 1992; Maselli et al., 2000), with limited relevance for comparison with our sub-field level results. In terms of accuracy, our results are comparable to the study in Mali by Lambert et al. (2018) who used peak LAI derived from Sentinel-2 imagery acquired at the end of August to estimate crop yield of millet, sorghum and maize. These authors report results from crop type specific regression models, with R^2 ranging between 0.48 and 0.80 and RMSE between 0.5 and 1.0 tons ha^{-1} . Other studies in Africa have also focused on single crop agricultural systems, mainly maize production, with varying results. For example, Jin et al. (2017) achieved R^2 between 0.28 and 0.36 for field level estimates of maize production in western Kenya using Sentinel-2 imagery but did not report RSME. In the same area, Burke and Lobell (2017) estimated maize production using Terra Belle high spatial resolution imagery and reported R^2 of 0.4 at field level. Other studies have estimated maize production at a higher level of spatial aggregation. Azzari et al. (2017) used peak VI from coarse spatial resolution MODIS data at province level in Zambia and reported R^2 of 0.55 and RMSE of 0.4 ton ha^{-1} . In Kenya and Tanzania, Jin et al. (2019) reported district level results with an accuracy of R^2 of 0.5 and RMSE of 0.39–0.54 ton ha^{-1} . Consequently, the accuracy of our estimates is in the same range as those previously reported even though a large proportion of the sampled fields in this study included both mixed crops and trees.

Effectiveness of Crop Production Estimators

We assessed several VI for their effectiveness in estimating crop production. Correlation analysis did not show substantial differences in the strength of the relationship between the different VI and AGB, in particular in 2017. In 2018, RENDVI 2 marginally outperformed the other VI and was therefore selected as the principle VI for crop production estimation in this study. The importance of the Sentinel-2 red-edge bands confirms previous research on crop mapping applications in the Sudano-Sahel (Forkuor et al., 2018; Lambert et al., 2018). A main reason for the high correlation between AGB and RENDVI 2 is likely the sensitivity of the Sentinel-2 red-edge bands, in our case particularly band 6, to key elements in photosynthesis, including canopy content of chlorophyll and nitrogen. However, the small differences in predictive performance suggests that the choice of VI does not appear to be a critical consideration in this particular agricultural system. The main exception was MTCI, which previously has been identified as an effective estimator of maize production (Jin et al., 2019), but was not in our study.

The effectiveness of the temporal metrics used as crop production estimators differed between the 2 years. In 2017, growing season peak values provided the strongest relationship between AGB for all VI, whereas median VI values were generally

the most effective estimator in 2018. This is likely caused by a combination of differences in i) availability of cloud free imagery between the years, and ii) local growing conditions related to rainfall amount and temporal distribution. The rainfall distribution differed substantially between 2017 and 2018, which may have affected both the total crop production, as well as the relationship between VI and AGB. Growing season peak values are the most commonly used estimator in comparable studies (Azzari et al., 2017; Lambert et al., 2018; Jin et al., 2019). However, our results suggest that the optimal estimator is likely a function of annual crop growth dynamics and crop density, which in the Sudano-Sahel is tightly coupled to the amount and distribution of rainfall (Rockström and de Rouw, 1997; Gibon et al., 2018).

Inter-Annual Consistency of VI-AGB Relationships

There was a relatively large disparity in estimation accuracy between the 2 years in this study with relRMSE ranging between 32.2–56.2% in 2017 and 47.5–63% in 2018. The rather high relRMSE is mainly an effect of the generally low AGB values resulting from unfavorable growing conditions (soil and rainfall) in the area. The higher relRMSE in 2018 can be explained by a combination of two factors: the substantially higher mean AGB and a weaker relationship between the VI based estimators and crop production. The large difference in AGB between the 2 years is the result of high climate variability that characterize this region (Sultan and Gaetani, 2016). In addition, clouds restricted the collection of imagery during late August and early September in 2018. This relatively short window of time appears to be crucial for achieving a strong relationship between VI and AGB at the EGS (see **Figure 5**), and likely corresponds to the period when crops reach maximum growth and greenness in this region (Lambert et al., 2018). From mid-September, the crops start to ripen with accompanying leaf senescence and chlorophyll breakdown, which results in reduced correlation between AGB and VI based estimators. Since the harvest of millet, sorghum and cowpea usually takes place between late October and early November, this time window opens up for using imagery from this period as an important source of information for predicting the harvest 1–2 months before the end of the growing season. Such information is a key component of early warning systems focusing on food security (Meroni et al., 2014a). However, the duration of this study is too limited to characterize the high inter-annual climate variability and its influence on crop development, as well as the ratio between AGB and grain yield. A longer time series of *in situ* crop production measurements is needed to derive well-founded conclusions.

In 2017, the use of RENDVI 2 from an individual date (September 7) resulted in slightly more accurate AGB estimates compared to using growing season peak values. When applying this method to map larger areas, the potential increase in accuracy achieved from using single date imagery needs to be contrasted with the possibility of increased data gaps caused by high cloud cover coinciding with the period of maximum vegetation growth. The use of a temporal metric, such as peak VI, has the benefit of limiting the impact of clouds by combining VI values from multiple

image acquisitions and thereby increasing the possibilities for deriving gap free data coverage.

The results from this study using 2 years of data represent conditions in a relatively small area, which needs to be kept in mind. Several factors may influence the timing of maximum crop growth and therefore the relationship between VI and AGB, including inter-annual rainfall variability, soil moisture, sowing date and differences between crop varieties or combinations (Meroni et al., 2014b; Sanon et al., 2014; Wolf et al., 2015). These factors are highly variable in space within the Sudano-Sahelian zone, which makes it difficult to predict optimal timing for image acquisition in a specific area. When applying this method for estimating crop production on a regional or national scale, it is therefore advisable to use temporal metrics as estimators instead of individual dates for image acquisition. Besides reducing the influence of clouds, the use of temporal metrics can also compensate for local differences in the timing of maximum crop growth, and consequently strengthen the relationship between VI and AGB. However, further research using both longer time series of *in situ* crop production, as well as sampling plots distributed in a larger area is needed to clarify this issue.

The collection of *in situ* crop production measurements is a work intensive and time demanding task. Hence, it would be advantageous if a crop production model developed for 1 year could be applied to estimate crop production for future years. Identifying a temporal metric that is stable through time and space will be an important task in this context in order to facilitate operational application of satellite-based crop production estimation. Our initial attempt to test this showed that reasonably accurate estimates in terms of R^2 and RMSE could be achieved when using the 2018 Sentinel-2 time series as input to the 2017 peak READV1 2 model. However, the results also showed that large inter-annual differences in the range of *in situ* AGB could cause problems for the estimation model. In our study, this generally resulted in severe underestimation ($\text{relRMSE} = 50\%$) of the 2018 crop production. Another explanation for the high relRMSE in 2018 when using the model developed in 2017 can be that the crop combination changed substantially between the 2 years. More accurate predictions may be possible in areas where the crop rotation cycle is more stable, and the same crops or crop combinations are grown in the fields in consecutive years.

Effects of Intercropping and Tree Cover on Crop Production Estimation

When considering that previous research reported accuracies for single crops or used crop specific prediction models, our results derived from a high proportion of intercropped fields are encouraging. Intercropping cereals with a significant proportion of legumes is a common agricultural strategy in Sudano-Sahel for improving food and nutrition security and replenish soil nitrogen (Franke et al., 2018; Kulkarni et al., 2018). We did not find evidence suggesting that a high degree of intercropping affected the relationship between VI and AGB, and consequently did not affect the ability

to estimate crop production from Sentinel-2 imagery. Crop specific regression models may increase estimation accuracy but require a land-cover map in which fields are classified based on crop type or crop combinations. This adds a layer of uncertainty that needs to be considered. In northern Burkina Faso, Forkuor et al. (2018) showed that it is possible to separate mono-crop fields with either cereals or legumes with relatively high accuracy using Sentinel-2 image classification. However, accurately distinguishing intercropped fields in remote sensing imagery is virtually impossible (Bégué et al., 2018). In addition, separation between millet and sorghum using image classification is generally not reliable due to their similar structure and spectral properties (Lambert et al., 2018). Taken together, this limits the applicability of crop specific regression models in smallholder agricultural systems in the Sudano-Sahel.

In addition to intercropping, the prevalence of a substantial tree cover in the fields is generally considered an important factor that restrict remote sensing for agricultural applications in African smallholder systems (Lobell, 2013; Sweeney et al., 2015). In this study, tree cover inside or in close proximity to the fields did not seem to influence the crop production estimation negatively. However, other sources of error may have disturbed the relationship between VI and AGB and reduced the accuracy of the estimations. Quantitative measures of weeds or shrub coppices in the field were not recorded during the *in situ* data collection. A high presence of such vegetation is likely to influence the performance of the satellite based estimation (Burke and Lobell, 2017), but could not be assessed here due to lack of this information in the collected reference data. The collection of such data is a key issue for future studies.

Potential Applications of Satellite-Based Crop Production Estimates

High-resolution crop production estimates of sufficient accuracy have several applications of prime importance, especially in an African context. Firstly, collection of field level data on crop production is expensive and seldom reported systematically in African countries, including in the Sudano-Sahel (Burke and Lobell, 2017). Lack of such data limits the possibilities to understand the causes of yield gaps in different types of agricultural systems. Knowledge about yield gaps and their causes is a key prerequisite for improving crop production and increasing food security through various interventions (Tittonell and Giller, 2013). In the agroforestry parkland system, such interventions could include different strategies for managing the tree cover, including crown pruning, tree species selection and modifying tree densities in the fields (Bayala et al., 2015). Detailed characterization of parkland tree cover, including canopy structure and species composition can be achieved using satellite remote sensing (Karlson et al., 2014, 2016). The combination of detailed and spatially explicit information on both trees and crops opens up new ways of studying the mechanisms of this important agriculture system.

Crop production statistics reported at district to national levels are generally considered unreliable in many African countries (Carletto et al., 2015b), including Burkina Faso. While limited in geographical scope, our results indicate that Sentinel-2 imagery may represent an important complementary data source to improve the situation. Other researchers have shown that the accuracy can be improved when crop production estimates are aggregated at a coarser spatial unit, including districts and provinces (Azzari et al., 2017; Jin et al., 2019). In Burkina Faso, national statistics of the main crops are based on field level estimates that are aggregated at regional and national level. These surveys are conducted throughout the different regions of the country and thereby captures the spatiotemporal variability in crop types and combinations, and crop production capacity. If quality of the surveys can be ensured and the field level measurements are accurately geo-references, these data could be used to calibrate Sentinel-2 based crop production estimation models, which allow national scale mapping. An important prerequisite for such an approach is, however, accurate maps of agricultural field boundaries. Previous research has demonstrated that such maps can be derived from optical satellite systems, such as Landsat 8 and Sentinel-2, even in the heterogeneous smallholder systems of Sudano-Sahel (Forkuor et al., 2018). Another option for this task may be high-resolution cubesat imagery with daily revisit periods provided by systems such as PlanetScope to capture this highly dynamic landscape where fields are regularly fallowed, and new land is subsequently cleared.

CONCLUSION

This study investigated the potential of Sentinel-2 data for mapping crop production at 10 m pixel resolution in a smallholder agroforestry area situated in central Burkina Faso. Several factors contribute to making this a challenging landscape for this type of mapping, including pervasive cloud cover during the growing season and heterogeneous agricultural fields in terms of size, shape and a high prevalence of intercropping of cereals and legumes, as well as interspersed trees and shrubs. Our results based on 2 years of *in situ* crop production data show that inter-annual variability in climate conditions can lead to relatively large differences in the relationship between crop development and VI, which constitutes the basis for estimating crop production from satellite data. This was manifested by interannual differences in the effectiveness of satellite data acquisitions and the method used for deriving VI temporal metrics. While the temporal dimension of crop production estimation appears to be highly influential on mapping accuracy, the choice between different VI appears to be of lesser importance in the agroforestry parkland system, which was in focus here. A way forward would be research targeting a wider area, additional years of *in situ* data and different crop combinations and densities to confirm these observations.

The method presented here based on freely available satellite data and processing tools was able to explain between 41 and 80% of the variability in the *in situ* crop production data, depending on year and approach used for deriving temporal metric from VI time-series. This relatively high mapping accuracy is encouraging, especially since a high proportion of the *in situ* data represented fields where cereals and legumes were intercropped. From an operational mapping perspective targeting regional or national scales, such field conditions are to be expected for large areas of the Sudano-Sahelian zone. These results contribute to a growing body of research showing that sufficiently accurate and spatially explicit estimates of crop production at landscape scale may be feasible not too far into the future, even for the challenging smallholder systems that dominates African agricultural systems. Such information is of prime importance to improve the quality, extent and resolution of national crop statistics, as well as for research analyzing the causes of yield gaps. While the quality may be questionable, field surveys collecting national crop statistics are regularly conducted in many African countries, including Burkina Faso. Relatively small modifications in such survey protocols to better align with the requirements of calibrating satellite-based crop production estimates could be an efficient investment to improve field data availability in this region.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

MK, MO, HB, HR, and JB conceived and planned the study. HB, AO, BS, and JS planned and carried out the field data collection. MK took the lead in analyzing the data, interpreting the results, and writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Amorphous Silica Controls Water Storage Capacity and Phosphorus Mobility in Soils

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Two problems currently facing agriculture are drought and the availability of mineable phosphorus minerals used for fertilization. More frequent and longer drought periods are predicted to threaten agricultural yields in the future. The capacity of soils to hold water is a highly important factor controlling drought stress of plants during the growing phase. High phosphorus availability in soils is also necessary for high agricultural yields, however, over application has also led to a range of environmental problems, foremost being the eutrophication of waterways. Amorphous silica (ASi) has been suggested as one solution to mitigate both water and phosphorus availability. In this work we analyzed the effect of ASi on phosphorus mobility and the soil water storage of a sandy soil. In a lysimeter experiment we found that ASi strongly increased the water storage capacity (WSC) of soils (up to 180% by addition of 3 wt.% ASi). Furthermore, the ASi is in direct competition with phosphorus for sorption sites on iron oxides and other soil minerals increasing nutrient mobilization and increasing potential bioavailability for plants. Following calibration to the lysimeter experiment a process based hydrological model was used to extrapolate experimental results to a sandy agricultural soil with and without ASi for 1 year. For the soil with ASi, the water storage capacities for the yearly scenario were up to 40 kg/m² higher compared to the untreated soil. Our results suggest that ASi enhances the WSC and phosphorus mobility in soil and that this may be one way to mitigate the predicted climate change related drought stress in sandy soils.

Keywords: amorphous silica, field capacity, nitrate, sulfate, water storage capacity

INTRODUCTION

Low phosphorus (P) availability as well as longer and more frequent droughts are known to reduce terrestrial ecosystem performance and crop production (Engelbrecht et al., 2005; Elser et al., 2007; Michaelian et al., 2011; Elser, 2012; Fahad et al., 2017). The P content of soils is not always low. But a high proportion of this P is stored in inaccessible forms such as organic P (Bünemann et al., 2010), or is bound/adsorbed as inorganic P to, e.g., aluminum, iron oxides, or calcium minerals, depending on soil pH (Beauchemin et al., 2003), soil diagenesis stage (Crews et al., 1995), and mineral composition. Inorganic P is predominately immobilized as calcium phosphate minerals at soil pH > 6.5. At lower pH values P tends to be bound/adsorbed by hydrous oxides of iron,

manganese, or aluminum (Brady and Weil, 2008). At neutral pH inorganic P reacts with silicate minerals (adsorption to weathered silicates like clay minerals; Rajan, 1975), but the P binding to those minerals is lowest compared with other minerals (Rajan, 1975; Brady and Weil, 2008). The different Si fractions in soils contain dissolved Si (free in soil solution or adsorbed to soil minerals), amorphous forms (e.g., the biogenic phytoliths or the minerogenic silica nodules), poorly crystalline forms (e.g., secondary quartz), and crystalline forms (the primary silicates like mica, feldspars, or quartz and the secondary silicates, e.g., clay minerals; Sauer et al., 2006). The availability of Si in soil pore waters varies over at least two orders of magnitude (0.01 to 2.0 mmol L⁻¹; Haynes, 2014), and is largely affected by vegetation type, parent material, and soil diagenesis stage (Derry et al., 2005; Struyf and Conley, 2009). Soils differ quite substantially in regard to amorphous Si (ASi) content in soils (0.1 to ~6%; Saccone et al., 2007) depending on climate regions, parent material, and vegetation cover.

It was recently shown that Si availability in soil is positively related to the plant P status (Neu et al., 2017) and to P availability in soils (Schaller et al., 2019; Hömberg et al., 2020). The study of Schaller et al. (2019) showed that increasing Si availability in soils lead to a mobilization of Fe(II)-P phases from mineral surfaces increasing P availability/mobility in soils (Schaller et al., 2019). However, it is currently not clear to which extent Si is able to inhibit P binding to soil minerals and if P is mobilized from secondary sources such as fertilizers.

Terrestrial ecosystems are suggested to be threatened from increased drought risks due to climate change on both the continental and the global scale (Lehner et al., 2006; Allen et al., 2010), negatively affecting ecosystem performance (IPCC, 2013). During severe drought periods plants suffer from drought stress and wilting, because the soil water storage decreases to values at which water is no longer available for plants, Anjum et al. (2011). Key parameters affecting the water supply to plants are the plant available water content in soils and the water storage capacity (WSC) at different water potentials (Saxton and Rawls, 2006). Soils differ quite substantial in WSC (Kern, 1995) and agricultural practice was shown to reduce soil water storage capacity (Mahe et al., 2005). Agricultural intensification also lead to decreasing ASi content of soils (Struyf et al., 2010; Vandevenne et al., 2012; Carey and Fulweiler, 2016) due to of yearly extractions of ASi by crop harvest (Vandevenne et al., 2012), as many crop plants are Si accumulators (Haynes, 2014).

Recently, it was shown that soil ASi content is an important factor for soil WSC and plant available water (Schaller et al., 2020). It was shown that an increase of ASi by 1% or 5% (by weight) increased the water content at any water potential and plant available water increased by up to >40% or >60%, respectively (Schaller et al., 2020). However, this effect of ASi on WSC and plant available water was only shown for small soil samples on laboratory scales with a soil depth of ~5 cm. It is currently not clear how ASi is able to increase WSC in the rooting zone of typical crop plants (50 cm).

In this work, we analyzed the effect of different soil ASi contents on both P mobility and WSC of soils using a lysimeter with a soil depth of ~50 cm. Lysimeter experiments were

modeled using a physically based hydrological model based on soil retention functions and soil characteristics published previously in Schaller et al. (2020). The model was then used to study the WSC and water availability in the crop rooting zone over 1 year using measured rainfall and soil properties from an agricultural area in Eastern Germany (Brandenburg). Our hypotheses were (i) Si will limit sorption of P to the soil matrix due to competition for soil sorption sites on iron oxides and oxyhydroxides and (ii) the WSC and water availability for plants is increased significantly in soils amended with ASi, and this is important on a yearly scale.

MATERIALS AND METHODS

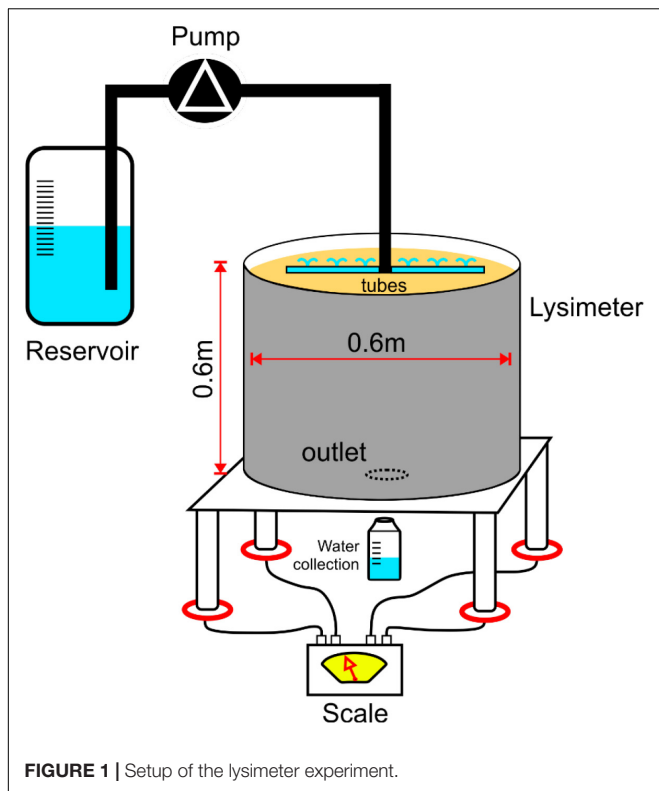
Analysis

The washed pure sand (Dorsilit, Dorfner GmbH, Hirschau, Germany) used in the experiments had a grain size between 0.1 and 0.5 mm. Samples taken from the sand were extracted using an *aqua regia* (9 mL HCl and 3 mL HNO₃) digestion using 500 mg sand at 160°C according to (DIN-EN-13346, 2001). The extract was analyzed for its Fe and P content with an ICP-OES (Varian, Vista-Pro radial). Additionally, the sand was examined for its N and C content using a Nitrogen and Carbon Analyzer (Thermo Quest, Flash EA 1112). Amorphous Si of the used sand was analyzed in a 0.1 M Na₂CO₃ solution at 85°C for 5 h according to DeMaster (1981), and available Si was extracted by the Mehlich-3 extraction (Mehlich, 1984).

Water samples were analyzed for Si by ICP-OES and for phosphate, nitrate, sulfate, and chloride by ion chromatography (Metrohm, 822 Compact IC plus; separation column: Metrosep A Supp 4) according to (DIN-EN-ISO-10305-1, 2008). Due to the phosphate concentration being too low for ion chromatography in case of the mobilization experiments P concentrations were analyzed using a spectrophotometer (Hach, DR 2800) and the molybdenum blue method. Therefore, 2.5 ml of the sample, 25 µl molybdate sulfuric acid, and 100 µl 10%-solution of ascorbic acid were poured into cuvettes and shaken. After a reaction period of 10 min the samples were measured in the spectrophotometer at a wavelength of 720 nm. Additionally, a standard with a Si-concentration of 30 mg l⁻¹ made from Na₂O₃Si · H₂O and ultrapure water was measured to check for an interference of Si with the molybdenum blue method. The phosphate concentration was calculated using the extinction measured by the spectrophotometer and a standard curve made beforehand.

Lysimeter Experiments

The setup of the lysimeter experiment is shown in **Figure 1**. The lysimeter was set up in a greenhouse and was operated from July to November. As there was only one scale each treatment was conducted as a separate part experiment one after the other. The experimental setting consisted of a scale and a cylindrical container with a diameter of 60 cm and a height of 60 cm. The soil container was connected to a scale to monitor the change in weight during the experiments. The scale was constructed of four load cells (Bosche, A30) with a maximum capacity load of 150 kg each. The container had an outlet at the bottom (2 cm radius),



where leachate water was discharged and collected at 1 L intervals in the beginning of the experiment and later at 5–10 L intervals. The discharged leachate water was not taken into account when weighing the lysimeter. A mass fraction of 0% (as control), 1%, and 3% by volume ASi (SiO_2 , Aerosil 300, Evonik Industries) was mixed with sand. After mixing the dry sand with the ASi the material was filled into the lysimeter to reach 55 cm thickness, each. The weight of the dry sand was 175 kg in the treatment with 0% ASi, 172 kg with 1% ASi, and 146 kg with 3% ASi. The pure sand had an ASi content of $25 \text{ mg kg}^{-1} \text{ DW}^{-1}$ and a Si availability of $10 \text{ mg kg}^{-1} \text{ DW}^{-1}$.

For irrigation of the sand a four-channel peristaltic pump (Ismatec, Model ISM945D) and silicone tubes (4 mm diameter, 1 mm wall thickness) with holes (0.5 mm diameter) were used. The lysimeter was watered at a rate between 3 ml min^{-1} and 5 ml min^{-1} , not differing between treatments. The irrigation water (deionized water) had a PO_4^{3-} concentration of $\sim 12.5 \text{ mg L}^{-1}$, a NO_3^- concentration of $\sim 55 \text{ mg L}^{-1}$, and a Cl^- concentration of $\sim 30 \text{ mg L}^{-1}$. There was no SO_4^{2-} in the used water. The irrigation water had a $\text{pH} \sim 8$, but weakly buffered. To reach those concentrations deionized water was mixed with 0.069 g NaNO_3 , 0.048 g NaCl , and 0.023 g $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$ per liter. The used sand had a Fe concentration of $299 \pm 74 \text{ mg kg}^{-1} \text{ DW}^{-1}$ and a P concentration of $162 \pm 30 \text{ mg kg}^{-1} \text{ DW}^{-1}$.

The load cells were logged using a BayEOS low power board that was read-out by a HX7 11 24 Bit AD-converter with an amplification of 128. The data was sent per RF24 to a router and from there transferred to the BayEOS server by BayEOS gateway.

After the breakthrough of the drainage water samples were taken every hour in the beginning and later in larger intervals. The samples were filtered with $0.2 \mu\text{m}$ syringe filters and analyzed using an ion chromatograph with suppressor module (see above).

After each experiment the rate of irrigation was determined by fitting a linear model of the form $y = mx$ to the weight data from the beginning of the experiment until the breakthrough. m is the irrigation rate, y is the weight, and x is the passed time.

Mobilization Experiment

Fifteen g of sand with 3% vol/vol ASi and 0% ASi for control were mixed with 30 ml of ultrapure water in 30 50 mL vials per treatment using a horizontal shaker (Edmund Bühler GmbH, SM 30) with a frequency of 125 min^{-1} at room temperature (replication of three). At each time step (after 0, 1, 2, 3, 8.5, 23.5, 30.5, 48.5, 96.5, and 168.5 h) three vials of each treatment were taken out and centrifuged for 3 min at 5,000 rpm (Thermo Scientific, Hareas Multifuge X3). The supernatant was then filtered using a $0.2 \mu\text{m}$ syringe filter and analyzed in the ion chromatograph and spectrophotometer by molybdenum blue method (see above).

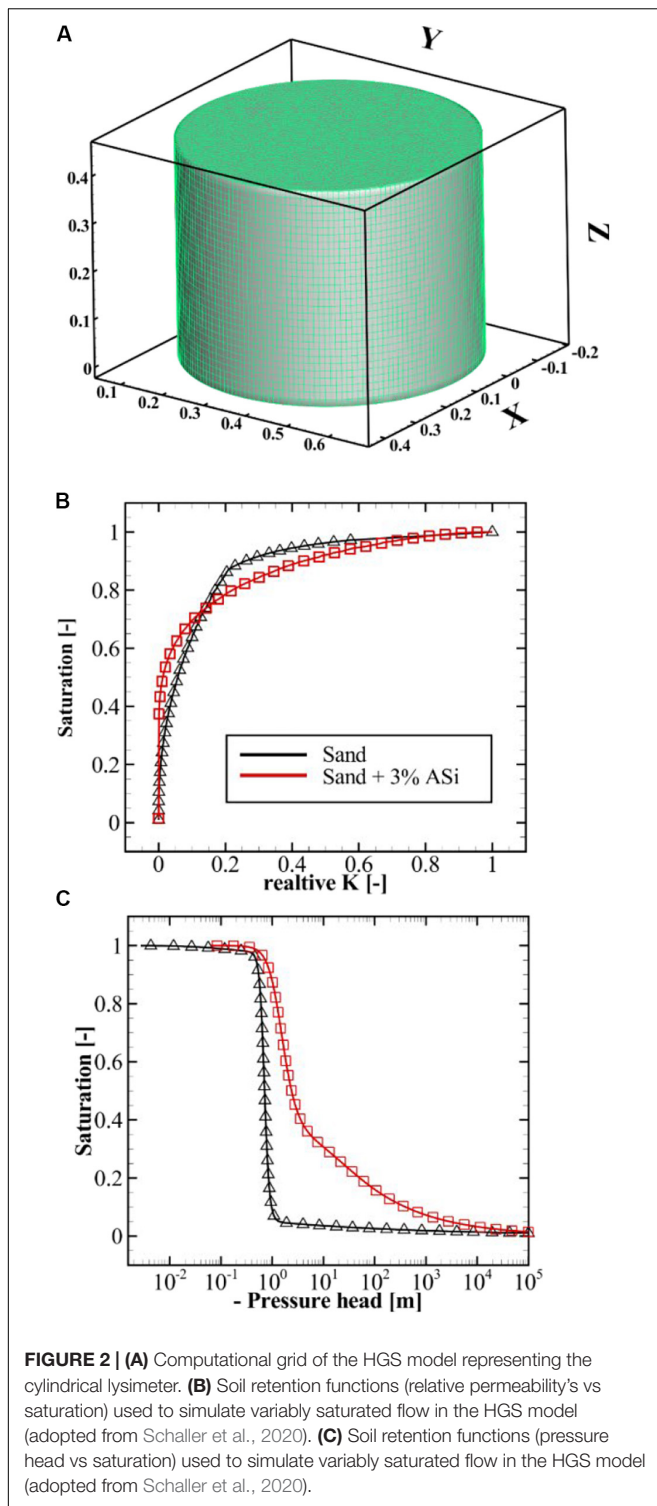
Statistical Analysis

Analysis of variance (ANOVA) was used to compare element concentrations between the different treatments using SPSS version 22.

Numerical Modeling

Variably saturated flow conditions for the lysimeter experiments were represented with the numerical flow model HydroGeoSphere (HGS; Aquanty and Inc, 2015). HGS solves the Richard's equation by applying a finite element/control volume discretization scheme. A computational grid was set up representing the dimensions of the lysimeter in 3D (Figure 2). The model domain was discretized using prismatic 6-node elements with a horizontal and vertical resolution of $\sim 2 \text{ cm}$ and 1.5 cm , respectively. Constant and variable rainfall application was implemented by assigning a specified flow boundary condition (type Neumann) to the upper elements of the model domain (Figure 2). A no flow boundary condition was assigned to the element interfaces representing the outer boundaries of the cylindrical lysimeter. A free drainage boundary condition was assigned to those elements at the lower boundary that represent the outlet of the lysimeter (Figure 1) to mimic percolating water. Soil retention functions for the pure sand and the sand containing 3% ASi (relative permeability vs. saturation and matrix potential vs. saturation as shown in Figure 2) as well as measured saturated hydraulic conductivities and effective porosities (Table 1) were adopted from Schaller et al. (2020). For the simulation of the breakthrough experiments the model was initialized by assigning a negative total head of -5 m to all computational nodes of the grid in order to represent the very dry initial state of the sand.

Saturated hydraulic conductivities for the pure sand and sand + 3% ASi were measured using a constant head darcy permeameter with 3 replica samples per soil types (mean values are listed in Table 1). As there usually is an uncertainty related



to the measurement of soil properties including the transfer of those parameters obtained from laboratory experiments to the field scale (or in our case to the scale of the lysimeter experiments) we decided to additionally derive a parameter set for K_{sat} and θ_{eff} from model calibration. For this the model was run to represent the WSC for the pure sand and sand with 3% ASi using the

TABLE 1 | Measure and calibrated soil characteristics for the pure sand and sand containing 3% ASi.

	Sand (measured)	Sand + 3% ASi (measured)	Sand (calibrated)	Sand + 3% ASi (calibrated)
Effective porosity θ_{eff} [-]	0.38	0.6	0.38	0.6
Saturated hydraulic conductivity K_{sat} [m d ⁻¹]	1.6	0.85	1.2	3.0

measured soil characteristics (Table 1) as initial values for the calibration procedure. Based on the observed WSC_m [M] and simulated WSC_s [M] an objective function (Φ) [M²] was defined as a quantitative measure for the model performance:

$$\Phi = (WSC_m - WSC_s)^2 \quad (1)$$

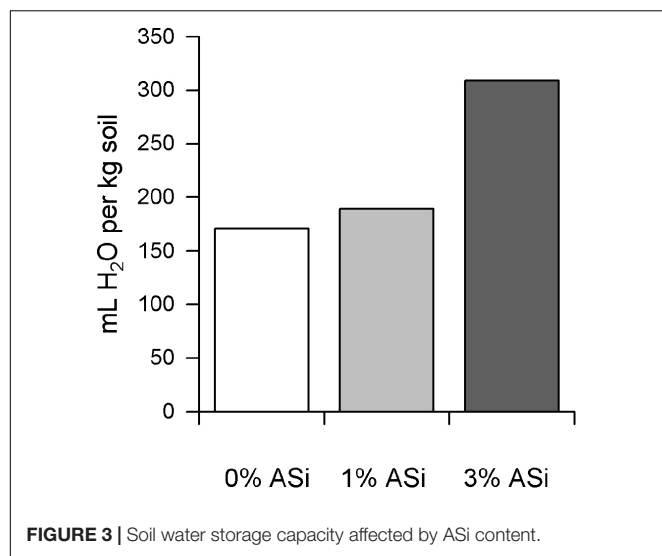
A best fit parameter set for the pure sand and with 3% ASi was derived by locally minimizing the objective function in Eq. 1 using a manual calibration scheme.

The best fit parameter set as well as the measured ones subsequently were used for a yearly scenario using an observed rainfall record. For the yearly scenario the free drainage boundary was extended to all interface elements of the lower boundary to create realistic gravity driven percolation of water for the entire lower interface area. For both soil types the yearly simulations were initialized using a spin up run using the same yearly rainfall record and where the final head distributions at day 365 were used as initial conditions for the main run. The yearly rainfall data was measured for the water year 2018 (November 1st 2017 to October 31st 2018) for the area around the town of Müncheberg, an agriculturally dominated region in eastern Germany (Brandenburg) that is predominantly characterized by sandy soils. With a total of 434 mm rainfall the water year 2018 was very dry compared to the average precipitation of 545 mm in this area. The eastern parts of Germany are predicted to suffer from extended drought period in the future and a decline of average rainfall rates over the next 50 years (Fischer and Knutti, 2016). The yearly simulations were performed according to the guidelines of virtual experiments (Weiler and McDonnell, 2004). Here the simulations were not intended to provide realistic predictions for a specific field site rather we wanted to show how the altered soil properties of the sand containing 3% ASi are affecting the WSCs during a variably rainfall scenario. An error analysis for the four yearly scenarios was performed where the simulations were checked for their accuracies in closing the dynamical water balance.

RESULTS AND DISCUSSION

Water Storage Capacity Dependent on Soil ASi Content

The WSC of the soil profile increased with increasing soil ASi content (Figure 3). The high surface area of the ASi increases the adsorption of water films on the particle surfaces. In addition, the



high porosity of the ASi may lead to the formation of silica gels which are known to have a water holding capacity of more than 500% (Iler, 1973). The finding that ASi is increasing the WSC of soils is in line with recent findings also showing an increase of soil WSC with increasing soil ASi content for the same material used (Schaller et al., 2020). However, compared to the 5 cm thick soil layer in the former study (Schaller et al., 2020) our current data showed little less WSC for 1% ASi and much higher WSC for the soil with 3% ASi. We conclude that ASi seems to be a highly important parameter for soil WSC, but the fundamental mechanisms of water storage are still poorly understood. The high WSC of the soil with 3% ASi is extremely high compared to that of clay minerals (Brady and Weil, 1999) or even Andosols (Fontes et al., 2004). It is clear that the different concentrations of ASi in soils in combination with different substrates have to be analyzed in future to obtain a more generalized picture on how ASi is affecting WSC of soils.

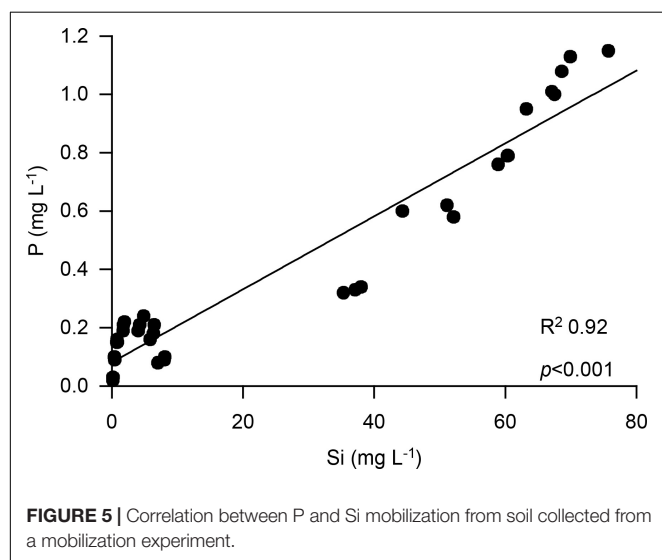
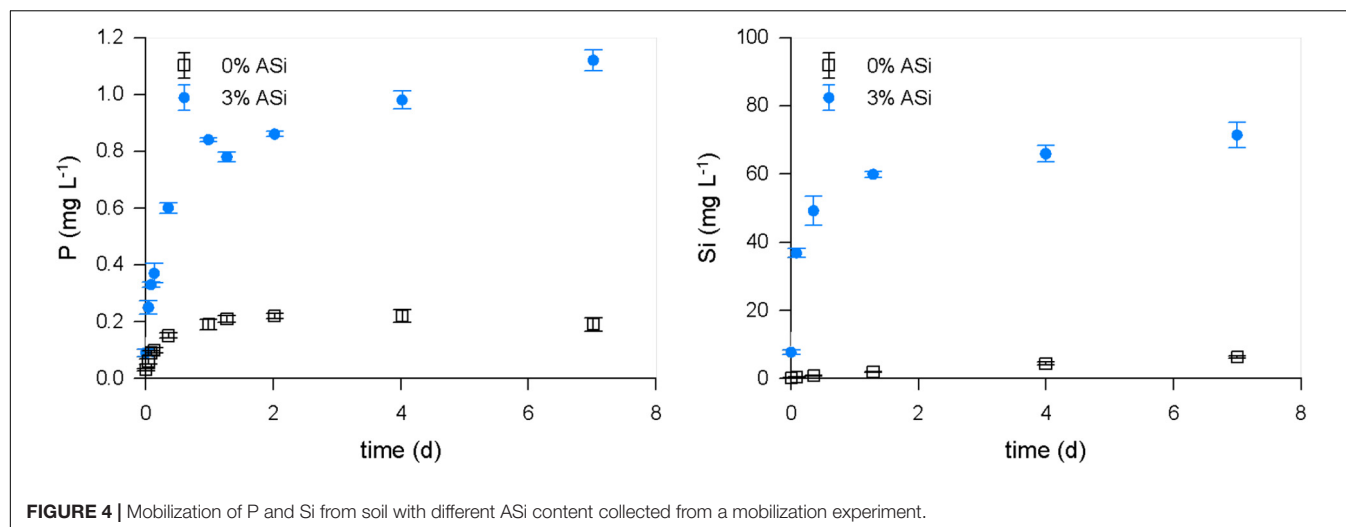
Nutrient Binding in the Soil Column Affected by Silica

Amorphous silica was able to reduce phosphate binding to soil minerals. Phosphate was also mobilized by ASi from binding to soil minerals. We found significant ($p < 0.001$, $df = 1$, and $F = 5685.959$) increased mobilization of phosphate in the treatment with 3% ASi compared to the control without ASi addition in the mobilization experiment (Figure 4). This can be explained by the simultaneously occurring significant [$p < 0.001$, $df = 1$, and $F = 5595.545$ ($p < 0.001$, $df = 1$, and $F = 5685.959$)] higher dissolution of ASi to silicic acid in the 3% ASi treatment compared to the control (Figure 4). A strong relationship (R^2 0.92, $p < 0.001$) between ASi silicic acid concentrations and phosphate mobilization from the soil minerals was found (Figure 5). Separated into the two experiments with 0% and 3% ASi addition the correlation between silicic acid and phosphate showed nearly the same relationship for the 3% ASi treatment with r 0.97 and $p < 0.001$ (Supplementary Figure S1). This

strong relationship of silicic acid and phosphate mobilization is in accordance with recent studies showing an increased phosphate mobilization with increasing ASi availability (Schaller et al., 2019). The same pattern was found for the lysimeter experiments. The Si concentrations in the lysimeter seepage water was below 10 mg L^{-1} for the control treatment (0% ASi), $\sim 70 \text{ mg L}^{-1}$ for the 1% ASi treatment and $\sim 80 \text{ mg L}^{-1}$ for the 3% ASi treatment (Figure 6A). The phosphate concentration in the seepage water at lysimeter breakthrough was $\sim 2 \text{ mg L}^{-1}$ for the control treatments with no ASi addition (0% ASi), $\sim 9 \text{ mg L}^{-1}$ for the treatments with 1% ASi, and $\sim 7 \text{ mg L}^{-1}$ for the treatment with 3% ASi. In the control treatment a slower increase in phosphate concentration was found, reaching $\sim 7 \text{ mg L}^{-1}$ after 23 days (Figure 6B). In the treatment with 3% ASi the seepage water reached the phosphate concentration of the initial irrigation water of $\sim 12.5 \text{ mg L}^{-1}$ after 12 days. The phosphate concentration in the treatment with 1% ASi reached 11.5 mg L^{-1} after 20 days. As the slope of the phosphate concentration in the treatments with 0% and 1% ASi was that low, the experiment was stopped before reaching the phosphate concentration of the initial irrigation water of $\sim 12.5 \text{ mg L}^{-1}$ (Figure 6B).

The temporal development of the phosphate breakthrough curves between the various experiments are consistent with our understanding of the competitive sorption of inner-sphere complexes to iron oxides. Silicic acid can be treated as a non-charged bidentate ligand at the experimental pH (< 7 ; Taylor, 1995). This means that the H_4SiO_4 molecule complexes covalently to oxygen atoms at the corners of edge linked iron oxide octahedra by π bonding of their p orbitals (Swedlund et al., 2010; Dol Hamid et al., 2011; Noritake and Kawamura, 2015). Adsorption is in ^2C coordination with ferrihydrite. In this coordination H_4SiO_4 is in direct competition for sorption sites with the monodentate and bidentate (depending on pH) ligands of the phosphate molecule (Figure 7; Taylor, 1995; Ahmed et al., 2019). In general the PO_4 molecule has a stronger binding energy to iron oxides than the silicic acid (Taylor, 1995), however, the high pore water concentrations of H_4SiO_4 in the ASi amended soils favor both (1) displacement of sorbed phosphate, as observed in the kinetic extraction experiments, and (2) occupation of iron oxide sorption sites by H_4SiO_4 ligands in the lysimeter experiment.

One potentially important aspect of surface siliceous acid chemistry is the propensity of the H_4SiO_4 to polymerize at high concentrations both in solution and on iron oxide surfaces forming Si-O-Si oligomer chains (Pokrovski et al., 2003; Hiemstra et al., 2007; Dol Hamid et al., 2011). It has been shown that polymerization of Si-O-Si occurs between H_4SiO_4 adsorbed to the iron oxide surface and the solution phase H_4SiO_4 and that this is favored at low pH and high Si/Fe ratios (Swedlund et al., 2010). Reduced iron surfaces, such as that of magnetite, also favor polymerization over more oxidized surfaces such as ferrihydrite (Elgaroshi et al., 2019). Once the solid-solution phase oligomer is formed the solution phase molecule tends to also bind to the iron oxide octahedral, again with ^2C coordination, increasing the coverage of H_4SiO_4 on the iron surface (Figure 7). The sorbed oligomer displays “condensed

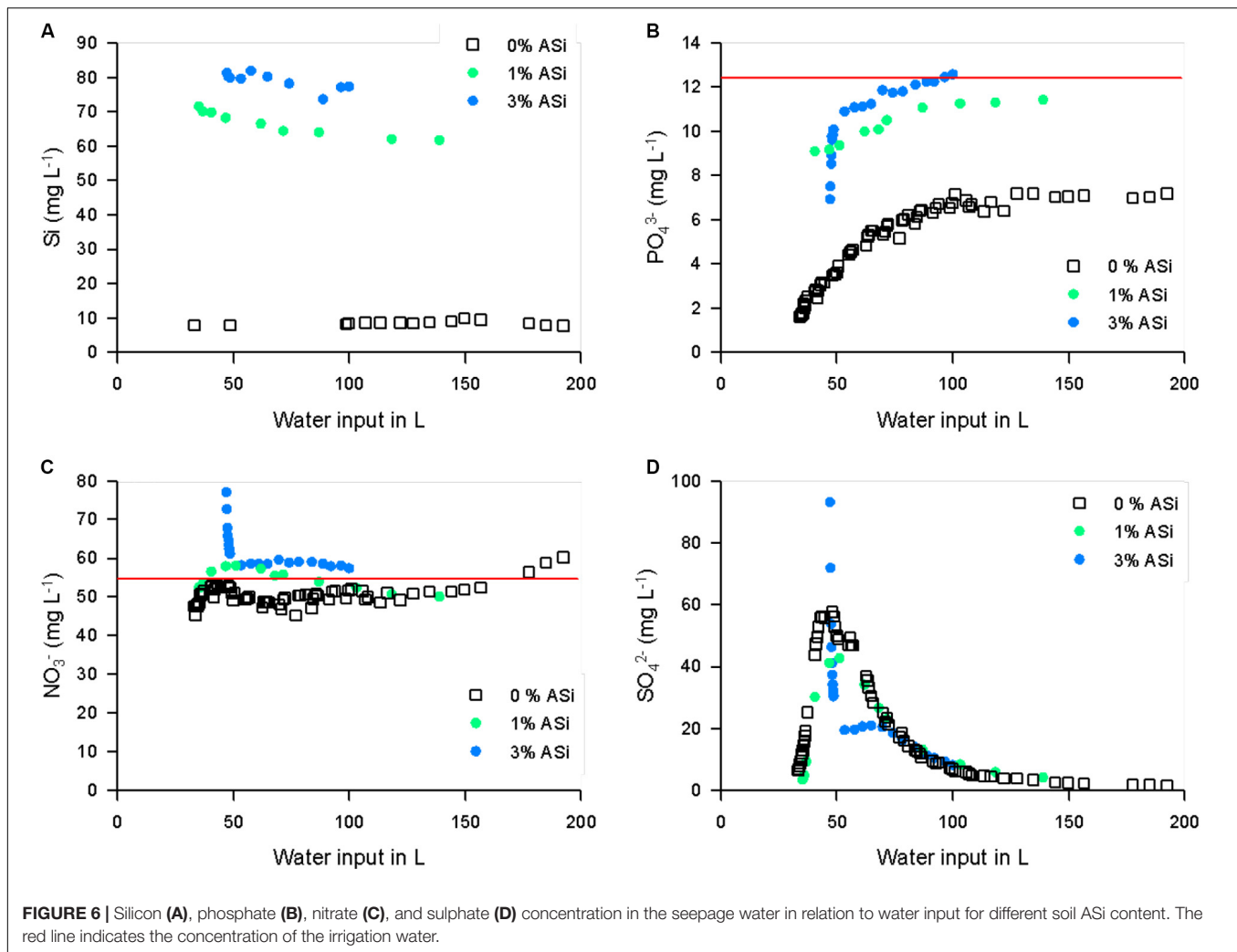


matter” properties with a pyroxene-like structure which may behave differently to the adsorbed monomer (Swedlund et al., 2010; Elgaroshi et al., 2019). The experiments conducted here had Si/Fe molar ratios of 0.53 which would favor the formation of condensed Si-O-Si oligomer surface structures on the iron oxides, especially in the 3 wt.% ASi experiment. While it is currently uncertain, we hypothesize that this condensed H_4SiO_4 surface oligomer will have a higher surface binding affinity to the iron oxides than the monomer alone because of the increased crystallinity of the tetrahedra chains, and thus reduce the probability of desorption as solution chemistry changes. In experiments studying sorption of CrO_4^{2-} to iron oxide surfaces Zachara et al. (1987) noticed that H_4SiO_4 hindered the adsorption of CrO_4^{2-} , and exchange was especially slow after iron oxides had been aged with H_4SiO_4 . At the time of Zachara et al. (1987) polymerization, condensation and crystallization of the silica oxide complex at the iron oxide surface was not known, but it seems likely that this process

reduced the rate of CrO_4^{2-} exchange between the aqueous phase and the iron oxide surface. This may mean that once H_4SiO_4 is sorbed to iron oxide surfaces it will be a long-lasting hindrance to phosphate immobilization, and thus make phosphate bioavailable in soils for longer than expected based on simple monomer chemistry. However, it is also clear that more work needs to be conducted on the fundamental process of competitive binding between iron oxides, phosphate and silicic acid beyond the double layer model and how this is affected by surface Si-O-Si polymerization and crystallization in the presence of phosphate.

Nitrate concentrations of the control treatment (0% ASi) started at $\sim 45 \text{ mg L}^{-1}$ followed by a period of values below the nitrate concentration of the irrigation water of $\sim 55 \text{ mg L}^{-1}$ and after water input of about 175 L the nitrate concentration increased to values little bit higher than those of the irrigation water (Figure 6C). The nitrate concentration of the 1% ASi treatment started at values of 50 mg L^{-1} . After water input of about 45 L the nitrate values in the seepage water were higher compared to the irrigation water until a water input of about 75 L. Afterwards, the nitrate concentration of the 1% ASi treatment decreased to values below the irrigation water (Figure 6C). The pattern of the nitrate concentration of the 3% ASi treatment was different with values of nearly 80 mg L^{-1} after a water input of about 50 L. This value dropped rapidly to values of $\sim 60 \text{ mg L}^{-1}$ and remained at this concentration (Figure 6C). Additionally, we found a mobilization of nitrate from the soil in the mobilization experiment (Supplementary Figure S2). These results with ASi increasing nitrate mobility are in line with finding from a peatland experiment showing increased nitrogen mobilization into pore waters after ASi addition (Reithmaier et al., 2017).

The sulfate concentration of the irrigation water was 0 mg L^{-1} for all treatments. Hence, all sulfate analyzed in the seepage waters was mobilized from the soils. The sulfate concentrations of both the control (0% ASi) and 1% ASi followed the same pattern with a start of $\sim 5 \text{ mg L}^{-1}$ followed by strong increase to values of 60 mg L^{-1} for the 0% ASi and 40 mg L^{-1} for

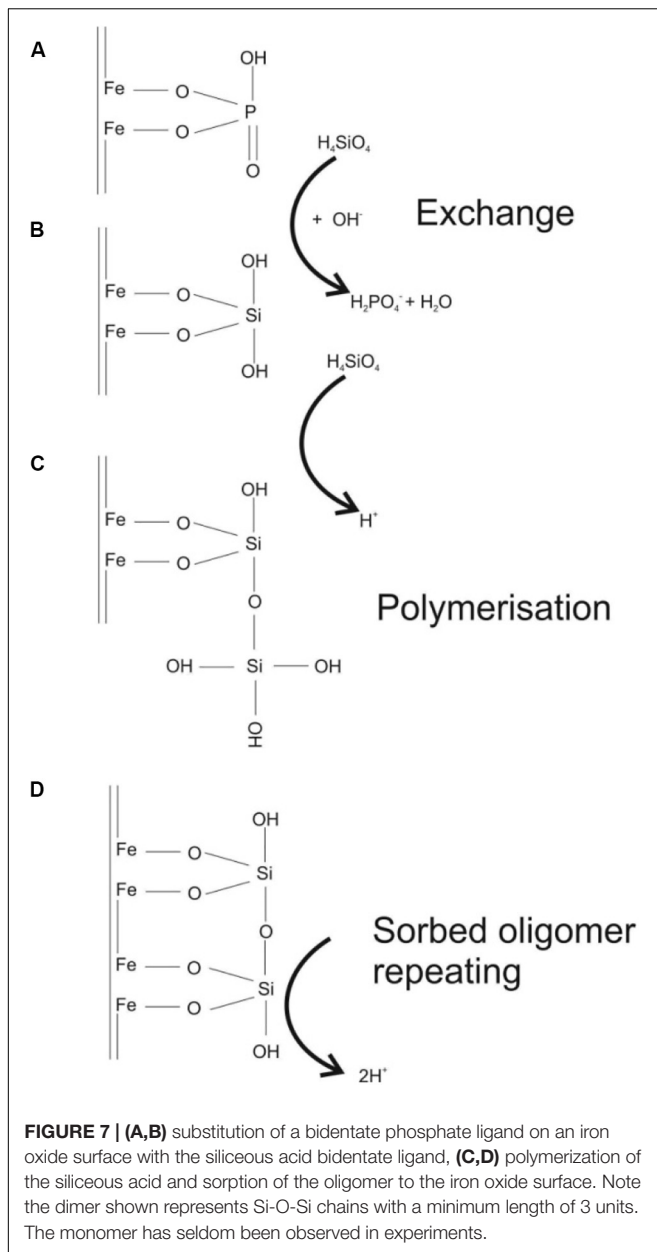


the 1% ASI treatment. Afterwards, the sulfate values dropped for both treatments to values of $\sim 4 \text{ mg L}^{-1}$ (Figure 6D). For the treatment with 3% ASI the sulfate concentration in the seepage water at lysimeter breakthrough was nearly 95 mg L^{-1} and dropped to values of 20 mg L^{-1} rapidly (Figure 6D). Afterwards, sulfate concentration in 3% ASI treatment decreased to values of the 0 and 1% ASI treatments. We also found a mobilization of sulfate from the soil in the shaker experiment (Supplementary Figure S2).

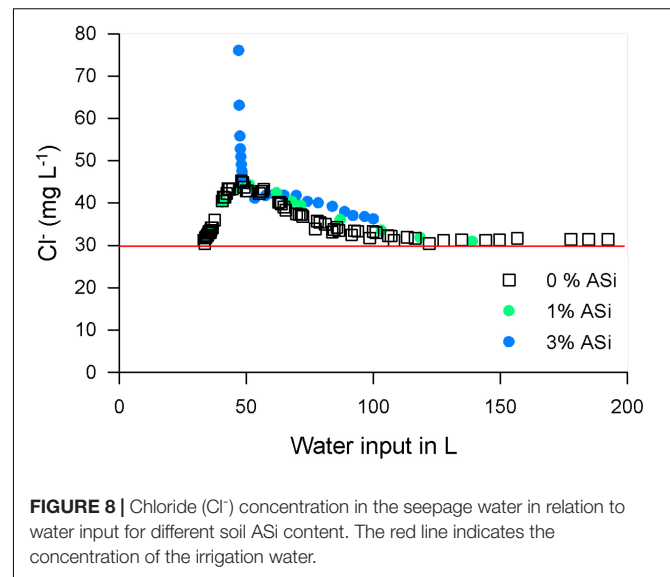
The chloride concentration followed the same pattern as for the sulfate. In all treatments the chloride concentration in the seepage water was always higher compared with the irrigation water (Figure 8, red line). The chloride concentration started at $\sim 30 \text{ mg L}^{-1}$ for both the 0 and 1% ASI treatments, increasing to values of $\sim 45 \text{ mg L}^{-1}$ and afterwards decreasing to values of $\sim 30 \text{ mg L}^{-1}$ again. The chloride concentration in the seepage water of the 3% ASI treatments was at lysimeter breakthrough $\sim 75 \text{ mg L}^{-1}$ and decreased rapidly to values of $\sim 40 \text{ mg L}^{-1}$ (Figure 8). Afterwards, chloride concentrations in 3% ASI treatment decreased to values of $\sim 36 \text{ mg L}^{-1}$. We found a tendency of increased mobilization

of chloride from the soil by ASI in the mobilization experiment (Supplementary Figure S2).

The temporal trends in nitrate, sulfate and chloride concentrations in the lysimeter can be explained by dissolution of salts from the soil matrix, with relatively little effect of sorption in the soils profile (Tyler and Thomas, 1977; Appelo and Postma, 2005). This is mostly clearly seen in the breakthrough curve of sulfate, as it was not added to the experiment. The temporal trend in these ions in the control and the 1 wt.% ASI amendment was very similar due to the minimal difference in WSC between these two experiments. In these experiments the contact time and therefore flow path length and water/sand ratio was similar, leading to a comparable dissolution of the salts (Cl^- , SO_4^{2-} , and NO_3^-) from the sand matrix (Berezniaik et al., 2018). In contrast, the 3 wt.% ASI experiment displayed a significantly longer water residence time in the lysimeter and a higher WSC than either of the other two experiments. This means that the water had a longer contact time with the sand matrix due to longer and more tortuous flow paths through the soil profile. This decreased the water/sand ratio and concentrated salts in the first part of the breakthrough



solution (Berezniak et al., 2018). In a real system the increased residence times in the soil profile are likely to have complex and possibly opposing effects (Raats, 1981; Lu et al., 2018). Increased water residence in the soil increases the contact time between redox sensitive elements such as nitrate and the organic substrate. This may favor chemical reduction and so loss (as N_2 or N_2O) of this nutrient from the soil (Jahangir et al., 2012). The longer residence time in the rooting zone may also lead to a higher availability of nutrients and uptake by vegetation before the nutrients are lost below the rooting zone, and ultimately into the groundwater (Zotarelli et al., 2008). This could have positive environmental effects as nitrate contamination of groundwater is a major concern for water resources and aquatic ecosystems. Overall there is little evidence



that the ASI influences and sorptive behavior of these ions, but indirect effects such as increasing the WSC of the soil and increasing the residence time in the rooting zone has the potential for positive environmental outcomes.

Simulated WSC

For the measured soil parameter set (**Table 1**) simulated WSCs under stationary conditions, achieved at the end of the breakthrough experiments, reached 32.9 kg of water for the pure sand and 42.4 kg for the sand containing 3% ASI. Best fit parameters obtained from model calibration (**Table 1**) that locally minimize the corresponding objective functions (**Figure 9**) resulted in a simulated WSC of 29.7 kg (sand) and 45.19 kg (sand + 3% ASI), respectively. Observed WSC for the pure sand was 29.9 kg and 45.2 kg for the sand containing 3% ASI. Parameter sets derived from calibration reflect a strong positive correlation between an increase in the effective porosity (0.38 to 0.6) and an increase in K_{sat} (1.2 m/d to 3.0 m/d) which is often described in literature (Suleiman and Ritchie, 2001; Urumović and Urumović Sr, 2014).

For the yearly scenarios using the measured and calibrated parameter sets, percolation (water that is leaving the model through the lower boundary) and rainfall rates are shown in **Figure 10A**. Maximum percolation rates for both parameter sets were simulated for the rainstorm event around day 250 and lowest rates for the extended drought period in-between day 100 and 130.

Simulations representing sand with 3% ASI (red solid and red dashed lines in **Figure 10A**) show higher percolation rates during dry conditions compared to the simulations for pure sand. Although, the effect was less distinct when the simulations were run using the calibrated parameter set compared to the measured ones (solid and dashed lines in **Figure 10A**). During rainfall events sand with 3% ASI is capable to store more water compared to the pure sand which can be seen for the measured parameter set (**Figure 10C**) and also to a lesser extend for the

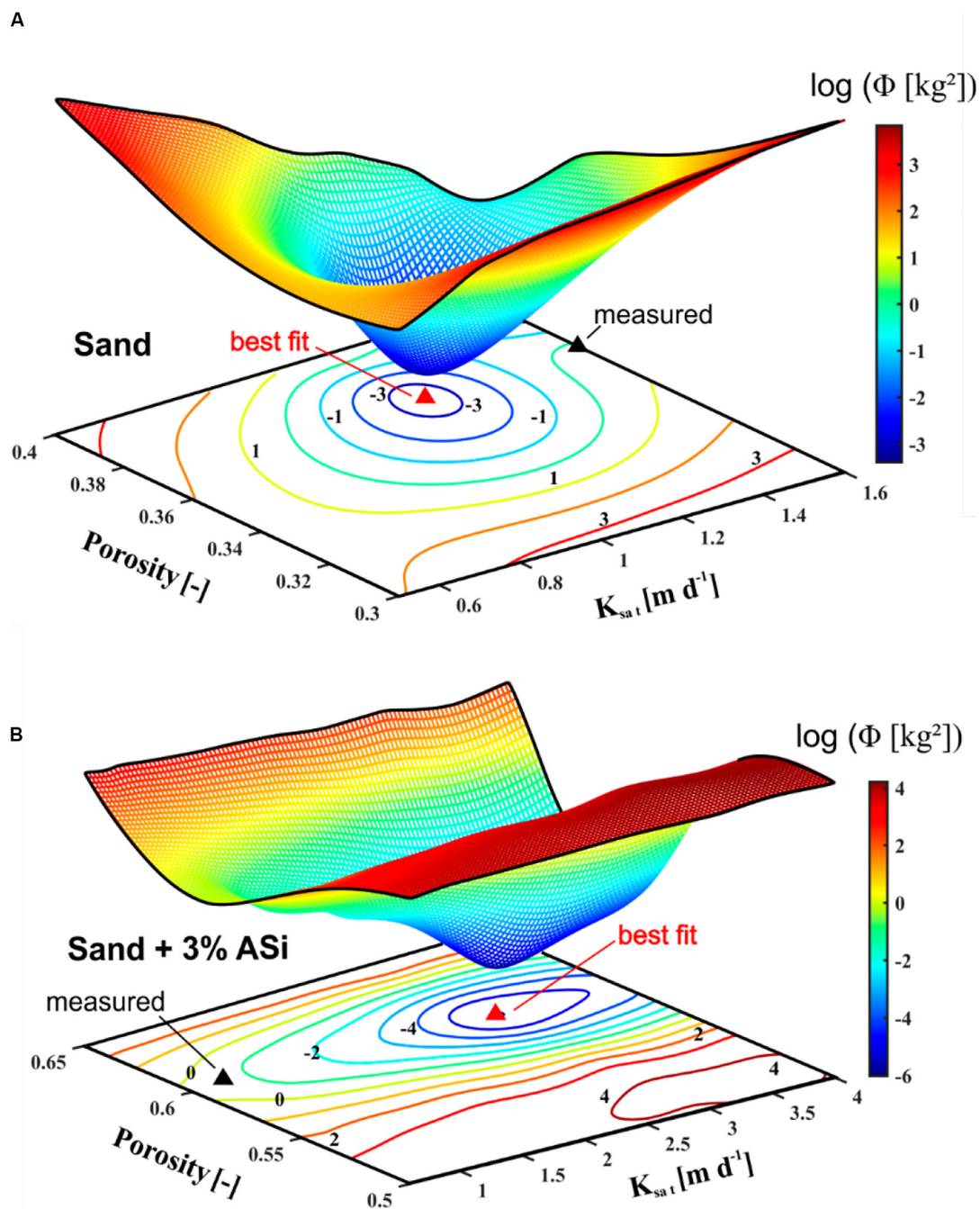


FIGURE 9 | Objective function for the simulations representing the pure sand **(A)** and sand containing in 3% ASI **(B)** defined in the K_{sat} /porosity parameter space. Measured parameter sets are indicated by black triangles and best fit sets, minimizing the objective functions, by red triangles.

calibrated parameter set (**Figure 10D**). The effect of the ASI to store more water during rainfall events and simultaneously hold it for a longer periods of time during dry conditions result in a higher mean saturation (mean = 0.59 and 0.38, **Figure 10B**) throughout the year compared to the pure sand (mean = 0.1, **Figure 10B**). All simulations were assessed and tested for numerical accuracy in solving the transient water balance. Numerical errors were lowest for the measured parameter set

(<1%) and slightly increased but still acceptable (<2%) for the calibrated parameters.

Although the effect of evapotranspiration was not explicitly considered in the HGS simulations, the simulations clearly indicated increased storage capacities of sand when mixed with ASI. This additional WSCs of sand containing ASI should have a positive effect on water availability for plants in the rooting zone of sandy soils which was indicated by the simulated higher

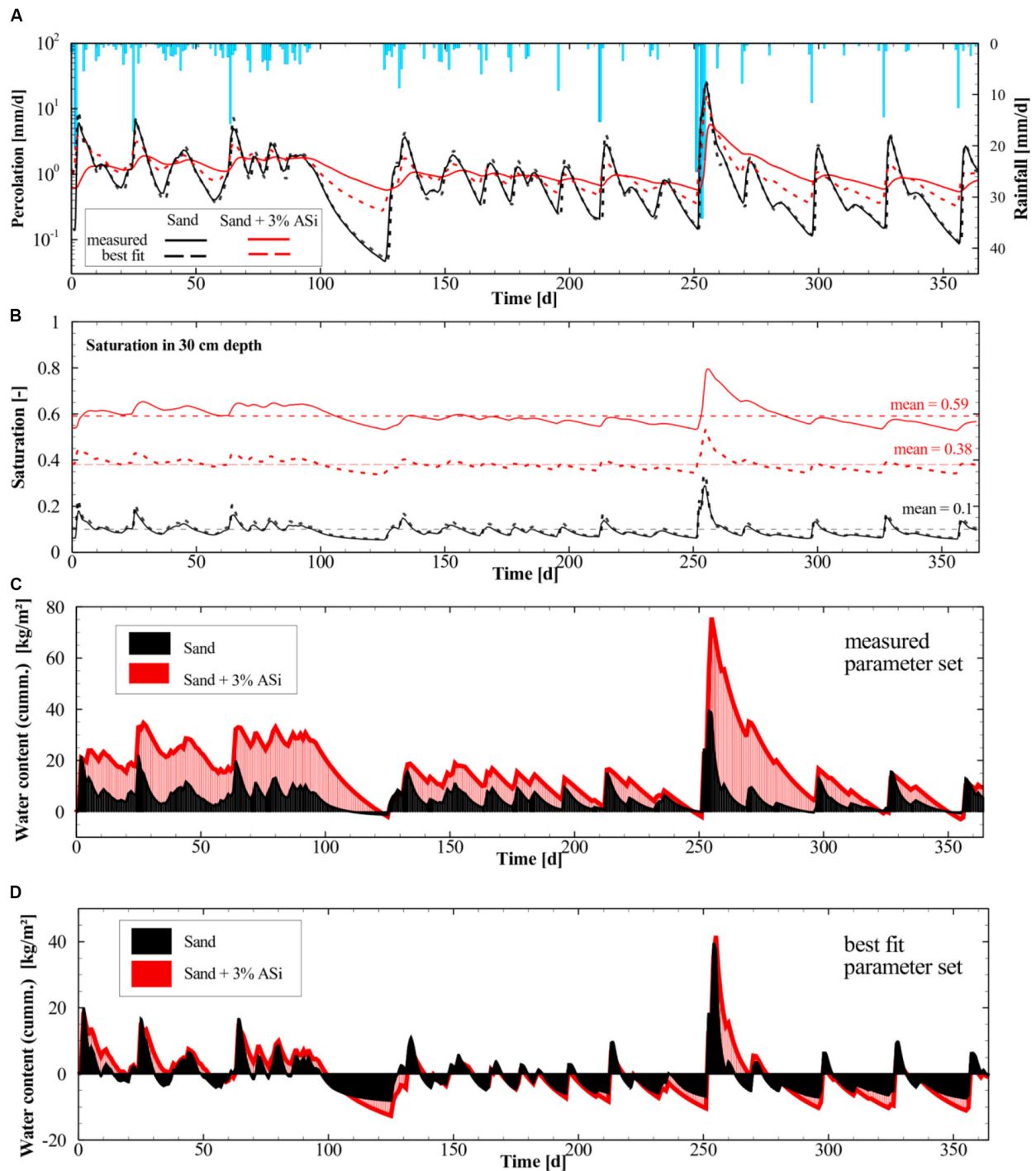


FIGURE 10 | (A) Applied rainfall rates for the yearly simulation scenario (November 1st 2017 to October 31st 2018) and the modeled percolation rates for the pure sand and the sand with 3% ASI. **(B)** Simulated water saturation at 30 cm depth for the measured and best fit parameter set. **(C)** Simulated cumulative water uptake in kg per unit area for the sand and sand with 3% ASI for the measured parameter set listed in **Table 1** (water uptake is relative to the initial water content; **D**) Simulated cumulative water uptake in kg per unit area for the sand and sand with 3% ASI for the best fit parameter set (water uptake is relative to the initial water content).

mean annual saturation. A more realistic simulation scenario would require accounting for evapotranspiration using various vegetation types in the hydrological model. However, such

simulations are beyond the scope of this study and probably will be performed in future once experimental data from field scale application of ASI is available.

CONCLUSION

The strong effect of ASi increasing the soil WSC and water availability for plants during drought periods may be used in future agriculture practices to mitigate plant stress due to water limitation and associated harvest losses. There may be a number of secondary effects of applying ASi that should be considered before widespread application, however. For example, an increase in WSC of soils may lead to lower groundwater recharge rates due to increased evapotranspiration, altering the water budget of stream systems connected to the regional aquifer. Also, the rapid increase in phosphorus mobility may lead to an excess of phosphate in the soil pore water. If plants uptake cannot quantitatively use this phosphate for growth there is the potential for increased phosphate levels in groundwater via leaching. There is, however, very little known about the potential side effects of ASi use, and they should be studied in the future at the plot scale. Despite these concerns, we feel that if done in a suitable manner the application of ASi can be considered a promising mitigating measure for increasing water and nutrient availability in agricultural systems. This may be particularly important in future as climate predictions are for increased extreme conditions, included more extreme, and longer duration of drought. It is expected that ASi will be especially effective in sandy soils where water storage capacities are low and interactions with organic carbon are less likely to compete with Si for sorption sites. Future work should look into more complex soil systems to understand interactions between soil solutions including organic matter and clay minerals, as this may

be important for both the WSC and sorption of phosphate in non-sandy soils.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

JS, SF, and BG had the idea and designed the experiments. LR conducted the experiments and did the measurements. JS, SF, and BG wrote the manuscript. All authors discussed the results and commented on the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2020.00094/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of Fungicide and Herbicide Chemical Exposure on *Apis* and Non-*Apis* Bees in Agricultural Landscape

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Fungicide and herbicide chemistries are commonly applied in agricultural production systems and other agricultural landscapes during flowering periods, which are concurrent with the timing of bee-dependent pollination services in many plant species. As a result, bees can be exposed to these pesticides while foraging crops and other flowering plants in the landscape where they have been administered. Laboratory and semi-field studies simulating these pesticide exposure scenarios have demonstrated lethal and sub-lethal impacts to both *Apis* and non-*Apis* species of domesticated bees. Exposure to fungicides and herbicides has also been attributed to bee genetic and molecular-level changes in some cases. Herbicides can also indirectly impact bees as a result of decreasing weeds and other flowering plants that serve as nutrient resources for foraging bees. We analyze a series of recent studies concerning the toxicity of fungicides and herbicides to *Apis* and non-*Apis* bees as a basis for forming our views on key priorities regarding the direction of future research initiatives in this area. Exploring the impacts of agricultural pesticides beyond insecticides to bees is timely given the documented bee declines in the last decade and the resulting widescale interest in identifying the different drivers of these declines among the biological and the ecological scientific communities.

Keywords: fungicides, herbicides, bees, pollination, crop production, fungi, weeds

INTRODUCTION

Bees are important pollinators of cultivated crops and wild flowering plants, and the ecological services that they provide are integral to plant reproduction and ecosystem foundation. Therefore, bees are a critical variable in the function of agricultural systems used to produce food and fiber crops. Rising awareness of population declines of bees and other invertebrate pollinators over the last decade has dramatically increased the amount of research devoted to this particular area of ecological science, and as a result, several key contributing factors have been identified and reviewed (Maini et al., 2010; Meeus et al., 2018; Belsky and Joshi, 2019). One of these factors is the increased frequency of insecticide applications for plant protection against herbivorous insect pests in commercial agricultural and ornamental crop production. Beyond insecticides, bees are exposed to a diversity of other agricultural chemicals, as demonstrated by Mullin et al. (2010) who found 121 different pesticides and metabolites in beehives and associated hive materials and Hladik et al. (2016) who detected 19 pesticides and degradates in 54 samples of native bees collected from wheat field and grassland land covers. Fungicides (commonly used in agricultural crop production for

controlling molds and fungal diseases) and herbicides (applied to reduce weeds and other unwanted plants) are among those other chemistries. Determining the impacts of fungicides and herbicides on bees is a new area of major scientific interest given that the global market value (Pesticides Industry Sales Usage, United States Environmental Protection Agency, 2017) and quantity of these chemicals sold and applied (in tonnage) outnumbers insecticides (Eurostat, 2018; Jess et al., 2018). Insecticide toxicity to bees has been extensively investigated over the last 15 years; however, comparatively little is known regarding the impacts of fungicides and herbicides to bees. Since they are often applied in agricultural crop production systems throughout the year, including periods of bloom (Bosch and Blas, 1994; Andert et al., 2016; Zubrod et al., 2019; Heller et al., 2020), *Apis* and non-*Apis* bee species can come into contact with these chemicals while actively pollinating. Simulating these scenarios, laboratory, and semi-field studies have demonstrated the impacts of fungicide and herbicide exposure (as both single chemicals and in combination with other pesticides) on lethal and sub-lethal toxicity to different species of managed bees (Fisher et al., 2017; Heard et al., 2017; Iverson et al., 2019). Several recent studies have also linked physiological changes at the molecular and the genetic levels in bees after exposure to these agricultural chemicals (Jumarie et al., 2017; Mao et al., 2017). Indirect effects on bees include diminished floral resources, resulting from herbicide usage on susceptible weeds and other flowering plants (Bohnenblust et al., 2016; Jacobson et al., 2018).

It is important to note that a small group of commercially bred *Apis* and non-*Apis* species comprises the principal agents of agricultural crop pollination. However, the plight of solitary wild bees is crucially important. Diverse pollinator communities are integral for the proper functioning of ecological systems (Ollerton, 2017; Winfree et al., 2018) and have been shown to drive increases in agricultural crop fruit set and yield, especially given the recent declines of domesticated honey bees (Klein et al., 2012; Blitzer et al., 2016; Garibaldi et al., 2016; Woodcock et al., 2019). Currently, less studies on pesticide toxicology to wild solitary bees have been done as a result of their not being regarded as major pollinators combined with the increased difficulty in administering pesticides to these bees (which are often smaller in size) in the laboratory and in the field. Despite this, future research exploring the differences in pesticide impacts among different bee species is warranted, given the discrepancies in the results of existing studies. Conflicting findings such as those suggesting differences in pesticide toxicology among bee species (Biddinger et al., 2013; Sgolastra et al., 2017) and those that do not (Spurgeon et al., 2016; Heard et al., 2017) must be further addressed, especially with regard to fungicide and herbicide chemistries. Although most of this work has and will continue to focus on the grouping of commercialized *Apis* and non-*Apis* species, taking this approach at least begins to expand into solitary bees. We address this subject along with disagreement in the discussion as to whether honey bees can serve as a surrogate for non-*Apis* species in pesticide toxicology testing.

Here we discuss recent studies that demonstrate the toxicity of fungicides and herbicides to commercialized *Apis* and non-*Apis* bee species. We also discuss current knowledge gaps and present

our views on future research areas and regulatory framework needs to appropriately assess the risks of bee exposure to fungicide and herbicide chemistries.

FUNGICIDE AND HERBICIDE CHEMISTRIES AND MODES OF ACTION

Fungicides are classified into several families. Sterol biosynthesis inhibitors (SBI) and demethylation inhibitors (DMI) inhibit the cytochrome P450 monooxygenase-dependent step in fungal ergosterol production and cell wall synthesis (Kuck et al., 2012; Casida and Bryant, 2017; Zubrod et al., 2019). Respiratory chain succinate dehydrogenase inhibitors of complex II impede respiratory chain succinate dehydrogenase (Angelini et al., 2014), while respiratory chain strobilurin-type quinone outside-site of complex III inhibitors inhibit mitochondrial respiration by interfering with proper electron transport from cytochrome b to cytochrome c₁ (Bartlett et al., 2002; Zubrod et al., 2019). Chlorothalonil is a chloronitrile fungicide that functions through multiple modes of action (Zubrod et al., 2019).

Herbicides are classified by their mode of action. 4-Hydroxyphenylpyruvate dioxygenase inhibitors impair carotenoid biosynthesis, causing plant bleaching and subsequent death (Hawkes, 2012), while protoporphyrinogen-IX-oxidase inhibitors facilitate the peroxidization of fatty acid unsaturated bonds within cell membranes (Theodoridis et al., 2012). Acetyl CoA carboxylase inhibitors prevent the normal metabolism of fatty acids (Bretschneider et al., 2012; Wenger et al., 2012). Cellulose biosynthesis inhibitors prevent the formation of crystalline cellulose fibers that are essential for the stability of plant cell walls (Dietrich and Laber, 2012). Acetolactate synthase inhibitors prevent the biosynthesis of branched amino acids, leading to cell division impairment (Jeschke, 2015). Auxin mimics function by mimicking the auxin hormone necessary for plant growth (Walsh and Schmitzer, 2012; Jeschke, 2015).

TOXICITY OF FUNGICIDES TO DIFFERENT SPECIES OF BEES

Apis Bees

Several recent studies have demonstrated the toxicity of field-realistic fungicide exposure to a variety of honey bee adult and larval health endpoints. The toxicities of both single active ingredient fungicides and the synergistic combinations of fungicides + other agricultural chemicals to honey bees have been demonstrated. Fungicide contact and oral LD₅₀ values and toxicity assessment to honey bees are presented by Stoner and Eitzer (2013) and Ladurner et al. (2005) and may vary across different compounds. For instance, upon investigating the foliar spray toxicity of formulated fungicides commonly used in almond production to honey bees (*Apis mellifera*), Fisher et al. (2017) showed that both exposure to Iprodione 2SE Select[®] (iprodione) alone and spray-tank mixes with either Pristine[®] (boscalid and pyraclostrobin) or Quadris[®] (azoxystrobin) significantly decreased forager survival. Using

technical-grade chemicals, Johnson et al. (2013) determined that four SBI fungicides increased the acute toxicity of the acaricide tau-fluvalinate to honey bee adult workers in a dose-dependent manner. This synergism is attributed to the inhibition of cytochrome P450 monooxygenase enzyme activity. An increase in the acute toxicity of the acaricides coumaphos and fenpyroximate was observed when honey bees were also exposed to the SBI fungicide prochloraz, while pre-treatment with the acaricide amitraz increased the acute toxicity of three other acaricides that are cytochrome P450-detoxified. Exposure to fungicides may also result in increased susceptibility to other pesticides, mainly insecticides. For instance, clothianidin and thiamethoxam became significantly more toxic to honey bee workers exposed to boscalid-contaminated pollen in maize fields by measuring of decreased LD₅₀ levels (Tsvetkov et al., 2017). Feeding honey bees with syrup contaminated with either copper oxychloride for 108 h and thiram for 168 h, Migdał et al. (2018) determined a daily fungicide intake of 4.75 and 16.78 mm³/bee, respectively. The daily mortality was 35.15 bees/cage for copper oxychloride and 5.17 bees/cage for thiram.

Several studies have also analyzed the impact of fungicides on honey bees at the molecular level. For example, adult consumption of flavonol quercetin mixed with the triazole fungicide myclobutanil resulted in the down-regulation of five of the six mitochondrion nuclear genes (Mao et al., 2017). This study also concluded that bees ingesting 0.25 mM quercetin combined with 5 ppm myclobutanil displayed depressed thoracic ATP levels compared to bees ingesting quercetin only. Exposing different developmental life stages of honey bees to the fungicide prochloraz and the acaricide coumaphos individually and in combination, Cizelj et al. (2016) documented differentiated molecular-level immune responses in brood *versus* adults. Specifically, by examining the expression of 17 immune-related genes by quantitative RT-PCR, gene downregulation was found in prepupae, while gene upregulation for most genes was found in adults following all treatments. In adult bees, prochloraz + coumaphos exposure resulted in the upregulation of the antimicrobial genes *abaecin* and *defensin-1*. In a similar study, Christen et al. (2019) observed the effect of orally exposing honey bees to the fungicides azoxystrobin, chlorothanolin, and folpet over 24, 48, and 72 h on gene transcription within their brains. They found that while azoxystrobin and folpet induced minor changes, including the downregulation of *hbg-3* and the induction of *ndufb-7*, respectively, chlorothanolin resulted in the major transcriptional down-regulation of genes related to oxidative phosphorylation and metabolism. In particular, chlorothanolin downregulated genes that include *cyp9q1*, *cyp9q2*, *cyp9q3*, *acetylcholine receptors alpha 1*, *hbg-3*, and *ilp-1*, all of which influence honey bee hormonal regulation and behavioral transition, such as nurse bees transitioning into foragers. Moreover, chlorothanolin exposure resulted in the largest overall impact on the transcriptional abundance of genes linked to honey bee energy production, metabolism, and the endocrine system, where the strongest effect was observed in the early spring as opposed to the early summer. Orally exposing Africanized honey bees to low concentrations (9 and 18 ppb) of the strobilurin fungicide

picoxystrobin continuously at 24–96 h, Batista et al. (2020) conducted a Malpighian tubule and midgut histopathological diagnosis and labeling of resulting cell death. While their results did not show any impact on the Malpighian tubules, data of the histopathological effects on the midgut were observed for bees consuming both concentrations. Indication of cell death and other morphological changes were recorded beginning at 24 h following initial exposure, thereby indicating that picoxystrobin exposure may compromise the ability of the honey bee midgut to absorb nutrients at a long term, which may result in malnutrition and impaired performance. In disagreement with these results, Decio et al. (2019) show that honey bee exposure to thiamethoxam + the fungicide carbendazim does not (a) increase the acute toxicity of thiamethoxam and (b) induce alternative splicing of the mRNAs examined. Specifically, they investigated three genes that are important in honey bee neuronal function: the stress sensor gene *X box binding protein-1*, the *Down Syndrome Cell Adhesion Molecule* gene, and the *embryonic lethal/abnormal visual system* gene. Therefore, they conclude that no honey bee cellular response is triggered as a result of exposure to this fungicide in the presence of thiamethoxam.

Fungicides may affect larval development or may alter the physiological developmental in bees. Larval oral consumption of technical-grade propiconazole or iprodione + chlorantraniliprole significantly reduced adult eclosion compared to a larval dietary intake of only chlorantraniliprole (Wade et al., 2019). Similarly, larvae fed with iprodione + methoxyfenozide or diflubenzuron alone or as a mixture with any of the tested fungicides reduced adult emergence compared to controls fed with acetone solvents only. Examining synergism between the formulated Pristine® and the spray adjuvant Break-Thru® (polyether-polymethylsiloxane-copolymer) on honey bee queen rearing success, Johnson and Percel (2013) determined that neither chemical nor their combination had a significant effect on immature queen survival. However, low levels of pyraclostrobin (50 ppb), but not boscalid, were found in royal jelly secreted by nurse bees feeding on treated pollen. Similarly, in feeding honey bee larvae different doses of technical-grade boscalid, Simon-Delso et al. (2017) quantified the safety levels of 741 ppm in pollen and 27 ppm in nectar, which are higher than 1 ppm isolated in pollen (Johnson et al., 2010; Stoner and Eitzer, 2013) and 1.43 ppm isolated in nectar (Wallner, 2010).

Non-*Apis* Bees (*Bombus* and Solitary Species)

Fungicides have been also demonstrated to impose toxicity to non-*Apis* bees by impairing colony performance, output and behavior for social species, and reproductive capacity and behavior for solitary species. For instance, synergized mortality following exposure to combinations of fungicides + insecticides and molecular-level impacts of fungicides on bumble bee flight have also been reported. Feeding *Bombus terrestris* adults with sugar syrup laced with the ergosterol biosynthesis inhibitor (EBI) fungicide imazalil + an insecticide, Raimets et al. (2018)

demonstrated that imazalil synergized the acute toxicity of fipronil, cypermethrin, and thiamethoxam. Therefore, the impact that EBI has in inhibiting P450 detoxification on bumble bees is different for individual pesticide chemistries. Conversely, feeding queenless *B. terrestris* microcolonies with pollen laced with captan at a field-realistic rate (10 mg/kg) had no effect on colony or individual worker longevity, consumption of pollen or sugar syrup, and normal larval development for all castes (Malone et al., 2007). Upon exposing queenless microcolonies of *B. terrestris* to honey water and pollen paste containing field-realistic average (23 mg/kg) or maximum (230 mg/kg) concentrations of the DMI fungicide propiconazole for 28 days, Elston et al. (2013) concluded that both concentrations reduced honey water consumption, while the smaller dose also decreased the quantity of wax cells produced. Separately, Bernauer et al. (2015) exposed five *Bombus impatiens* colonies to flowers treated with chlorothalonil at a field-realistic rate (20 g/L) over the course of 1 month. The treated colonies produced less than one-third as many workers, contained less than 50% of bee biomass, and were queenless by mothers with 50% of body mass compared to controls. Considering the findings of these researchers, it is important to characterize the mode of action of fungicides in bees. In a recent study, exploring the sensitivity of *B. terrestris* flight muscle mitochondrion to 16 fungicides, Syromyatnikov et al. (2017) determined disconnected respiration following exposure to diniconazole and fludioxonil and inhibited respiration following exposure to dithianon and difenoconazole. These researchers found the inhibition of electron transport at complex I and glycerol-3-phosphate dehydrogenase by difenoconazole exposure. Diathianon also inhibits succinate dehydrogenase and the oxidation of both nicotinamide adenine dinucleotide substrates and glycerol-3-phosphate while not impacting complex I like difenoconazole (Syromyatnikov et al., 2017).

Exposure to fungicides may also result in increased susceptibility to other pesticides, mainly insecticides. An experimental design comparing the impact of oral consumption of insecticide + fungicide mixtures on three bee species (*A. mellifera*, *B. terrestris*, and *Osmia bicornis*) found a small but maintained increase in clothianidin toxicity when combined with propiconazole, which was especially pronounced for the two non-*Apis* species with an almost three-fold increase for *O. bicornis* (Robinson et al., 2017). In a similar study, contact exposure of insecticides + fungicides, approximating LD₂₀ values and maximum sublethal levels, respectively, resulted in synergistic toxicity to *B. impatiens* workers (Iverson et al., 2019). Specifically, a synergistic interaction was noted for the triazole SBI fungicide difenoconazole when mixed with the pyrethroid bifenthrin, resulting in a maximum synergy ratio of 1.48. A separate synergistic interaction was observed for mixtures of the triazole fungicide myclobutanil with both bifenthrin and thiamethoxam, resulting in maximum synergy ratios of 11.0 and 2.38, respectively.

Although less examined, the toxicity of fungicides to commercially available solitary bees has also been assessed. Specific findings show synergisms arising from exposure to fungicide + insecticide combinations, differential toxicities of

fungicides to solitary bees compared to honey bees, and direct impacts of fungicides on solitary bee female nest construction and provisioning. Feeding *O. bicornis* females with a sugar solution containing either propiconazole or clothianidin, or a combined mixture, Sgolastra et al. (2018) noted a significantly lower feeding for the combined mixture, which decreased as emergence time increased. A significant synergistic interaction was determined for clothianidin + propiconazole on day 4 of treatment by means of a survival curve analysis. Post-exposure assessment at 3 days also revealed a significant synergistic interaction for the combined mixture, where *O. bicornis* exposed to this mixture had shorter oocytes compared to bees in all other treatments. Similarly, comparing the toxicity of these same chemicals and their combination to three bee species, Sgolastra et al. (2017) identified significant synergism by the measure of mortality for all bee species exposed to clothianidin + propiconazole. However, this synergism only lasted for the duration of the experiment (96 h) for *O. bicornis*. Comparing the exposure of *Osmia lignaria* and *A. mellifera* to five fungicides in cage studies, Ladurner et al. (2005) determined that the oral ingestion of propiconazole was acutely toxic to both species, while oral and contact exposure to captan was chronically toxic to *O. lignaria* only by severely impacting survival between 72 h and 7 days after exposure. In another study, Biddinger et al. (2013) showed synergistic toxicity to *Osmia cornifrons* when the fungicide fenbuconazole was mixed with commonly used neonicotinoid insecticides.

Comparing fungicide spray toxicity to *O. lignaria* and *Megachile rotundata* in semi-field cage studies, Artz and Pitts-Singer (2015) document *O. lignaria* exposure to technical-grade iprodione, and a premix fungicide with boscalid pyraclostrobin active ingredients and the non-ionic spray adjuvant (polyethoxylated non-ylphenol) negatively affected female nest recognition ability. Similarly, *M. rotundata* female nest recognition was impacted following exposure to a treatment containing fungicide premix (boscalid and pyraclostrobin) and polyethoxylated non-ylphenol adjuvant. Conversely, in a separate semi-field cage study, Ladurner et al. (2008) concluded that *O. lignaria* foraging and nesting behavior were unaffected when sprayed with one of the fungicides iprodione, propiconazole, benomyl, and captan.

EFFECTS OF HERBICIDE EXPOSURE TO BEES

Apis Bees

Herbicide exposure has been found to negatively impact different aspects of honey bee life, including drone sperm count, adult worker survival, and larval midgut composition. Impaired honey bee metabolic activity has also been demonstrated following exposure to single herbicides and or combinations of herbicides + metal mixtures. Testing the impact of glyphosate on honey bee drone spermatozoa, Hoopman et al. (2018) determined an LD₅₀ of 0.31 mg/ml, where the number of dead spermatozoa significantly increased with both exposure time and glyphosate concentration. Honey bee consumption of sugar syrup laced with bentazone and metamitron over a duration

of 168 h resulted in a daily herbicide intake of 16.16 and 13.87 mm³/bee, respectively (Migdal et al., 2018). Daily mortality of 3.21 bees/cage and increased aggression were noted for bentazone, while metolachlor exposure leads to a daily mortality of 13.00 bees/cage and low to moderate levels of increased bee aggression and mobility. Chronic *in vitro* larval exposure to glyphosate traces (1.25–5.0 mg/L of food) resulted in reduced brood weight and delayed molting (Vázquez et al., 2018). The authors acknowledge that while this experimental design does not account for nurse-bee feeding of brood, it does enable the researchers to control the glyphosate dose in the larval food and hence have it tested. Similarly, larval consumption of technical-grade glyphosate revealed significantly decreased survival (4 and 20 mg/L doses) and larval weight (0.08 and 4 mg/L doses) (Dai et al., 2018).

Several projects have also demonstrated the impacts of herbicides on impairing honey bee learning, sensory abilities, and navigation. For example, in analyzing the effects of chronic exposure to field-realistic concentrations of imidacloprid + glyphosate on honey learning, Mengoni Goñalons and Farina (2018) show reductions in sucrose responsiveness and impaired olfactory learning. Larva reared on food contaminated with glyphosate ate less, thereby further supporting the results of Dai et al. (2018) and Vázquez et al. (2018). Testing whether sub-lethal glyphosate concentrations affect honey bee homeward foraging and navigation, Balbuena et al. (2015) showed that bees fed sugar syrup laced with 10 mg/L (0.500 µg per bee) at a feeder spent more time flying home compared to controls or bees fed lower concentrations of 2.5 and 5 mg/L (0.125 and 0.250 µg per bee). Honey bees fed sugar syrup with the higher glyphosate concentration also performed more indirect flights; following a second release, the proportion of accurate homeward flights increased for controls, but not for treated bees. This result demonstrates that oral consumption of field-realistic doses of glyphosate (simulating that found in nectar) can impair the ability for honey bee foragers to learn a navigational task such as flying back to their hive.

An analysis of larval midgut bacteria composition demonstrated a significant decrease in beta diversity following an uptake of 20 mg/L glyphosate compared to controls (Dai et al., 2018). Confirming these results, Vázquez et al. (2018) also found that immune and detoxifying gene transcription within larval guts was variable among the numerous colonies studied following their exposure to glyphosate. Exposing adult bees to syrup contaminated with 0.12 mg/L glyphosate, atrazine, and 0.03 mg/L cadmium alone or in combination revealed that consuming herbicide + metal mixtures decreased the levels of alpha- and beta-carotene (Jumarie et al., 2017). Metabolically, herbicide + metal mixtures were indicative of impacting processes downstream of retinaldehyde formation and vitamin A metabolism and significantly increased thiobarbituric acid reactive substances (TBARS) levels compared to controls. Similarly, honey bee's consumption of sugar solution over 10 days significantly decreased β-carotene and all-*trans*-retinol (*at*-ROH) when spiked with atrazine and increased lutein and *at*-ROH when spiked with metolachlor (Helmer et al., 2015). Glyphosate-spiked solutions significantly decreased β-carotene

as doses increased and significantly increased the mean value of α-tocopherol at the dose of 1.25 ng/bee. Metolachlor ingestion of 20 ng/bee significantly reduced the TBARS levels compared to controls, oral uptake of glyphosate significantly decreased the protein levels as doses increased, and the highest dose of atrazine (5 ng/bee) significantly altered bee mass compared to controls. Similarly, Motta et al. (2018) show that honey bees exposed to field-realistic concentrations of glyphosate have decreased abundances of dominant species of the gut microbiota. In this study, young worker bees exposed to glyphosate, after being exposed to the pathogen *Serratia marcescens*, displayed higher levels of mortality. Moreover, this study confirmed that different species of honey bee gut microbiota responded differently in their susceptibility to glyphosate; this difference was largely driven by their possession of either 5-enolpyruvylshikimate-3-phosphate synthase (ESPS) class I (glyphosate-sensitive) or class II (glyphosate-insensitive). Subsequent *in vitro* experiments cloning the ESPS gene from honey bee gut bacteria into *Escherichia coli* further confirmed this difference – namely, reductions in most strains of the honey bee bacterial gut species *Snodgrassella alvi* were a consistent outcome. However, it was also found that some *S. alvi* strains are tolerant of glyphosate, indicating that they have an alternative resistance mechanism, warranting further investigation. In contrast, Decio et al. (2019) show that honey bee exposure to thiamethoxam + glyphosate does not (a) increase the acute toxicity of thiamethoxam and (b) induce the alternative splicing of the mRNAs examined. Specifically, they investigated three genes that are important in honey bee neuronal function: the stress sensor gene *X box binding protein-1*, the *Down Syndrome Cell Adhesion Molecule* gene, and the *embryonic lethal/abnormal visual system* gene, and they concluded that no honey bee cellular response is triggered as a result of exposure to glyphosate in the presence of thiamethoxam.

Non-*Apis* Bees (*Bombus* and Solitary Species)

In the recent past, the different toxicity of herbicides to non-*Apis* bee species, compared to honey bees, has also been determined. Stingless bee species oral ingestion resulted in paraquat inducing higher mortality for *Tetragonisca fiebrigi* and nicosulfuron inducing higher mortality in *Tetragonisca angustula* (Fermino et al., 2011). Increased superoxide dismutase isoenzyme activity was observed in both species after exposure to paraquat and is believed to be physiologically related to paraquat detoxification. A separate study observing the effect of oral and contact exposure to glyphosate, 2,4-D, picloram, and glyphosate + 2,4-D on the survival of *Melipona scutellaris* showed no decrease in longevity, following the application of 50% of the recommended field dose for all compounds (Nocelli et al., 2019). However, decreased *M. scutellaris* longevity was observed following a contact topical application of 100% of the recommended field dose for glyphosate + 2,4-D. Oral exposure of both 100% and twice the recommended field dose reduced the longevity for all compounds except for 2,4-D at twice the recommended field dose. Interestingly though, when separately evaluating the impact

of these herbicides on *M. scutellaris* temperature control and colony weight in semi-field conditions, Nocelli et al. (2019) found no differences between control and exposure treatments.

A comparative toxicity study of 2,4-dichlorophenoxyacetic acid to three bee species over 10 days of *ad libitum* feeding found the same LC₅₀ for *A. mellifera* and *B. terrestris* (>900 mg/L) and a higher LC₅₀ for *O. bicornis* (>1,437.5 mg/L) (Heard et al., 2017). Although the premise of this study is that honey bees can serve as a surrogate for non-*Apis* bees, this discrepancy supports a separate assessment of individual bee species. Exposing *A. mellifera* and *Hypotrigena rufipes* to plant material sprayed with 1 × and 2 × the label-recommended dose of Sunphosate 360 SL® (360 g/L glyphosate in 480 g/L isopropylamine salt) significantly impacted bee mortality as measured at 24 h after the treatment (Abraham et al., 2018). Similarly, significantly higher mortality was recorded at 24 h for *H. rufipes* contacting dried filter paper drenched with the recommended dose of Sunphosate 360 SL® – namely, these studies bolster support for the notion that *Apis* species cannot serve as a surrogate for non-*Apis* species in assessing the impacts of herbicide toxicity, as has been previously shown for insecticides (Biddinger et al., 2013; Sgolastra et al., 2017). As a result, they strongly support the need for future research that specifically investigates the impacts of herbicides on the non-*Apis* species in question.

Indirect Effects of Herbicide Use: Decreasing Floral Resources in Farmscape

Multiple interactions between bees, weed abundance, and pollination have been identified in the recent past. For example, weeds are a quantitative limiting resource for honey bees in between massive pollination events and a qualitative limiting resource for wild bees that are specialist foragers (Bretagnolle and Gaba, 2015). Changes in New Hampshire bumble bee species richness have been linked to habitat degradation, agricultural practices, and urban development which reduce floral resources (Jacobson et al., 2018). Specifically, *Bombus vagans* has been ecologically replaced by the generalist *B. impatiens*, while the range of *Bombus terricola* has shifted to higher elevations in more northern latitudes. Examining genetically modified herbicide-tolerant canola and adjacent wildflower strips, O'Brien and Arathi (2018) concluded that these plants provide wild bees refugia habitats by calculating a higher species diversity index for a wildflower plot compared to two canola plots.

Evaluating synthetic-auxin herbicides, Bohnenblust et al. (2016) show that dicamba application, at doses simulating particle drift, delayed and reduced plant flower production and resulted in diminished pollinator visitation. Surveying herbicide presence in native bee tissue from samples collected in two ecologically distinct habitats, Hladik et al. (2016) detected maximum concentrations of atrazine (99 ng/g) and metolachlor (13 ng/g) in 19 and 9% of samples, respectively. County peak application levels for the period 2008–2012 were 28,000 kg for atrazine and 22,000 kg for metolachlor (Baker and Stone, 2013). Assessing honey bee pollen pesticide contamination over a 16-week period, Long and Krupke (2016) note that herbicides

and fungicides comprised the most common pesticides. In non-agricultural areas, 83.3% of pollen samples contained metolachlor; adjacent to untreated maize, 75 and 54% of pollen samples contained metolachlor and atrazine, respectively, and adjacent to treated maize, 34–87.5% of pollen samples contained metolachlor, atrazine, and acetochlor.

CONCLUSION AND RECOMMENDATIONS

Pesticide toxicology studies on bees have primarily emphasized insecticides, given their specific design to kill insect species. As a result, a large quantity of data detailing if and how insecticides impact bee survival, at acute exposure, and behavior and normal function, at chronic exposure and sub-lethal doses, has been generated. However, in comparison, fairly little is known regarding the toxicity of other commonly used agricultural chemicals (namely, fungicides and herbicides) to bees. Moreover, the discovery of multiple dimensions of fungicide and herbicide toxicity to *Apis* and non-*Apis* species (as detailed in this review) elevates their likelihood of being potentially harmful to bees. For fungicides and herbicides, cases of direct acute mortality and sub-lethal impairment of normal biological behavior have been documented. Application of fungicides (unlike many insecticides) is widely permitted during crop bloom when bees actively pollinate, thereby exacerbating this situation for fungicides. Beyond directly imposing toxicity and impairment of normal functioning to bees, herbicides can also indirectly harm bees by decreasing the available forage resources. Weeds and other non-crop flowering plants provide essential food resources for bees. However, many of these naturally occurring flowering plants commonly foraged by bees are not genetically modified to tolerate herbicides. As a result, herbicide application often kills these flowering plants. Therefore, the importance of future research studies that build upon the results of current studies by continuing to investigate how exposure to these agricultural chemicals affects bees is highly elevated.

From our preliminary analysis of the published data on fungicide and herbicide toxicity to bees, we have identified several knowledge gaps that should be addressed in future work. First off, we explore how the plight of commercialized non-*Apis* species and specifically solitary bees should be addressed going forward. Currently, most research have focused on honey bees, which is reasonable given their widescale commercialization and usage in pollination events. However, for insecticides, it has been demonstrated that *Apis* and non-*Apis* species can react differently (Biddinger et al., 2013; Belsky, 2018), which may in part be explained by differences in how these bee species interact with their environmental surroundings where these insecticides are applied (Boyle et al., 2019; Hinarejos et al., 2019). Conversely, honey bees have been demonstrated to be suitable surrogates for non-*Apis* species in tier I insecticide assessments as in Thompson and Pamminger (2019). Although some work have investigated the impacts of fungicides and herbicides on non-*Apis* species, future studies should directly compare their acute and sub-lethal impacts on *Apis* and non-*Apis* species simultaneously in

both lab and field settings. Additionally, extrapolation on how differences in environmental interaction between *Apis* and non-*Apis* species influence their exposure to fungicide and herbicide chemistries and, hence, resulting susceptibility or resistance is needed. It is important to note that most of these studies will address a handful of non-*Apis* species that are commercially bred for pollination services. Regarding the majority of solitary species remaining, although they are not utilized for commercial pollination, their plight is equally as important. These species are often specialist pollinators that only pollinate a small group of plants that flower in synchrony with their pupal eclosion (Wcislo and Cane, 1996). Therefore, work investigating how usage of fungicides and herbicides in agricultural environments indirectly impacts these bees by means of killing the plants they pollinate is a future direction for research efforts. Also, since most solitary bees construct their nests in the soil (Cane, 2001), an assessment of how they encounter fungicide and herbicide chemistries on these substrates would be useful and builds upon the findings of Boyle et al. (2019) and Hinarejos et al. (2019). Additionally, several non-commercialized bees such as *Xylocopa*, *Exomalopsis*, *Centris*, *Oxaea*, and many Halictidae species have been documented to contribute to eggplant pollination (Patricio et al., 2012), while Halictidae and Andrenidae species were found to be the predominant pollinators of blueberries (Tuell and Isaacs, 2009). New projects can explore how fungicide and herbicide application in agricultural crop production systems at least partially pollinated by non-commercialized bee species impacts their abundance and pollination performance.

Other knowledge gaps in our current understanding of how fungicide and herbicide chemistries impact bees relate to aspects of experimental design. While most studies have examined acute toxicity in a laboratory setting and a few have brought this initial work into field settings, more field work is needed. Specifically, semi-field studies exposing bees in tunnels or greenhouses and true field studies exposing bees in their natural environments will aid in our understanding of how bees encounter fungicides and herbicides through interacting with their environmental surroundings. Although some work have addressed chronic toxicity by feeding bees fungicide- or herbicide-laced syrup for multiple days and sub-lethal toxicity through mapping changes in bee functioning and behavior following exposure, this is only the beginning. Pinpointing the duration of exposure for bee chronic toxicity onset and determining precisely when sub-lethal exposure translates into bee behavioral changes will aid pesticide applicators in making improved decisions regarding the timing of fungicide and herbicide administration. Using these data, pesticide applicators will be able to incorporate procedures to protect non-target bees from these agricultural chemicals into their application protocols. A few studies have explored how fungicides and herbicides impact honey bee brood (and specifically larvae) (Dai et al., 2018; Vázquez et al., 2018). More studies in this direction that expand into non-*Apis* brood (probably starting with the domesticated *Bombus* species) are warranted. Another aspect that has briefly been investigated are the impacts of fungicides and herbicides on bees at the molecular and the genomic levels. Specifically, future work should build upon results such as those of Dai et al. (2018)

and Motta et al. (2018) by mapping physiological changes at the molecular genomic level in more precise detail following contact with fungicides, herbicides, and their combination with insecticides or diseases. It is plausible that exposure to these chemicals alters normal gene expression and microbial composition (as has been demonstrated in several studies), which inhibits the ability for bees to detoxify an insecticide or combat a malady.

Findings of synergism arising from a combined contact with insecticides + fungicides or herbicides suggest that exposure to these chemicals in combination might in part instigate their observed hazard to bees. Therefore, the degree to which the potency of fungicides and herbicides to bees results from interactions with other stressors in their environments should be extrapolated in a future work. Most projects investigating herbicides have focused on glyphosate, which is a great start, however, there are a multitude of other herbicide chemistries that are commonly used to control weeds in agricultural settings. Therefore, future research endeavors should prioritize an examination of the toxicity of these different herbicide chemistries to bees.

Building upon our current data regarding insecticide toxicity to bees, the next wave of pesticide research on bees should strongly incorporate fungicides and herbicides. This work is timely given the widescale usage of fungicide and herbicide chemistries in agriculture by measure of market value (Pesticides Industry Sales Usage. United States Environmental Protection Agency, 2017). Moreover, the quantity of these chemicals sold and applied (in tonnage) outnumbers insecticides (Eurostat, 2018; Jess et al., 2018). Since fungicide and herbicide chemistries are commonly applied in environments where domesticated bees are placed for pollination services or that native bees inhabit, analyzing their impact on these organisms is of rising importance. Future research efforts moving in this direction will further illuminate our understanding (as scientists, growers, beekeepers, and pesticide applicators) of the consequences of bee interactions with the full spectrum of agricultural chemicals. Obtaining and utilizing this information will enable our ability to cohesively unify in modifying fungicide and herbicide application protocols to ensure that they do not impact non-target pollinating bees by means of appropriately reflecting the most recent data findings.

AUTHOR CONTRIBUTIONS

JB and NJ conceived the plan of this study. JB prepared the draft with inputs from NJ. Both authors contributed to the article and approved the submitted version.

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Effects of Revegetation on Soil Physical and Chemical Properties in Solar Photovoltaic Infrastructure

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Solar photovoltaic (PV) technology is being deployed at an unprecedented rate. However, utility-scale solar energy development is land intensive and its large-scale installation can have negative impacts on the environment. In particular, solar energy infrastructure can require extensive landscape modification that transforms soil ecological functions, thereby impacting hydrologic, vegetative, and carbon dynamics. However, reintroducing native vegetation to solar PV sites may be a means of restoring their soils. To this end, we investigated critical soil physical and chemical parameters at a revegetated photovoltaic array and an adjacent reference grassland in Colorado, United States. Seven years after revegetation, we found that carbon and nitrogen remained lower in the PV soil than in the reference soil and contained a greater fraction of coarse particles. We also found that the PV modules introduced heterogeneity in the soil moisture distribution, with precipitation accumulating along the lower edges of panels. The redistribution of soil moisture by panel arrays could potentially be used in concert with planting strategies to maximize plant growth or minimize soil erosion, and should be considered when evaluating the potential to co-locate vegetation with solar infrastructure.

Keywords: land use change, infiltration, soil moisture, renewable energy, agrivoltaics, co-location

INTRODUCTION

Solar photovoltaic (PV) technologies have been among the fastest growing energy technologies in recent years due to technological advancements and favorable government policies (U. S. Department of Energy, 2012; Breyer et al., 2017). Further, solar power has the greatest technical potential among renewable energy technologies (Jacobson, 2009; Edenhofer et al., 2011; De Marco et al., 2014; Ellabban et al., 2014) and is expected to fulfill 20–29% (32,700–133,000 GW) of global electrical demand by 2100 (Breyer et al., 2017). Although solar generation of electricity has many advantages, including low emission rates, an ability to reclaim degraded land, and an ability to improve quality of life in off-grid rural areas, deploying large-scale solar PV infrastructure can have negative impacts on ecological functions including carbon sequestration (Ravi et al., 2014; Hernandez et al., 2019). Moreover, solar PV is space-intensive, with the large-scale, non-integrated deployment of solar PV estimated to require more land area than coal,

nuclear, or natural gas technologies (Fthenakis and Kim, 2009). Under an optimistic scenario for global solar energy deployment by 2050 (8500 GW_p) (IRENA-International Renewable Energy Agency, 2018) and assuming utility-scale PV installations require 3 ha per MW_p of capacity-weighted average direct land use (Ong et al., 2013), around 25 million hectares of land will be transformed in the next 30 years if all arrays are ground-mounted. Further, requirements for access roads, transmission lines, etc. can raise the total land area transformed by solar PV installations substantially (Ravi et al., 2014).

Conventional, utility-scale solar energy infrastructure modifies landscapes extensively through the site preparation process: native vegetation is removed, the ground surface is graded, and fill is added and compacted (Hernandez et al., 2014). Such modifications transform soil physical, chemical, and biological properties, thereby impacting moisture and nutrient dynamics, and thus soil's ability to support vegetation and perform a host of associated ecological processes. Strategies to mitigate the cumulative impacts of solar PV installation and operation on landscape function have therefore been the focus of recent research (Turney and Fthenakis, 2011; Phillips, 2013; Hernandez et al., 2014, 2015; Armstrong et al., 2016; Walston et al., 2016). One promising strategy is to integrate other, vegetated land cover types (e.g., crop cultivation or native grassland) into PV facilities, as these may increase their ability to support ecosystem services like pollination and carbon sequestration (Dupraz et al., 2011; Macknick et al., 2013; Hernandez et al., 2014; Ravi, 2015; Hoffacker et al., 2017; Walston et al., 2018). The concept of co-locating solar PV and agriculture (i.e., "agrivoltaics") was first suggested by Goetzberger and Zastrow (1982). Since then, a number of modeling (Dupraz et al., 2011; Ravi et al., 2014, 2016) and field studies (Marrou et al., 2013b,c; Beatty et al., 2017; Barron-Gafford et al., 2019) have investigated the technological, economic, and environmental feasibility of co-location approaches.

Assessing the impacts of PV deployment on land surface characteristics can inform guidelines for site preservation and help determine the optimum environmental and financial outcomes co-location strategies could bring about. Ultimately, such information could be used to determine if proposed co-located PV installations are likely to be successful. Prior studies of the environmental impacts of solar infrastructure have been focused on modeling runoff and monitoring microclimatic impacts (Cook, 2011; Marrou et al., 2013a; Barron-Gafford et al., 2016). For example, one review found that site preparation for PV arrays typically remove vegetation and degrade soil, resulting in significant increases in onsite runoff and soil erosion (Cook, 2011). Further, changes in structure of the terrain, vegetation cover, and albedo can raise air temperatures over PV arrays relative to surrounding natural areas (Barron-Gafford et al., 2016). However, other studies have identified effects of PV infrastructure that are likely to be favorable for co-located vegetation; these include increases in soil moisture content, biomass, and plant water use efficiency (Marrou et al., 2013a; Hassanpour Adeg et al., 2018; Barron-Gafford et al., 2019). In addition, lower PV panel temperatures have been observed in arrays with underlying agricultural crops vs. those without

(attributed to greater evapotranspiration rates), highlighting the potential of co-location to increase electrical generation in arid regions (Barron-Gafford et al., 2019).

Despite the above-mentioned advances, it is unclear if reintroducing native vegetation at existing solar PV sites can successfully mitigate changes to soil hydrology and ecology. In cases where natural land is leased for solar projects, leases typically span 20–30 years; during this time soil hydrological and ecological processes may be negatively impacted or made spatially heterogeneous. In addition to diminishing the landscape's ability to support ecosystem services during the solar facility's lifespan, these changes may leave legacy effects that persist long after the installation is removed. Although many studies have quantified the environmental footprints of photovoltaic facilities (Fthenakis and Kim, 2010, 2011; Sherwani et al., 2010; Turney and Fthenakis, 2011; Fthenakis et al., 2012; Perez and Fthenakis, 2012; Leccisi et al., 2016), there have been few field-based investigations of the impacts of PV arrays and co-location strategies on soil properties (e.g., hydrology, geomorphology, and chemistry) (Armstrong et al., 2014; Hassanpour Adeg et al., 2018). Given this, we investigated whether revegetation of a PV facility is able to engender soil physical and chemical properties similar to those at an undisturbed reference site and, further, if the PV modules introduce spatial variation in soil properties. We did this at a PV installation that had been experimentally revegetated with native grasses. Soil properties at the PV site were compared (a) to those in an adjacent, undisturbed grassland characteristic of the region's native land cover and (b) spanning the range of location types under and between PV modules.

MATERIALS AND METHODS

Site Description

This study took place at a 1.1 MW solar PV facility in the National Renewable Energy Laboratory's National Wind Technology Center. This facility is located on the eastern slope of the Front Range in northern Jefferson County, Colorado, USA and has a mean annual precipitation of 374 mm (Beatty et al., 2017). When the site was constructed in 2009, the topsoil was largely removed and the site was graded, leveled to < 1% slope, and compacted (Beatty et al., 2017). Surficial soils at the site are paleosols derived from unsorted alluvial/colluvial deposits; soil-forming processes have extensively oxidized native iron and leached clay minerals (Beatty et al., 2017). The area encompassing the site was historically a xeric tallgrass prairie dominated by big bluestem grass (*Andropogon gerardii*). Grazing and other activities have altered the region's vegetation while leaving the paleosols and clay-enriched subsoils intact (Beatty et al., 2017). Following the construction of the solar PV array, a portion of the disturbed area was revegetated with a variety of native grasses (e.g., *Bouteloua gracilis* and *Poa compressa*) to determine, in part, if soil alteration could be mitigated (Beatty et al., 2017). Revegetation occurred in 2010, 7 years prior to the activities described below.

Study Design

A campaign to collect soil samples and take field measurements was carried out in the revegetated portion of the solar PV facility and in a nearby grassland in July 2017. This portion of the PV facility had four rows of PV modules, each 49 m in length (**Supplementary Figures S1A,B**). PV panels were mounted on single-axis solar-tracking systems 1 m above ground-level (Beatty et al., 2017). Soil measurements and samples were taken along three transects in the revegetated portion of the solar PV site (hereafter, solar transects/soils) and along a fourth transect in an adjacent, undisturbed grassland (hereafter, reference transect/soil) that is representative of the area's vegetation (**Supplementary Figure S1A**). Solar transects ran perpendicular to the rows of PV modules, spanning four rows and the adjacent interspaces. The reference transect was 50 m east of the nearest point in the PV array, across an unpaved road (**Supplementary Figure S1A**). Soil measurements and sampling took place at four locations on solar transects: below the east edge of each panel (EE), beneath the center of each panel (BP), below the west edge of each panel (WE), and in the uncovered interspace adjacent to each panel (IS) (**Supplementary Figure S1C**). Transects thus consisted of 16 sampling locations, spaced ~2.5 m apart, and were ~10 m in length. Sampling/measurement locations on the reference transect were likewise spaced 2.5 m apart, with the transect extending 10 m. In total there were 48 solar PV sampling locations and 16 reference locations.

Field Measurements

Volumetric soil moisture (VSM) content and infiltration rates were measured at each sampling location. VSM was measured using a 15-cm long soil water content reflectometer (HydroSense II, Campbell Scientific, Logan, United States). In addition to the primary set of VSM measurements, a second set of measurements was made the next day, after an overnight rainfall event. This event delivered 6.6 mm of rainfall and the wind direction ranged from southwesterly to northwesterly. Precipitation and wind data were collected from a meteorological station located ~200 m northwest of the PV array. The solar array was facing west at the end of the field campaign and during the precipitation event that evening.

Infiltration rates were measured along two of the three solar transects, as well as the reference transect, with two measurements made per sampling location. This modification to

the sampling design was deemed appropriate given the spatial variability of infiltration in combination with the limited period of access to the site. A mini disk infiltrometer (METER, Pullman, United States) was used for measurements; it had an adjustable suction (1 cm was used) and a small footprint (5-cm diameter disk). The mini disk infiltrometer exerts a negative tension on the soil surface, thereby excluding macropores and only measuring flow in the soil matrix; the resulting data better represent properties of the soil itself. Infiltration data were used to calculate unsaturated hydraulic conductivity (K_{unsat}) for each sample following Zhang (Zhang, 1997).

Laboratory Measurements

Surficial soil samples (0–5 cm) were taken at all sampling locations along the four transects. After air-drying, soil samples were sieved to 2 mm, passed through a riffle sampler, and sub-sampled. One sub-sample per sample was used to characterize the particle size distribution (PSD) while another was used for chemical analysis. PSDs were measured with a laser diffraction particle size analyzer (LS 13-320 with a tornado dry powder module; Beckman Coulter, Brea, United States); the dynamic grain size ranged from 0.4 to 2000 μm . Total soil carbon (C) and nitrogen (N) were determined by Brookside Labs (New Bremen, United States) using the combustion method (McGeehan and Naylor, 1988; Nelson and Sommers, 1996).

Statistical Analysis

Data were analyzed using mixed- or fixed-effects linear models (**Table 1**). Separate models were generated for each response variable, which included VSM before and after the precipitation event, K_{unsat} , total carbon, total nitrogen, mean particle diameter, and the coarse particle fraction ($> 2 \text{ mm}$). In all models, the location category (EE, BP, WE, IS, and Reference) was the only fixed explanatory variable. When likelihood ratio tests indicated that it was warranted (**Table 1**), random effects were included to account for the spatial clustering of samples along transects and/or beneath panels. *F*-tests, based on Type II sums of squares, were used to determine if response means differed across location categories. When they differed, pairwise comparisons were made using estimated marginal means with Tukey adjustments applied to *P*-values. All analyses were carried out in R 3.6.1 (R Core Team, Vienna, Austria) with $P < 0.05$ considered statistically significant.

TABLE 1 | Statistical models and results for evaluations of whether soil properties differed by location type.

Response	Fixed effect			Random effects		R^2 Fixed	R^2 Full
	Location type	<i>F</i>	<i>P</i>	Transect	Panel		
Moisture before	•	13.1	< 0.001	•	•	0.48	0.82
Moisture after	•	108.1	< 0.001	•	•	0.84	0.93
K_{unsat}	•	5.1	0.001			0.18	0.18
Mean particle diameter	•	23.9	< 0.001			0.61	0.61
Coarse fraction	•	13.8	< 0.001			0.54	0.54
Carbon	•	28.5	< 0.001			0.64	0.64
Nitrogen	•	10.3	< 0.001		•	0.74	0.83

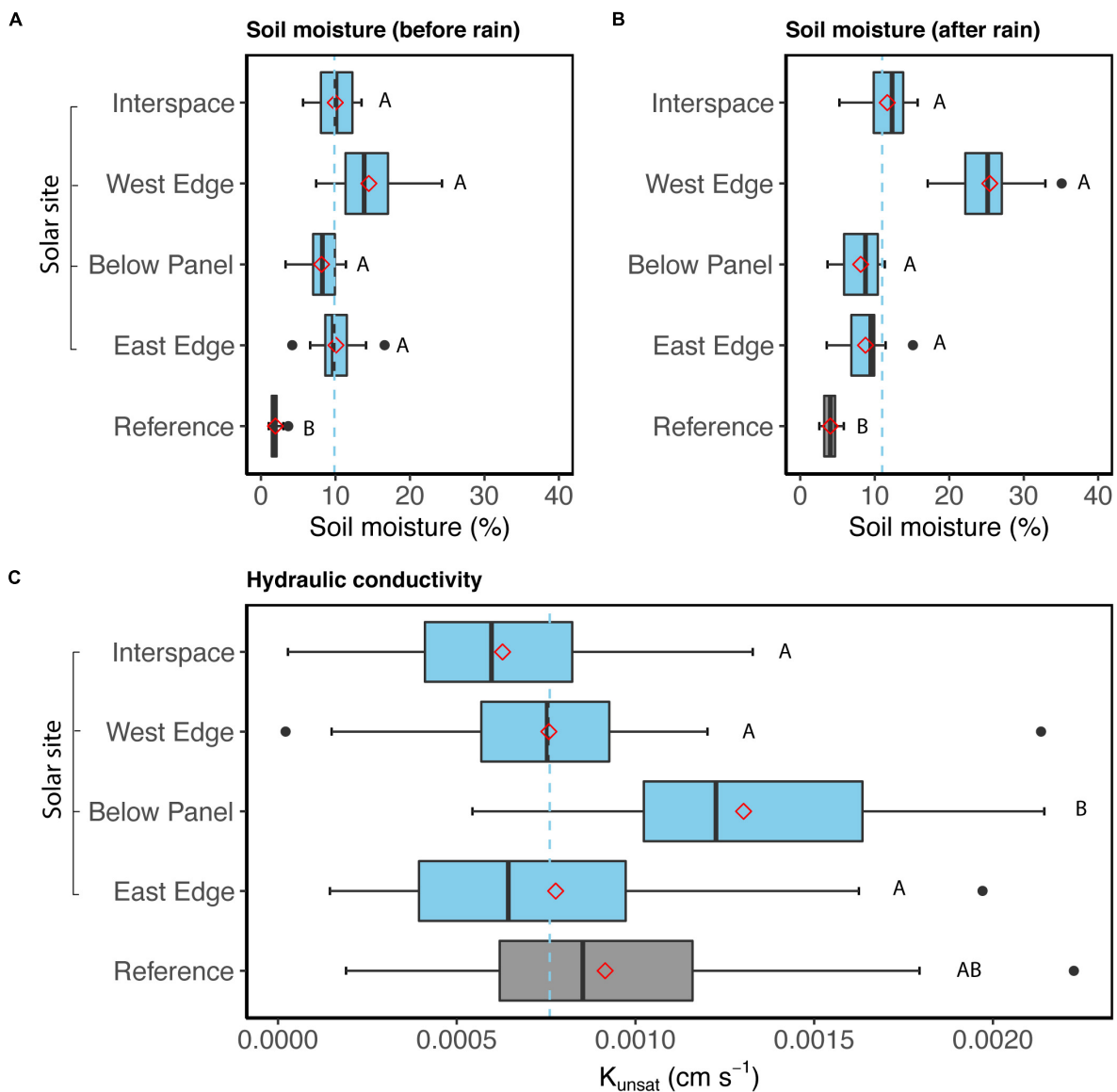


FIGURE 1 | Soil moisture (A) before and (B) after a precipitation event in the reference grassland and in locations beneath solar modules. (C) Unsaturated hydraulic conductivity (K_{unsat}) in the reference soil and in locations beneath solar modules. Box plots show medians (solid lines) and interquartile ranges (box enclosures), with whiskers extending to the most extreme observation $\leq 1.5 \times$ the interquartile range beyond the box. Also shown are means (red diamonds) and individual observations beyond whiskers (black circles). Location categories share letters if pairwise comparisons did not identify significant differences between means.

RESULTS

Soil Moisture

The reference soil had the lowest sample mean during both measurement periods, though population means were not statistically differentiable between the reference and nearly any PV location; the exception was west edges after the rain event. Soil moisture was elevated in west edge locations in PV soils, both before (Figure 1A) and after (Figure 1B) the rain event (Table 1). Moreover, mean moisture levels increased from 14.5 to 25.4% at these locations following the rain event, whereas they increased from 9.4 to 9.5%, on average, in other soils at the PV site.

Hydraulic Conductivity

Mean K_{unsat} was highly variable, ranging from 0.2×10^{-4} to $21.4 \times 10^{-4} \text{ cm s}^{-1}$ in the PV array and 1.9×10^{-4} – $22.3 \times 10^{-4} \text{ cm s}^{-1}$ in the reference. Given the substantial overlap, K_{unsat} was not significantly different in reference vs. PV soils but was greater beneath panels than at edge locations or in interspaces (Figure 1C and Table 1).

Particle Size

Soil at the PV site had a greater coarse-particle ($> 2 \text{ mm}$) mass fraction than did the reference soil (Figure 2A); the fraction was similar across the four PV location categories. Correspondingly,

mean particle diameter was greater in soils (< 2 mm) from the PV site than those at the reference site (**Figure 2B**). Moreover, the reference soil had a more heterogeneous grain size distribution than did soil from the solar site, with the degree of sorting roughly proportional to the mean particle diameter [ϕ size-scale = $-\log_2$ (diameter in mm)] (**Figure 2C**). No difference in particle size characteristics were observed among locations in the PV site.

Carbon and Nitrogen

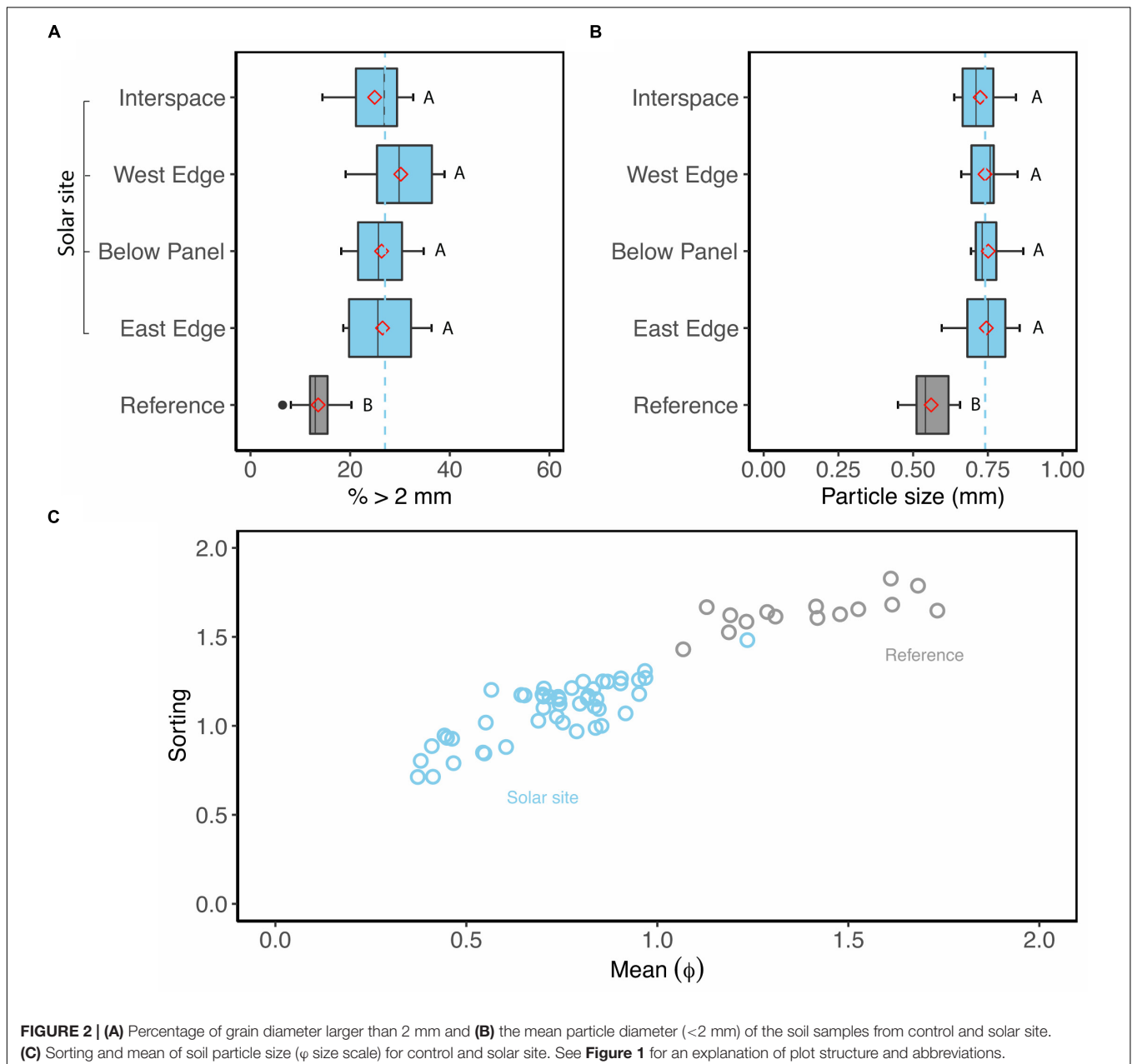
Soils at the solar PV site contained significantly less carbon (38%) and nitrogen (50%) than did the reference soil (**Figure 3** and **Table 1**). However, there was no evidence of

differences in mean carbon or nitrogen content among location categories at the PV site.

DISCUSSION

Soil Moisture

Although statistically equivocal, our results suggested that soil moisture may have been greater at the solar PV site compared to the reference soil. If real, this could have been due to shading and wind sheltering by the PV array, as these have been found to decrease actual evapotranspiration by 10–40% (Marrou et al., 2013a). Our results also demonstrate that



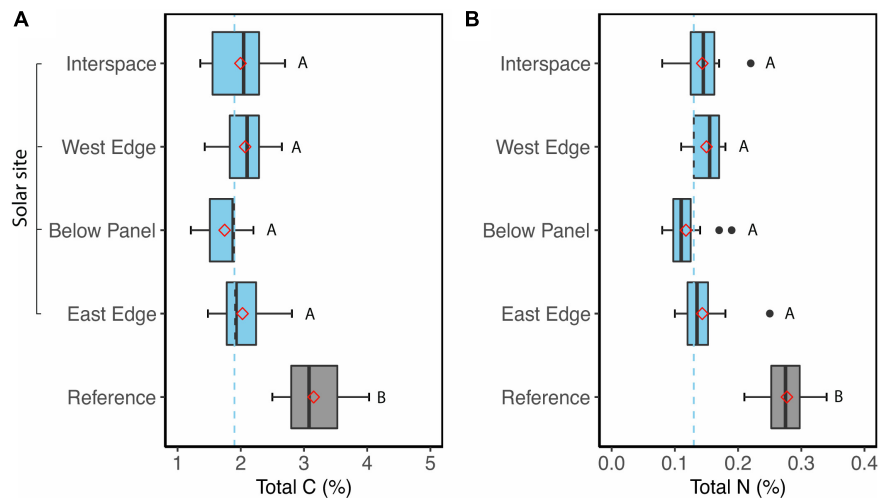


FIGURE 3 | (A) Total carbon and **(B)** total nitrogen for the reference soil and in locations beneath solar modules. Also shown are means (red diamonds) and individual observations beyond whiskers (black circles). See **Figure 1** for an explanation of plot structure and abbreviations.

PV arrays can introduce considerable heterogeneity in the spatial distribution of soil moisture. Specifically, the presence of PV modules can concentrate moisture along their low-lying edges, which were to the west in this study. While this spatial pattern was greatest following a rain event, it was also evident before the event, which was 3 days after the prior (5.6 mm). There may be important implications of soil moisture heterogeneity for plant growth in PV arrays co-located with crop or forage plants; these would be especially magnified in arid regions, where small variations in water availability could have large effects on plant growth (Noy-Meir, 1973; D'Odorico et al., 2007).

Hydraulic Conductivity and Particle Size

Even though soil grain size distribution is one of the determinants of infiltration rates (Mazaheri and Mahmoodabadi, 2012), the difference in grain size distribution did not result in measurable differences in K_{unsat} between the solar PV site and reference soils (**Figure 1C**). However, when comparing K_{unsat} among the location types within the solar site, K_{unsat} was elevated immediately below panels, even though the particle size distribution in these locations were similar to those at other locations (Arya et al., 1999). This discrepancy between expected and observed hydraulic conductivity below panels may be attributable to reduced exposure to site maintenance activities that cause compaction, as compaction decreases hydraulic conductivity (Vomocil and Flocker, 1961). Differences in compaction may likewise explain the lack of difference in K_{unsat} despite the difference between particle size distributions between PV and reference soils (**Figures 2A,B**).

It is likely that the disparity in particle size between reference and PV soils arose during the PV facility's construction phase. As documented by other studies (Mohammad and Adam, 2010; Hernandez et al., 2014), disturbance to topsoil, the addition of

fill, and the removal of native vegetation during construction can accelerate the erosion of fine particles, both by wind and water action. In the long-term absence of vegetation – a major soil erosion control – accelerated erosion results in loss of soil carbon, nutrients, and water holding capacity, leading to an overall decrease in the ability of soil to sustain vegetation (Lal, 2003; Li et al., 2008). Dust particles resulting from wind erosion can be transported long distances (Field et al., 2010; Ravi et al., 2011) or even deposited locally on solar PV panel surfaces, resulting in significant losses in solar power generation and additional operational costs for cleaning panels (Mani and Pillai, 2010; Mejia and Kleissl, 2013).

Carbon and Nitrogen

The significantly lower total carbon and nitrogen levels in the solar PV soil vs. in the reference soil (**Figure 3**) was likely caused by the removal of topsoil during the array's construction. This is consistent with prior studies showing reduced soil organic matter and plant production in experiments simulating topsoil erosion by artificially removing it (Larney et al., 2000, 2016; Hölzel and Otte, 2003). Reduced C and N levels suggest that nutrient cycling had not fully reestablished 7 years after PV construction and, further, that the PV soil's ability to sequester carbon was diminished relative to the native soil. In order to minimize the loss of these key soil functions, appropriate site preservation practices should be adopted to minimize topsoil disturbance during construction. Total N and C content for the reference site were similar to those reported by prior studies in the region (e.g., Golubiewski, 2006).

Implications

The revegetation of our study site was intended, in part, to render the soil at a solar PV facility similar to those of the

region's grasslands. A number of the investigated soil characteristics differed between reference and PV array, indicating that this was not fully achieved in 7 years. Further, the presence of photovoltaic arrays induced spatial heterogeneity in soil moisture and K_{unsat} , specifically coupling soil moisture to the position and orientation of the PV modules. Despite these persistent changes to soil properties, other studies indicate that co-location can restore certain ecological functions. For example, a previous study at our site indicated that the revegetation was sufficient to suppress erosion and provide wildlife habitat (Beatty et al., 2017).

The environmental heterogeneity introduced by PV modules may have some advantages, even if soil properties differ from those of native grasslands. For instance, it may be possible for co-located PV panels to be strategically oriented in a manner that would direct intercepted rain to or away from the adjacent soil, depending on the irrigation requirements of the plants. This control over rainfall distribution could benefit vegetation types with specific watering requirements, like many agricultural crops. Further, the rapidly-draining soils beneath PV modules could be suitable for cultivating drought-resistant crops (Ravi et al., 2016). Co-locating vegetation would also suppress dust emissions, reducing rates of dust deposition on panels (Ravi et al., 2016; Elamri et al., 2018a,b). Conversely, crops that require protection from excessive rainfall at the seedling stage could be placed under the PV panels during the seedling stage and replanted on the side of the lower edge of the PV array in their adult stage to receive more water (Choi, 2019). Thus, understanding the timing and direction of rainfall is crucial to placing and orienting PV panels in order to bring about a controlled and favorable soil moisture distribution.

The effects of panel orientation on precipitation redistribution by solar panels indicate that solar arrays could be used to manipulate soil moisture distribution, an important factor when evaluating the potential to co-locate crops with solar infrastructure. Further, the environmental consequences of PV installation like those identified here should be quantified and modeled to inform site preservation, to evaluate long-term impacts of onsite management strategies, and to optimize soil

moisture, nutrient, and sunlight availability when co-locating natural vegetation or crops with photovoltaic arrays.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

CC, AC, JM, and SR conceived and planned the study. CC, AC, and JM carried out the field data collection. CC, SR, JC, and DB conducted the laboratory and data analyses. CC, SR, JC, and JM interpreted the results. CC, SR, and JC wrote the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2020.00140/full#supplementary-material>

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The Relative Role of Climate Variation and Control Interventions on Malaria Elimination Efforts in El Oro, Ecuador: A Modeling Study

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Malaria is a vector-borne disease of significant public health concern. Despite widespread success of many elimination initiatives, elimination efforts in some regions of the world have stalled. Barriers to malaria elimination include climate and land use changes, such as warming temperatures and urbanization, which can alter mosquito habitats. Socioeconomic factors, such as political instability and regional migration, also threaten elimination goals. This is particularly relevant in areas where local elimination has been achieved and consequently surveillance and control efforts are dwindling and are no longer a priority. Understanding how environmental change, impacts malaria elimination has important practical implications for vector control and disease surveillance strategies. It is important to consider climate change when monitoring the threat of malaria resurgence due to socioeconomic influences. However, there is limited assessment of how the combination of climate variation, interventions and socioeconomic pressures influence long-term trends in malaria transmission and elimination efforts. In this study, we used Bayesian hierarchical mixed models and malaria case data for a 29-year period to disentangle the impacts of climate variation and malaria control efforts on malaria risk in the Ecuadorian province of El Oro, which achieved local elimination in 2011. We found shifting patterns of malaria between rural and urban areas, with a relative increase of *Plasmodium vivax* in urbanized areas. Minimum temperature was an important driver of malaria seasonality and the

association between warmer minimum temperatures and malaria incidence was greater for *Plasmodium falciparum* compared to *P. vivax* malaria. There was considerable heterogeneity in the impact of three chemical vector control measures on both *P. falciparum* and *P. vivax* malaria. We found statistically significant associations between two of the three measures [indoor residual spraying (IRS) and space spraying] and a reduction in malaria incidence, which varied between malaria type. We also found environmental suitability for malaria transmission is increasing in El Oro, which could limit future elimination efforts if malaria is allowed to re-establish. Our findings have important implications for understanding environmental obstacles to malaria elimination and highlights the importance of designing and sustaining elimination efforts in areas that remain vulnerable to resurgence.

Keywords: malaria, environmental change, elimination, vector control, climate, spatio-temporal model

INTRODUCTION

Malaria is the most important vector-borne disease worldwide, with 228 million cases reported in 2018 (WHO, 2019). Despite global elimination and eradication efforts, progress toward elimination is stalling in some endemic countries. In particular, increases in malaria incidence have been observed in some South American countries since 2014 including Venezuela, Peru, Colombia, and Ecuador, where movement of infected individuals between neighboring countries in the region can result in a resurgence of cases in local populations, threatening progress toward malaria reduction for other countries (Grillet et al., 2019; WHO, 2019). Malaria is highly sensitive to environmental conditions, including climate variability and land use practices (Moreno, 2006; Caminade et al., 2014), which along with socioeconomic influences can act as significant barriers to elimination. In addition, the lapse in control and surveillance efforts combined with global environmental changes also poses a threat to malaria elimination efforts in South America (Alimi et al., 2015; Conn et al., 2018).

Variation in meteorological conditions, particularly temperature, and rainfall, determines the spatiotemporal patterns of malaria through their effects on both the *Plasmodium* parasite and the *Anopheles* vector. Warmer temperatures can decrease the extrinsic incubation period (EIP), the time taken for the malaria parasite to complete its development inside the mosquito. A shorter EIP increases the transmission intensity of malaria by allowing mosquitoes to become infectious more quickly. The EIP for the *Plasmodium falciparum* parasite is longer (~10 days) than that for *Plasmodium vivax* (~8 days) and *P. vivax* can also develop at relatively cooler temperatures (minimum 15°C), compared to *P. falciparum* (minimum 18°C) (Patz and Olson, 2006). Temperature also impacts the mosquito vector, by affecting both larval and adult survival and longevity (Beck-Johnson et al., 2013), mosquito development (Bayoh and Lindsay, 2003), and vector population dynamics (Beck-Johnson et al., 2013). However, the effect of temperature on malaria transmission is non-linear, with the optimal temperature for malaria transmission estimated to be 25°C with transmission declining above 30°C (Paaijmans et al., 2009; Mordecai et al.,

2013). Rainfall is also important for malaria transmission as it determines the availability of mosquito breeding habitat and thus mosquito abundance, although heavy and persistent rainfall can wash out larval habitats (Galardo et al., 2009; Olson et al., 2009; Prussing et al., 2019; Wolfarth-Couto et al., 2019).

Anthropogenic activities, such as urbanization and agricultural land use can also change malaria transmission patterns over longer time scales by creating more or less favorable microclimatic conditions and habitats for malaria vectors, depending on the species (Conn et al., 2002; Wayant et al., 2010; Tucker Lima et al., 2017). For example, urban development can eliminate mosquito habitat subsequently decreasing malaria incidence or in some cases increases in peri-urban malaria can occur as a result of economic migration and the creation of novel breeding habitats (Padilla et al., 2015). In addition, deforestation can facilitate malaria transmission due to the creation of new mosquito habitats on forest fringes (Vittor et al., 2006; Stefani et al., 2013). Increasing environmental suitability of malaria transmission, associated with climatic change and land use modifications, has important implications for malaria elimination efforts in South America, as these changes could compromise elimination efforts (Recht et al., 2017).

Previous studies have identified several environmental risk factors for malaria transmission that may impede elimination efforts in South America. For example, large-scale climate patterns such as the El Niño Southern Oscillation have been associated with large outbreaks of malaria in Peru, due to flooding conditions and in Colombia and Venezuela due to drought conditions (Gagnon et al., 2002). In highland areas of Colombia, warmer years were associated with an increased number of malaria cases occurring at higher altitudes (Siraj et al., 2014). Studies assessing spatial heterogeneity in malaria risk in South America have found deforestation and agricultural development have altered anopheline vector ecology in the Amazon region through creation of new *Anopheles* habitats and increased human-mosquito interactions (Moreno et al., 2007; Vittor et al., 2009; Hahn et al., 2014; Burkett-Cadena and Vittor, 2017).

When assessing the role of environmental factors on malaria incidence it is important to simultaneously consider the relative impact of control interventions, especially in elimination

settings. Studies that evaluate the effectiveness of malaria control programs often consider environmental factors, most commonly rainfall and vegetation indices. Some studies used geostatistical modeling and after adjusting for climate conditions estimate the contribution and effectiveness of malaria control measures, including indoor residual spraying (IRS) in sub-Saharan Africa, relative to the impacts of climate variability (Graves et al., 2008; Giardina et al., 2014). Other studies often treat climate and environmental variables as confounders and do not apportion variation in malaria incidence due to climate (Aregawi et al., 2011; Bennett et al., 2014). There has been a limited assessment of how the combination of environmental factors, elimination efforts and socioeconomic pressures influences long-term trends in malaria and the route toward elimination. Understanding how malaria elimination efforts might be hindered by environmental changes has implications for targeting of vector control and disease surveillance, and is important especially given the possibility of shifting vector distributions and increasing climate suitability in South America (Pinault and Hunter, 2012).

According to the Pan-American Health Organization (PAHO), Ecuador is in the malaria pre-elimination phase, but has fallen short of its target to achieve elimination by 2020 (WHO, 2018). Since 2016 malaria has increased significantly in the country, mainly in the Amazon region, with more cases being detected than expected for a country on the verge of malaria elimination (PAHO, 2017; Sáenz et al., 2017). Recent surges of malaria in other South American countries, primarily Venezuela, are threatening current elimination efforts in Ecuador due to regional migration (Daniels, 2018; Grillet et al., 2019). This study focuses on Ecuador's southern province, El Oro, a high-risk region, which previously eliminated malaria but recently recorded indigenous cases, in addition to being vulnerable to the introduction of infections via imported cases due to high rates of human movement associated with its location on a strategic migration route (Jaramillo-Ochoa et al., 2019). Here we seek to identify threats to malaria elimination, with a view to prevent lapses in control efforts and the re-establishment of malaria in an area currently considered malaria-free but historically endemic for malaria. We advance the existing knowledge of the malaria elimination success in El Oro (Krisher et al., 2016) by quantifying the associations between malaria, climate and control interventions in a region vulnerable to resurgence.

MATERIALS AND METHODS

Study Area

El Oro province (latitude: 3°5'45.20"S–4°11'3.06"S, longitude: 79°43'10.92"W–80°50'37.96"W) is located in southern Ecuador on the Pacific Coast and shares a border with the Tumbes region of northern Peru (Figure 1A). El Oro covers 5,870 km² and is divided into 14 cantons, which range in size from approximately 70 km² up to 900 km². Population densities per canton range from 7 people per km² in rural areas and up to 760 people per km² in the province capital, Machala.

Plasmodium vivax and *P. falciparum* are the malaria-causing parasites in the region (WHO, 2019) and the primary mosquito vectors transmitting malaria in El Oro are *Anopheles albimanus* and *Anopheles punctimacula* (Ryan et al., 2017).

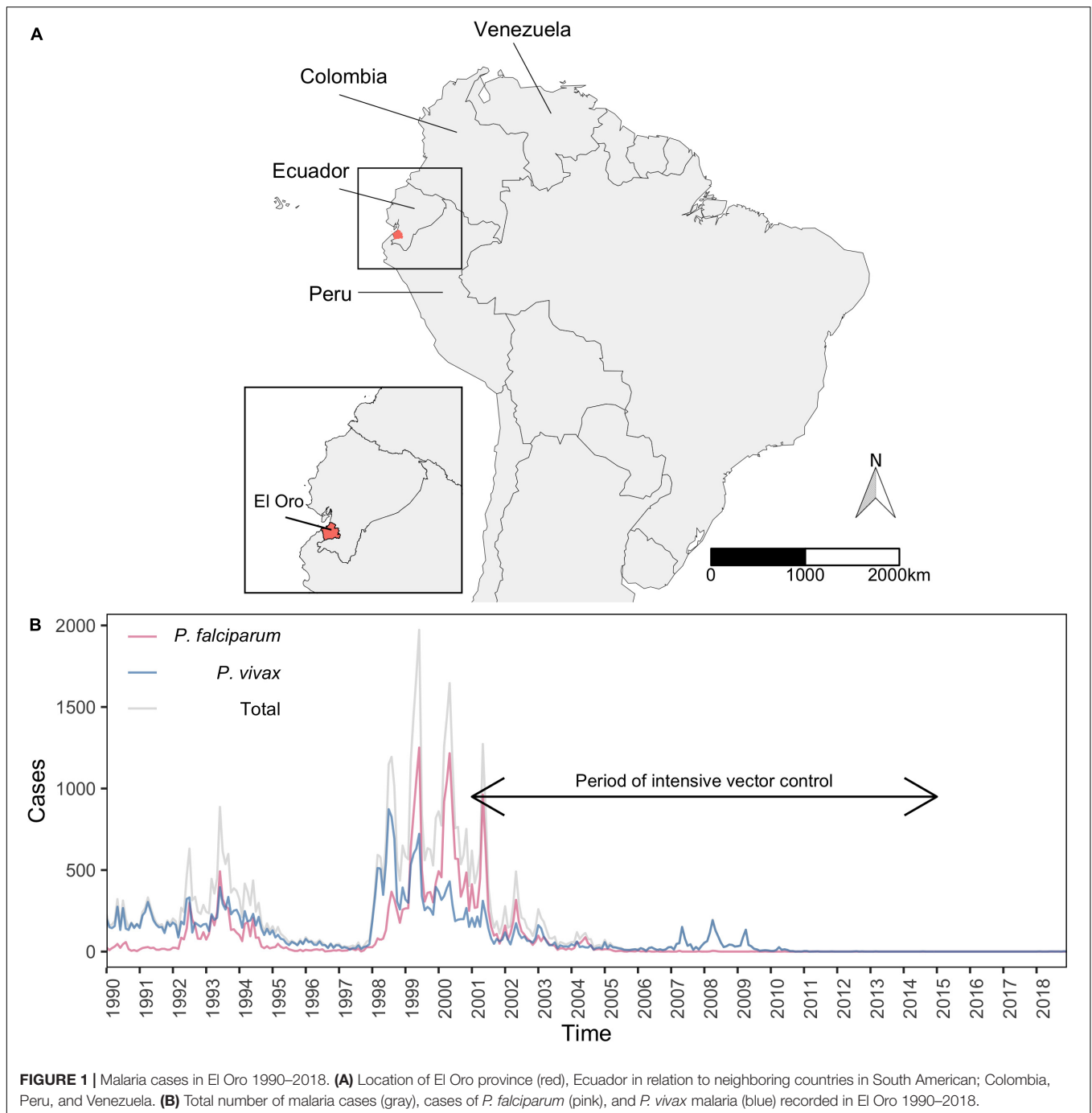
During the mid-1990s public health authorities in El Oro and neighboring Tumbes unified to implement an effective binational collaboration to tackle the surge in malaria transmission in the region (Krisher et al., 2016). El Oro has been free of locally acquired malaria infections since 2011 although malaria has been increasing elsewhere in Ecuador (WHO, 2019). In 2018 seven malaria cases were recorded in El Oro (6/7 were imported cases) during a period of elevated migration of Venezuelan citizens associated with the social and economic crisis in their country (Jaramillo-Ochoa et al., 2019). El Oro's border location, along the Pan American highway, a route used by many migrants, makes it particularly vulnerable to malaria resurgence. In addition, El Oro is one of the most hazardous coastal zones in Latin America and the Caribbean (Calil et al., 2017). Vulnerable populations are susceptible to the effects of the El Niño Southern Oscillation, which intensifies annual flooding events during the rainy season (Lowe et al., 2017; Tauzer et al., 2019). Lapses in surveillance and control efforts and reductions in funding following local elimination also means malaria resurgence is likely (PAHO, 2017; Sáenz et al., 2017).

Data Sources

Monthly counts of blood smear confirmed cases of *P. falciparum* and *P. vivax* malaria for each canton in El Oro, January 1990–December 2018, were provided by the Ecuadorian Ministry of Health, where malaria is a mandatory notifiable disease. Cases were recorded at local clinics across El Oro and collated by the Ministry of Health. Population data, available for each canton, were sourced from the national census in Ecuador, from the Instituto Nacional de Estadística y Censos (INEC¹) for 1990, 2001, and 2010. Data for 2011–2018 were provided by INEC as annual population projections. Population values between census years 1990, 2001, and 2010 were estimated by interpolating assuming linear growth, to provide annual population estimates for each canton. To estimate the proportion of the population in poverty per canton in El Oro, we used Unmet Basic Needs (UBN) sourced from the 2010 census, an indicator based on measures including housing quality, education and access to water and sanitation. We used UBN data from 2010 as a socioeconomic covariate in all our models.

Between 2001 and 2015 an intensive period of vector control was carried out across El Oro following a resurgence of malaria cases and monthly canton-level data for three control measures were available from the Ministry of Health. Monthly estimates per canton of the number of households treated by IRS with insecticides (deltamethrin 5% concentrated suspension, deltamethrin 2.5%, malathion 50%, alphacypermethrin 10% concentrated suspension, and betacypermethrin 2.5%) were available for January 2001–September 2013. The number of neighborhoods that were treated with insecticide via ultra-low volume (ULV) fumigation, which is performed by spraying

¹<http://www.ecuadorencifras.gob.ec/institucional/home/>



entire neighborhoods with 96% malathion from trucks were available between January 2004 and May 2015. Finally, the number of households space-sprayed with 2.5% deltamethrin concentrated emulsion, using a backpack fogger that creates a fog insecticide to treat both inside and outside the home, were available between January 2004 and May 2015 (**Supplementary Figure S1**). For each control measure we tested for lagged relationships, up to 3 months, in order to account for delays between implementation and impact on measurable malaria cases (**Supplementary Table S1**).

Other malaria control interventions including fumigation with DDT (until banned in 1996), elimination of larval habitats, and bed net provision occurred during the study period, but no detailed data for control measures were available prior to 2001.

Monthly climate data, for temperature and precipitation, between January 1990 and December 2018 were sourced from TerraClimate, a high-spatial resolution (~4 km) dataset that uses climatically aided interpolation to produce monthly estimates (Abatzoglou et al., 2018). To obtain climate estimates for each

canton in El Oro, TerraClimate variables were aggregated by taking mean grid cell values using the *raster* package in R.

Annual land cover maps at a 300 m spatial resolution generated by the European Space Agency (ESA) Climate Change Initiative (CCI) were compiled for 1990–2018² (European Space Agency, 2019). For each annual map, the number of grid cells in each canton that were classified in the urban category were extracted, and from this the proportion of urban grid cells per canton were calculated to give a continuous variable of urbanization (**Supplementary Figure S3**). All continuous variables were scaled by subtracting the variable mean from each value and dividing this by the standard deviation. This transformation allowed for the direct comparison of continuous variables and enabled their relative importance to be determined in the model.

Statistical Analysis

Bayesian hierarchical mixed models are powerful statistical models that can be used to estimate the marginal posterior distributions of multiple covariates whilst simultaneously accounting for multiple sources of uncertainty that can arise from disease count data. Here, spatiotemporal models were constructed, for each malaria parasite, *P. falciparum* and *P. vivax*, to explore the contributions of climate and control interventions to variations in monthly malaria incidence in El Oro between January 1990 and December 2018 (348 months).

We fitted zero-inflated negative binomial models that take into account excess zeros that cannot be explained by a standard negative binomial distribution (**Supplementary Appendix S1.1**). Where μ_{st} is the mean number of malaria cases in each canton ($s = 1, \dots, 14$) for each timestep ($t = 1, \dots, 348$), the hierarchical spatiotemporal model is defined as:

$$\log(\mu_{st}) = \log(P_{st}) + \log(\rho_{st})$$

Annual population estimates per 1,000 inhabitants for each canton in El Oro, $\log(P_{st})$, were included as an offset in the model to account for canton-level differences in the annual parasite incidence (API). The estimated API, $\log(\rho_{st})$ is then made up of a combination of climate covariates, including mean monthly minimum temperature and precipitation for each canton. Other explanatory variables considered included socioeconomic factors (poverty rates), level of urbanization and vector control interventions (IRS, ULV fumigation and space-spraying).

To account for the seasonality in malaria incidence, a monthly random effect was introduced using a first order autoregressive prior (m_t), which allows malaria in 1 month to depend on incidence in the previous month. Independent random effects for each year (y_t), 1990–2018, were included to allow for additional sources of variation due to unobserved confounding factors such as variations in healthcare access, case reporting and diagnostics.

Spatially structured random effects, v_s , were introduced into the model to allow for correlated heterogeneity in malaria incidence across cantons in El Oro. This spatial dependency

structure was accounted for by assuming a conditional intrinsic Gaussian autoregressive (CAR) model prior distribution for the spatial effects, which takes into account the neighborhood structure of the area (Besag et al., 1991). We also included spatially unstructured random effects in the model to allow for additional uncorrelated spatial variation across cantons, v_s , which were assigned independent diffuse Gaussian exchangeable prior distributions (Lowe et al., 2016). Separate models were constructed for both *P. falciparum* and *P. vivax* malaria due to their intrinsic differences, which include diagnostic potential, development time inside the mosquito vector (extrinsic incubation period, EIP), the differential impact of vector control on transmission, infection reservoirs of *P. vivax* hypnozoites and the presence of asymptomatic cases.

Detailed data on interventions was only available for the period January 2001–December 2015. Therefore, we formulated separate sub-models for each malaria type to investigate the relative impact of the control measures implemented between 2001 and 2015 (see **Supplementary Appendix S2**), herein referred to as “intervention models.” The intervention models, fitted to data for the period 2001–2015 included the same explanatory variables and random effects as the “full” models (fitted to data for the period 1990–2018) with the addition of the three control measures. We examined differences between the random effects structures for the full models and the intervention models, to see how much variation in malaria due to interventions could be accounted for by random effects in the absence of intervention data for the entire time period.

Posterior distributions of model parameters were estimated using Integrated Nested Laplace approximations (INLA) (Rue et al., 2009). INLA provides a computationally more efficient alternative to Markov Chain Monte Carlo (MCMC) methods, by using numerical approximations of model parameters (see **Supplementary Appendix S1.2**). Covariate time lags and the most parsimonious models were selected using the deviance information criterion (DIC) (Spiegelhalter et al., 2002) and the Watanabe–Akaike information criterion (WAIC) (Watanabe, 2010), which are Bayesian methods of model comparison that trade off model adequacy against model complexity. Models with lower DIC and WAIC indicate a more parsimonious model. We also used the logarithmic score to assess model fit, which is based on the conditional predictive ordinate (CPO) leave-one-out cross-validation score, where a smaller value indicates a greater predictive power of the model (Gneiting and Raftery, 2007).

Covariates were added iteratively to the models starting with a baseline model, which included spatial and temporal structured and unstructured random effects and retained if model fit was improved, assessed through a decrease in DIC and WAIC. The most appropriate temperature variable, maximum or minimum temperature was selected using DIC and WAIC (**Supplementary Table S2**). We tested different time lags (from 0 to 3 months) to take into account the lagged effect of climate factors on malaria transmission, including development time of mosquitoes and parasites as well as the time between malaria diagnosis and case recording (**Supplementary Table S2**). We also tested non-linear functions of temperature and precipitation (**Supplementary**

²<https://www.esa-landcover-cci.org/>

Table S3). We used the root mean square error (RMSE) to assess the extent to which models of malaria incidence for 2001–2015 were improved by the inclusion of each control measure in turn. RMSE is a measure of the standard deviation of the model residuals, with smaller values indicating better model fit to the observed data.

RESULTS

Urban and Rural Malaria Incidence in El Oro

Between 1990 and 2018, 62,120 cases of malaria were recorded in El Oro, with 54% of infections resulting from *P. falciparum* malaria and 46% from *P. vivax*. Malaria transmission in El Oro was historically high, reaching up to 2,000 total annual cases in 1999 and an API of 61 cases of *P. falciparum* and 35 *P. vivax* cases per 1,000 people recorded in Huaquillas canton along the Peruvian border. Between 1998 and 2002 large outbreaks, particularly of *P. falciparum* malaria occurred across the province before declining to low levels of incidence after the period of more intensive vector control (**Figure 1B**). A small outbreak of *P. vivax* malaria occurred between 2007 and 2009 before malaria incidence was dramatically reduced and remained at a low and stable level (**Figure 1B**). The highest malaria incidence was concentrated in the western part of the province, along the Peru-Ecuador border (**Supplementary Figure S4**).

Between 1990 and 2018, outbreaks of both *P. falciparum* and *P. vivax* malaria occurred roughly at the same time. Prior to the decline in cases in 2001–2015 there was a greater difference between *P. falciparum* and *P. vivax* incidence in rural areas, compared to urbanized areas (**Supplementary Figure S5**). During the elimination phase (2001–2010) the incidence of *P. falciparum* and *P. vivax* malaria in rural areas was reduced much more than malaria in urbanized areas, where outbreaks of *P. vivax* were still observed. There was also a greater overall reduction of *P. falciparum* malaria in rural and urbanized areas.

In the models of malaria incidence in El Oro between 1990 and 2018, we found that areas that were more urbanized were associated with greater incidences of *P. falciparum* malaria (**Figure 2A**). To examine how the epidemiology of malaria in El Oro had changed with the three vector control interventions, we tested whether there was a difference in the relationship between malaria and level of urbanization in El Oro, before and after the interventions were implemented. We found a statistically significant interaction between level of urbanization in El Oro and the period the control measures were implemented (2001–2015) for *P. vivax* malaria and no significant interaction for *P. falciparum* malaria (**Supplementary Figure S6**). Prior to 2001, more urbanized areas of El Oro were associated with decreased *P. vivax* malaria and after 2001 this relationship reversed with urbanized areas associated with more *P. vivax* malaria.

During the period of malaria decline (post 2001) there was also a reduction in the seasonal pattern of both *P. falciparum* and *P. vivax* malaria in El Oro especially for *P. vivax* malaria incidence, which ceased to peak between June and July (**Supplementary Figure S7**). For *P. falciparum* a seasonal peak in

incidence during this period was evident between June and July, but less distinct than before 2001.

Climate Variability and Malaria Incidence in El Oro 1990–2018

Minimum temperature was the best temperature variable in the full models (**Supplementary Table S2**) and in the 3 months prior to case reporting was an important predictor of the spatio-temporal distribution of *P. falciparum* and *P. vivax* malaria in El Oro (**Figure 2A**). Warmer temperatures in El Oro between 1990 and 2018 were associated with increases in malaria incidence and greater increases in *P. falciparum* malaria than *P. vivax* malaria (**Figure 2A**). Precipitation lagged by 3 months for *P. falciparum* and 1 month for *P. vivax* were selected as the best time lags although when included in the full model, precipitation was not significant in driving malaria incidence in El Oro (**Figure 2A**). Introducing a non-linear relationship between malaria and climatic variables improved model fit by decreasing model DIC and RMSE for *P. vivax* but not *P. falciparum* malaria incidence (**Table 1** and **Supplementary Figure S8**). We also found that higher temperatures were associated with larger increases in malaria incidence (**Figure 2B**).

We also investigated the influence of minimum temperature on the unexplained variation in *P. falciparum* and *P. vivax* incidence in El Oro between 1990 and 2018. We compared the monthly and interannual random effects of models with and without temperature to determine the extent to which temperature accounted for the seasonal pattern of malaria incidence. The monthly random effects of the model for *P. falciparum* incidence that included minimum temperature were near zero, showing that temperature accounted for all of the seasonal variation in *P. falciparum* malaria in El Oro 1990–2018 (**Figure 3A**). In contrast there was minimal reduction in the random effects for the *P. vivax* model with minimum temperature, indicating the seasonal pattern was driven by other factors (**Figure 3B**). The proportion of the variation accounted for by the yearly random effects in a model without minimum temperature was greater than the proportion of the variation accounted for by the monthly random effects in a model without minimum temperature. This suggests that temperature is the main driver of malaria seasonality and other interannual signatures as well as temperature contribute to the interannual variability of malaria in El Oro. For example, for some years the proportion of variation accounted for by the yearly random effects decreased when minimum temperature was included into models of malaria incidence 1990–2018. A reduction in the value of the random effects can be seen for 1998, when a strong El Niño event occurred (**Supplementary Figure S9**). Reductions were also particularly apparent for *P. falciparum* malaria between 2001–2004 and 2009–2011, suggesting that some additional variation was explained by minimum temperature. In contrast for some years there was no reduction for example in 2008, when an outbreak of *P. vivax* malaria occurred suggesting the outbreak was not driven by a climate event.

We explored how climate suitability for malaria transmission had changed over the time period 1990–2018 in El Oro,

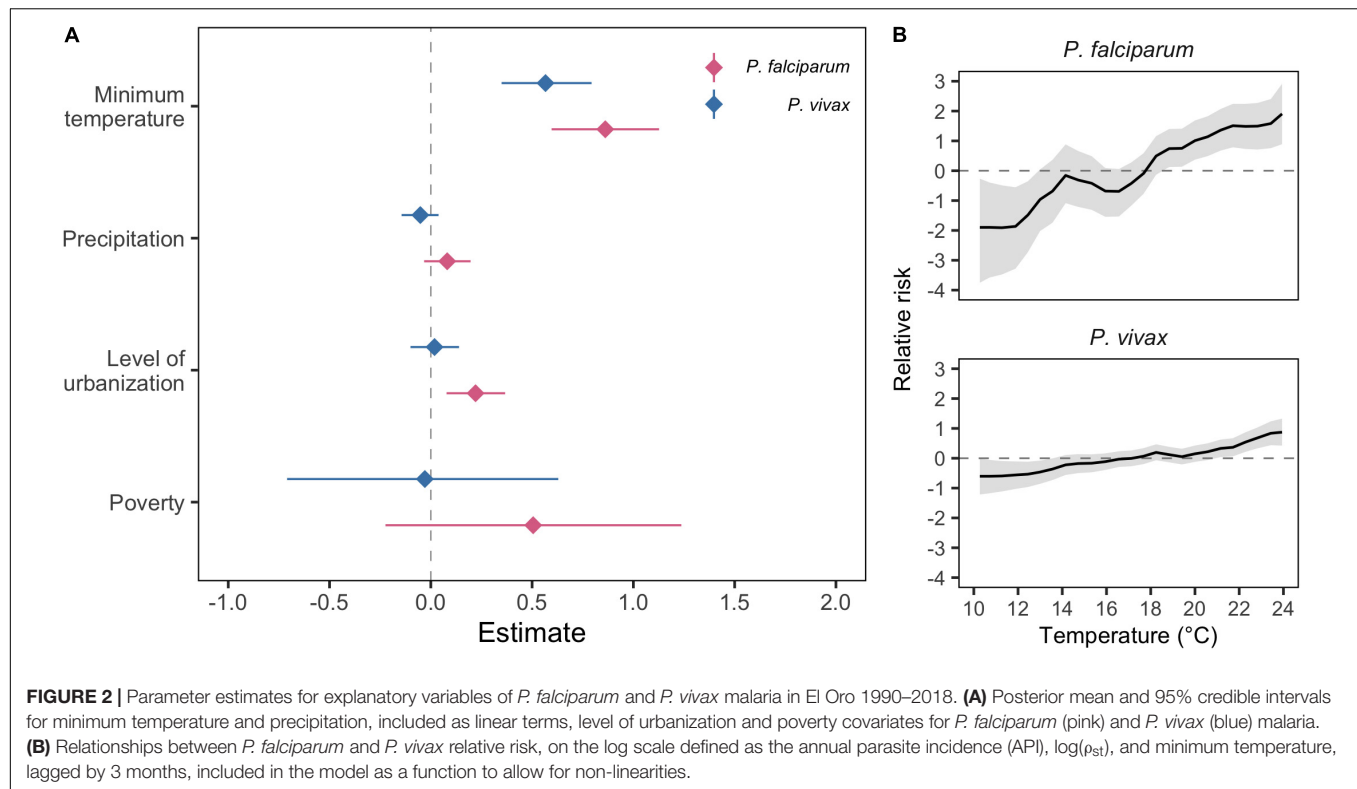


TABLE 1 | Adequacy results, deviance information criterion (DIC), Watanabe–Akaike Information Criterion (WAIC) and cross-validated log score for full models of *P. falciparum* and *P. vivax* malaria in El Oro 1990–2018.

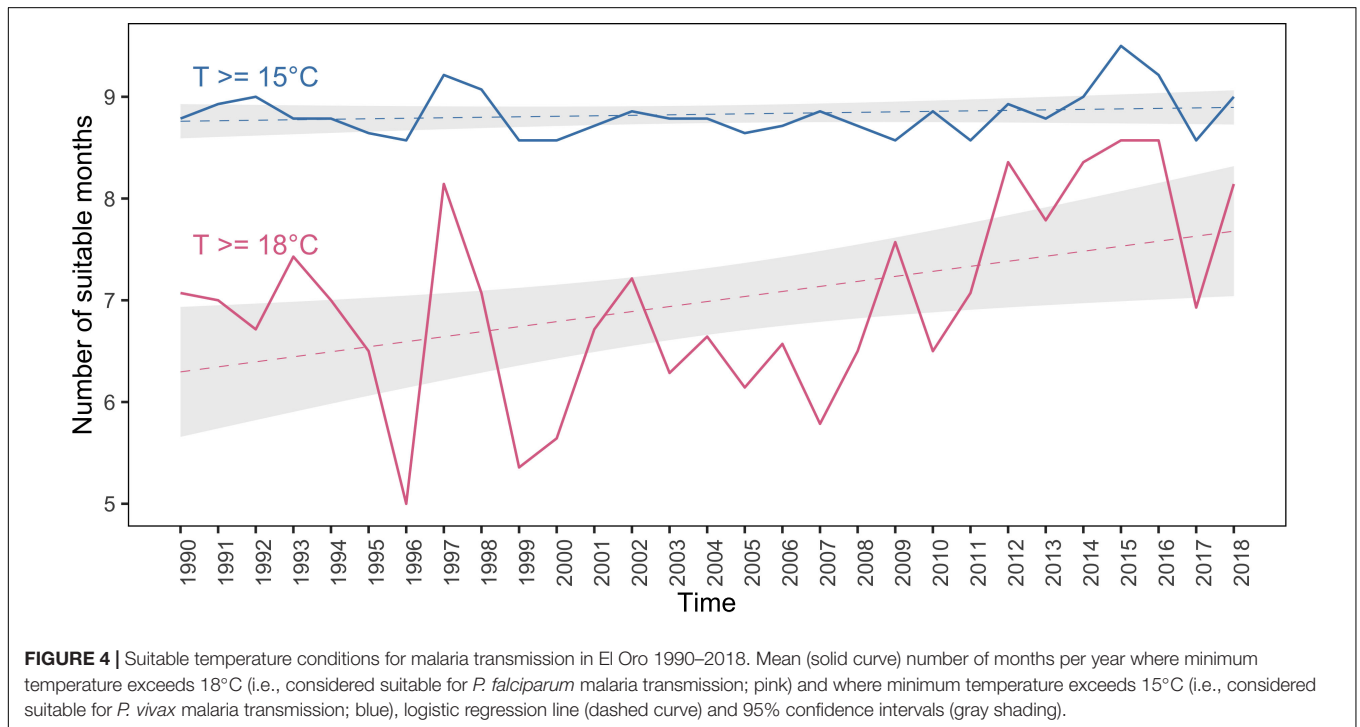
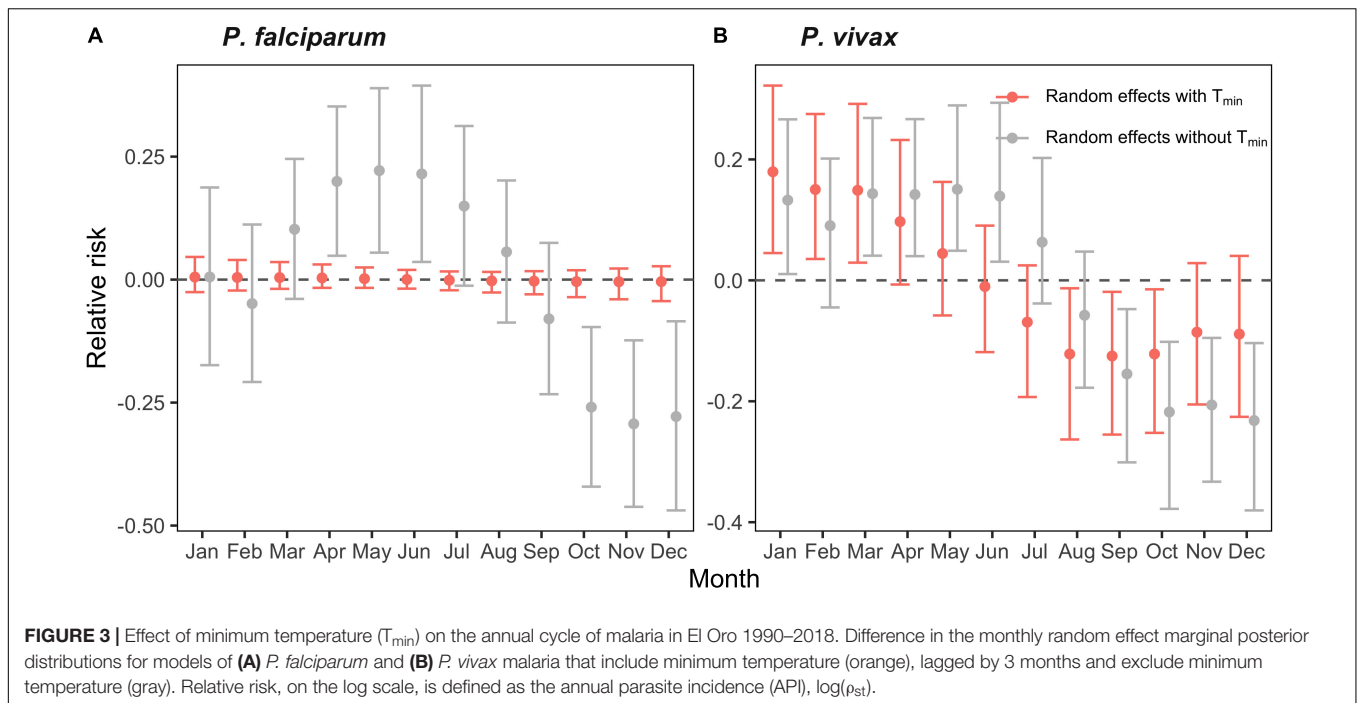
Model	Parasite	DIC	WAIC	Log score
Baseline spatial seasonal $\log(\rho_{st}) = \nu_s + \nu_t + m_t$	<i>P. falciparum</i>	12640.97	12646.73	1.38
	<i>P. vivax</i>	18745.44	18743.44	2.05
Unstructured yearly random effects $\log(\rho_{st}) = \nu_s + \nu_t + m_t + y_t$	<i>P. falciparum</i>	11947.63	11970.4	1.31
	<i>P. vivax</i>	17887.75	17894.17	1.96
Socioeconomic effects $\log(\rho_{st}) = \nu_s + \nu_t + m_t + y_t + x_{1s}$	<i>P. falciparum</i>	11946.75	11970.10	1.31
	<i>P. vivax</i>	17887.61	17893.23	1.96
Urban effects $\log(\rho_{st}) = \nu_s + \nu_t + m_t + y_t + x_{1s} + x_{2st}z_i$	<i>P. falciparum</i>	11935.89	11958.12	1.31
	<i>P. vivax</i>	17823.38	17831.99	1.95
Temperature effects $\log(\rho_{st}) = \nu_s + \nu_t + m_t + y_t + x_{1s} + x_{2st}z_i + x_{3st}$ (linear) $\log(\rho_{st}) = \nu_s + \nu_t + m_t + y_t + x_{1s} + x_{2st}z_i + f(x_{3st})$ (non-linear)	<i>P. falciparum</i>	11926.14	11941.98	1.31
	<i>P. vivax</i>	17797.49	17806.2	1.95
Precipitation effects $\log(\rho_{st}) = \nu_s + \nu_t + m_t + y_t + x_{1s} + x_{2st}z_i + x_{3st} + x_{4st}$ (linear) $\log(\rho_{st}) = \nu_s + \nu_t + m_t + y_t + x_{1s} + x_{2st}z_i + f(x_{3st}) + f(x_{4st})$ (non-linear)	<i>P. falciparum</i>	11924.91	11941.77	1.31
	<i>P. vivax</i>	17795.36	17804.2	1.95

Covariates were added iteratively, starting with a baseline model that included structured and unstructured spatial random effects, and a seasonal term. The level of urbanization was interacted with a categorical variable indicating a decline in malaria incidence post 2001. The most parsimonious models included climate (temperature and precipitation) as linear terms for *P. falciparum* and as non-linear functions for *P. vivax* malaria.

specifically for minimum temperature since it was an important predictor. To do this, we calculated the number of months that had suitable temperature conditions for transmission of each malaria parasite. We used the lower temperature limits of the range for which the development of the malaria parasites inside

the mosquito vector is considered most suitable for transmission, as an indicator of this suitability.

For *P. falciparum* a lower temperature limit of 18°C was used and for *P. vivax* 15°C (Gilles, 1999; Watts et al., 2019). Between 1990 and 2018, the number of months with



suitable temperatures for *P. falciparum* transmission showed an increasing trend (Figure 4), with noticeable peaks of around 8 months of suitable temperature conditions occurring between 1997 and 1998 and between 2014 and 2016. This trend of increasing temperatures for *P. falciparum* transmission coincides with warmer temperatures throughout the year in El Oro and increasing temperatures between 1990 and

2018 (Supplementary Figure S10). In contrast, temperature conditions for *P. vivax* malaria transmission showed no increasing trend in El Oro between 1990 and 2018 (Figure 4), although similar peaks in suitability were observed in 1997–1998 and in 2015–2016.

The most parsimonious models of malaria in El Oro 1990–2018 included minimum temperature and precipitation as

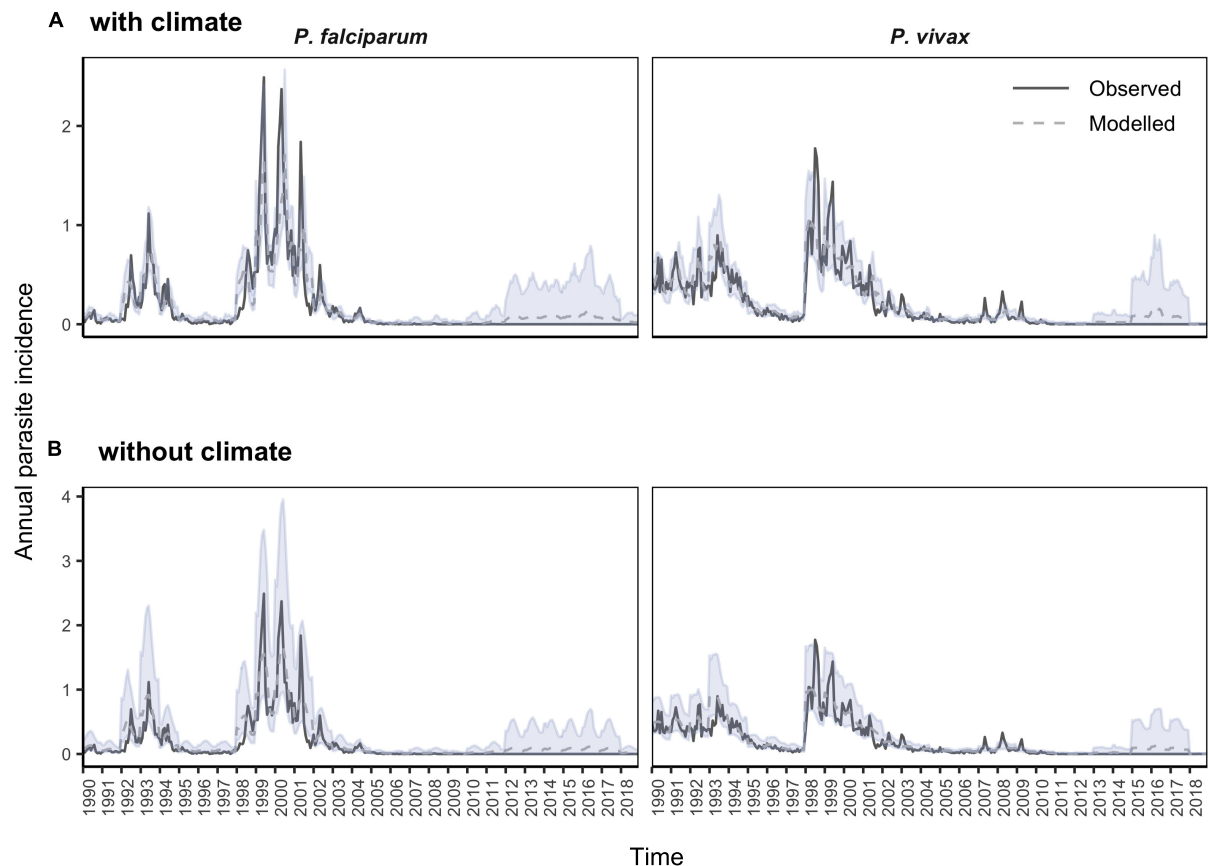


FIGURE 5 | Model posterior distributions with and without climate information for *P. falciparum* and *P. vivax* malaria in El Oro 1990–2018. Observed (gray solid line), posterior mean (blue dashed line) and 95% credible intervals (blue shading) for annual parasite incidence (API) for (A) models that include minimum temperature, lagged by 3 months and precipitation, lagged by 3 months for *P. falciparum* models and 1 month for *P. vivax* models and (B) without climate information. For models of *P. falciparum* malaria minimum temperature and precipitation were included as linear terms. For *P. vivax* malaria models, model fit improved when including non-linear functions of minimum temperature and precipitation.

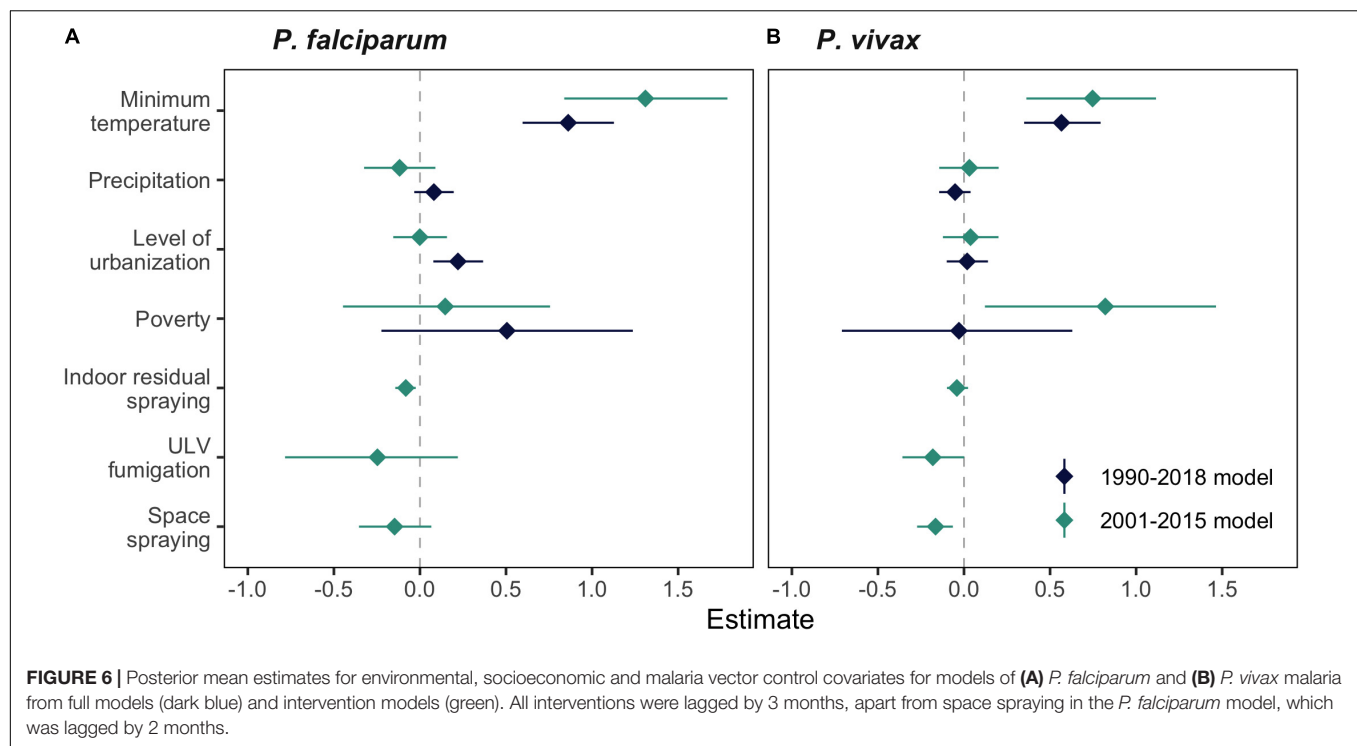
linear terms for *P. falciparum* and as non-linear functions for *P. vivax* malaria, poverty rates, level of urbanization including an interaction with the period when the vector control measures were implemented, and spatial and temporal random effects (Table 1). Although in these models the credible intervals for precipitation and poverty rates contained zero, the addition of these covariates to the models decreased DIC values and increased model fit for *P. falciparum* and *P. vivax* malaria incidence in El Oro between 1990 and 2018. Accounting for poverty rates (socioeconomic effects) across El Oro also decreased DIC and WAIC for models of *P. falciparum* and *P. vivax* malaria. Including meteorological variables (as linear terms for *P. falciparum* and as non-linear functions for *P. vivax* malaria) decreased the uncertainty of the model posterior distributions, in comparison to distributions from models that excluded meteorological variables (Figure 5). In particular, model uncertainty was reduced more for *P. falciparum* malaria, especially during the large outbreaks that occurred in 1992–1994 and 1998–2002. Interestingly during the later years of the study period, model posterior distributions show an upsurge in malaria incidence, particularly for *P. vivax* incidence

between 2015 and 2018 although this upsurge was not observed in case reports.

Control Interventions and Malaria Incidence in El Oro 2001–2015

We fitted separate sub-models for incidence of *P. falciparum* and *P. vivax* malaria in El Oro between 2001 and 2015, to evaluate the impact of the three vector control measures (IRS, space spraying and ULV fumigation) that were implemented during this period. These intervention models included the same covariates as the full models (linear and non-linear climate information and socioeconomic effects) and random effects but with the addition of the intervention data that were available for 2001–2015.

By comparing the differences in the covariate parameter estimates from the full models for the entire time series 1990–2018 and the intervention models for the period 2001–2015, we found that posterior mean estimates were roughly similar across the different models (Figure 6 and Supplementary Tables S4, S5). However, there was more uncertainty in the



estimates for the environmental covariates in the intervention models compared to the 1990–2018 models, particularly for minimum temperature. We also found that between 2001 and 2015 areas in El Oro with greater levels of poverty were associated with higher incidences of *P. vivax* malaria. In the intervention models, IRS implemented in El Oro 2001–2015, 3 months prior to case detection was associated with decreased incidence of *P. falciparum* malaria but was not associated with significant decreases in incidence of *P. vivax* malaria. Space spraying was associated with decreased *P. vivax* malaria incidence in the following 3 months but not *P. falciparum* malaria in the following 2 months. ULV fumigation, also lagged by 3 months was not statistically significant (i.e., the credible intervals contained zero).

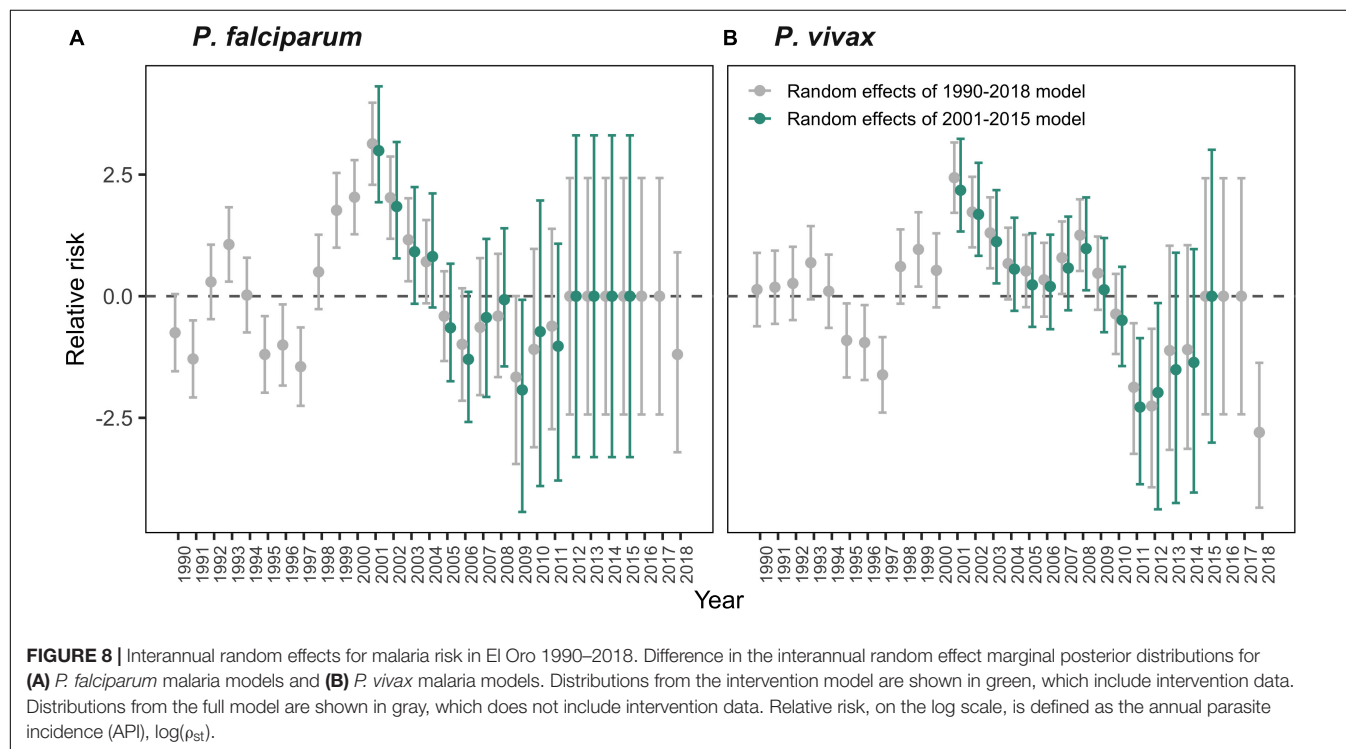
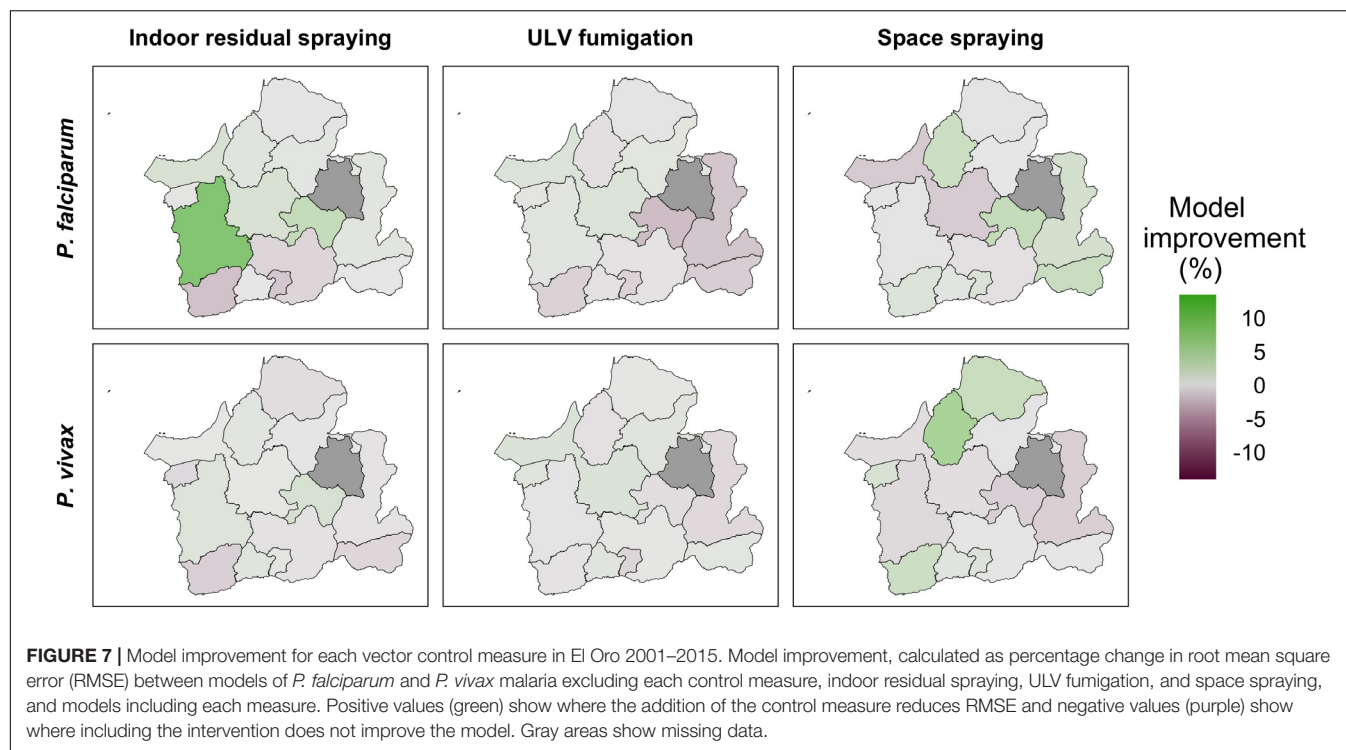
We also assessed which vector control measure implemented in El Oro between 2001 and 2015 provided more valuable information to the intervention models in explaining malaria incidence across the province. We found there was considerable heterogeneity in the model improvement for each control measure, as measured by RMSE difference (Figure 7). IRS improved the model for *P. falciparum* malaria in the coastal northwest of El Oro and in cantons along the Ecuador-Peru border, improving the model by up to 14%. In contrast, there was little improvement in model fit for *P. vivax*. Space spraying improved models of both *P. falciparum* and *P. vivax* in the province capital, Machala, with a greater improvement for *P. vivax*. ULV fumigation provided minimal or no model improvement for either parasite.

Finally, we explored if the variation in malaria incidence in El Oro attributed to the vector control measures in the intervention model could be captured in the random effects structure of the

full models. We compared the yearly random effects of the full models (fitted to data from 1990 to 2018), which did not include intervention data, to the yearly random effects of the intervention models (fitted to data from 2001 to 2015). We found that for some years (2001–2008) there was a reduction in the magnitude of the random effects for the 2001–2015 models, suggesting the control measures accounted for some of the unexplained variation in malaria incidence in the model for 1990–2018 during those years. However, a considerable portion of the random variation was likely due to other unmeasured factors. Between 2012 and 2015 the random effects of the *P. falciparum* models are close to zero, indicating that variation in malaria incidence for those years was well captured by factors included in the model (Figure 8). We found a similar pattern for *P. vivax* malaria, with a reduction in the value of the random effects for the intervention model, compared to the full model during the early years of the interventions 2001–2009. This reduction indicates that the control measures influenced the interannual variation of malaria risk during these years.

DISCUSSION

For regions approaching malaria elimination and where funding and disease surveillance are limited, it is important to understand the drivers of malaria incidence in order to prevent disease re-establishment and to ensure that elimination efforts are sustained. In this study, we developed a statistical modeling framework to disentangle the relative role of multiple explanatory variables in driving variation in malaria incidence, while accounting for unobserved heterogeneity. We found that minimum temperature



was an important driver of malaria incidence in El Oro. We also found that control measures had a differential impact on the two types of malaria. After the intensive period of vector control in El Oro between 2001 and 2015 malaria incidence in rural areas declined more than malaria in urbanized areas and *P. vivax*

malaria became more dominant. Incidence of *P. falciparum* malaria was also reduced more than *P. vivax* malaria and relatively more *P. vivax* cases were reported in urbanized areas during this time period. The greater reduction in *P. falciparum* malaria is expected given that malaria control measures deployed

during this time, are primarily designed for use in rural settings and target mosquito vectors that are exophagic such as *An. albimanus*, a dominant malaria vector in El Oro.

Indoor residual spraying has largely been shown to be effective in reducing malaria transmission in settings with high prevalence and when applied on a large scale, especially in African countries (Pluess et al., 2010). In this study we found that IRS was effective at reducing the incidence of *P. falciparum* malaria in El Oro between 2001 and 2015, but not *P. vivax* malaria. Conventional control methods are less effective in reducing transmission of *P. vivax* compared to *P. falciparum* malaria because of the ability of *P. vivax* to cause multiple relapsing malaria episodes after the initial infection, from the activation of dormant liver stages (hypnozoites). In addition the *P. vivax* parasite develops more rapidly in the mosquito than *P. falciparum* and so control methods such as IRS deployed in El Oro, which aim to shorten the mosquito lifespan are less effective (Mendis et al., 2001; Bassat et al., 2016). The rapid development of *P. vivax* and hypnozoite stage also means that drug treatments, which are often of a longer duration are less effective in reducing transmission as mosquitoes can become infected during a pre-symptomatic period, allowing for onward transmission before drug treatment is initiated (White, 2008; McCarthy et al., 2013).

We found that IRS only reduced the incidence of *P. falciparum* malaria and in the west of the province. IRS is most effective against endophilic mosquitoes, which rest indoors following a blood meal. The two main malaria vectors in El Oro, *A. albimanus* and *A. punctimacula*, are most commonly observed biting outdoors and mainly rest outdoors (Ryan et al., 2017). As a result, IRS and other conventional control methods may not be suitable for reducing malaria transmitted by these mosquitoes.

In contrast we found that space spraying was effective in reducing incidence of *P. vivax* in El Oro, especially in the capital, Machala and by a larger amount than IRS. Evidence for the success of space spraying is limited and it is currently only recommended during outbreaks. In our study, space-spraying is possibly targeting outdoor resting mosquitoes, such as *A. albimanus*, especially in peri-urban areas where housing conditions are poor and outdoor exposure is higher. There is evidence of a positive effect of space-spraying on malaria incidence in India, but the current evidence base is limited (Pryce et al., 2018).

The *P. vivax* malaria parasite is able to develop at lower temperatures than *P. falciparum* (Nikolaev, 1935; Moshkovsky, 1946; Oliaro et al., 2016; Ohm et al., 2018), enabling its persistence in less favorable environmental conditions (Mendis et al., 2001). We found a stronger association between minimum temperature and *P. falciparum* malaria than *P. vivax*, and all of the seasonality in *P. falciparum* malaria was explained by temperature. A greater association between minimum temperature and *P. falciparum* malaria compared to *P. vivax* has been previously identified in China (Bi et al., 2013), but to our knowledge this difference has not been previously quantified in South America. The stronger effect of temperature on *P. falciparum* malaria is due to the greater dependence of *P. falciparum* transmission on the mosquito vector, which is sensitive to climate conditions such as temperature. In contrast

P. vivax transmission is less influenced by the mosquito vector and can be sustained by unpredictable relapsing infections, which have been suggested to be explained by other factors such as systemic illness in humans (White, 2011). Similarly, between 2001 and 2015 we found that areas in El Oro with higher levels of poverty were associated with increased incidences of *P. vivax* malaria, which suggests that socioeconomic conditions, such as limited access to healthcare, may have influenced relapsing *P. vivax* infections during this time.

Warmer minimum temperatures in El Oro were associated with increases in *P. falciparum* and *P. vivax* malaria incidence 3 months later. This finding is in agreement with other studies in South America that have found a similar relationship between higher temperatures and malaria incidence (Poveda et al., 2000; Basurko et al., 2011; Laneri et al., 2019). This relationship can be attributed to the physiological effects of temperature on both the mosquito vector and parasite that increases transmission. For example warmer temperatures shorten the development time of the parasite inside the mosquito, the EIP and increase mosquito larval reproduction thus increasing disease transmission (Bayoh and Lindsay, 2003; Blanford et al., 2013; Mordecai et al., 2013). We also found a trend of rising minimum temperatures in El Oro between 1990 and 2018, with an increase in the number of months that have suitable minimum temperature conditions for *P. falciparum* malaria transmission, which suggests a lengthening of the malaria transmission season. Large peaks in the transmission suitability of both *P. falciparum* and *P. vivax* malaria are evident between 1997–1998 and 2014–2016, when major El Niño events occurred. El Niño conditions bring warmer and wetter conditions to southern Ecuador, conditions that are favorable for malaria transmission and have previously been suggested to have caused a peak in cases observed during this time (Krisher et al., 2016). Increasingly suitable temperature conditions and large-scale climate events such as El Niño pose a real threat for the re-establishment of malaria in El Oro if cases are allowed to return, surveillance is not maintained, and interventions are not deployed appropriately. Our model posterior distributions for 2012–2018 show increases in malaria incidence despite zero or few cases being reported, which is possibly due to warmer temperatures and the large El Niño event that occurred during this time. This highlights how incidence could rise if risks, such as relaxing control efforts are not mitigated.

Rainfall is essential for providing suitable habitats for mosquito breeding (Thomson et al., 2005; Parham and Michael, 2010) and is thus considered to be a dominant factor in driving malaria transmission. Studies in the Amazon and Argentina have found that rainfall was important in determining malaria incidence (Dantur Juri et al., 2009; Olson et al., 2009). In this study, rainfall was not a statistically significant explanatory variable for malaria incidence in El Oro, possibly because ample larval habitat was already available. Two to three months prior to the peak malaria season (March–June), monthly rainfall accumulation reaches 80 mm across El Oro, which is considered to be suitable conditions for malaria transmission (Watts et al., 2019), and up to 530 mm rainfall in the southeast of the province. In addition, much of El Oro is rural with extensive mangroves

in the northwest coastal regions, which provide habitat suited to *A. albimanus* mosquitoes (Pinault and Hunter, 2012). Therefore, our results suggest that rainfall is not a limiting factor for malaria transmission in El Oro.

The addition of temporal random effects for each year in the full models for 1990–2018 accounted for some of the variation in malaria incidence due to the control measures that were explicitly accounted for in the intervention model for 2001–2015, particularly for *P. falciparum* malaria, which appeared more sensitive to interventions. The use of temporal random effects also allowed us to pick up variation in malaria in El Oro due to other unobserved factors. For example, the large El Niño events mentioned previously likely caused an increased number of cases and a reduction in the unexplained yearly variation during 1997–1998 and between 2014 and 2016. However, we expect that the temperature and precipitation variables included in the models to pick up some variation to due climatic anomalies from El Niño events. The Cenepa War, a period of political instability across the Ecuador-Peru border (Krisher et al., 2016) likely hindered malaria control efforts and case reporting during 1995, and there is evidence of reduced malaria risk during this time in the interannual random effects, which is likely explained by reduced reporting. In addition we found that the outbreak of *P. vivax* in 2008 was not driven by a climatic event and may instead be driven by a political event or lapse in control efforts.

Despite the considerable length of the dataset, there are some limitations to consider. In Ecuador a high number of malaria cases are asymptomatic. Low levels of parasitemia, which act as a reservoir of transmission, are difficult to diagnose (Sáenz et al., 2017). It is likely that many cases of *P. vivax* malaria were missed and not reported during the study period. In addition, many *P. vivax* cases reported between 1990 and 2018 are likely to be relapses from the same initial infection, which could mask the true climate-malaria relationship. We also found that urban malaria was approximately 70% higher than incidence of malaria in rural areas. However, this may be a product of migration from neighboring countries and cases being imported from rural areas and subsequently recorded as originating in urban areas, as was found in Colombia (Molina Gómez et al., 2017). We could not quantify the amount of malaria variation explained by migration owing to the lack of detailed migration data for the whole time period. Due to El Oro's location on a key migratory route, including for Venezuelan refugees, it is likely that human movements are influential in driving case reporting and malaria incidence. In addition, outbreaks in northern Peru can increase the risk of malaria transmission in El Oro due to cross border human movement. Other factors that likely contributed to the spatiotemporal variation in malaria incidence in El Oro that were accounted for through the use of random effects include variations in case reporting and surveillance, as well as health seeking behavior in El Oro.

The incompleteness of the intervention data for the study period is also a limiting factor in this study. We were only able to evaluate the role of the three control measures implemented

between 2001 and 2015 although there were other important elimination efforts that were carried out in El Oro between 1990 and 2018. For example, the use of DDT was widely used until 1996 and a campaign to distribute insecticide-treated bed nets (ITNs) was initiated in 2004, although no detailed data exist for this in El Oro, or for other forms of personal protection that would have contributed to local elimination. However, by examining the interannual random effects of our models we observe a reduction in *P. falciparum* malaria risk between 2004 and 2006, which could be attributed to the use of ITNs. In 2005, the Ministry of Health suspended the use of chloroquine for treatment of *P. falciparum* malaria, adopting the recommended change to artemisinin-based combination therapy (ACT) amid reports of drug resistance, which local partners reported led to a major decline in transmission (Krisher et al., 2016). The introduction of ACT in Colombia and Peru at similar times contributed to significant reductions in malaria case numbers (Rodríguez et al., 2011; Quispe et al., 2016). ACT introduction is also likely to have contributed to local malaria elimination El Oro as we observed declining malaria risk from this time through the use of interannual random effects in our models. At the same time in El Oro, a new shorter treatment regime for *P. vivax* malaria was adopted to ensure patients completed their treatment course, which was also reported to have contributed to declining transmission (Krisher et al., 2016).

In summary, we have developed a modeling framework for exploring the barriers to malaria elimination in a low-transmission setting. Accounting for unobserved interannual variation via yearly random effects is useful for investigating malaria variation in many different contexts where detailed data, such as intervention information, may not be available. Here, we used available intervention data to explore the relative impact of three vector control measures on *P. falciparum* and *P. vivax* malaria in El Oro. We also provide an assessment of where control measures improved models of *P. falciparum* and *P. vivax* incidence, which will prove useful for targeting future malaria control efforts in the region. We have shown that warmer temperatures increase incidence of *P. falciparum* malaria, which is important to consider in light of global environmental change and increasing climate suitability for malaria transmission (Laporta et al., 2015; Watts et al., 2019). In addition, with a global warming of 1.5°C above pre-industrial levels it is predicted that the western coast of South America, including southern Ecuador, will experience temperatures between 2 and 3°C warmer and precipitation could increase by up to 20% (IPCC, 2018), providing climate conditions that are highly suitable for malaria transmission. In contrast, we show that *P. vivax* malaria is less sensitive to temperature variations and shows more complex transmission patterns, making it particularly challenging to eliminate (Feachem et al., 2010). An assessment of the environmental obstacles to elimination efforts in Ecuador is timely and important. Lapses in control efforts should be avoided, especially as it becomes harder to prevent and detect cases due to limited funding for surveillance and control. Recently, indigenous cases of malaria have been detected in El Oro and re-introduction of malaria parasites along with warming temperatures threaten current elimination progress.

DATA AVAILABILITY STATEMENT

The data and code used to produce the analysis are available on GitHub via the following link: https://github.com/isabelfletcher/malaria_eloro.

AUTHOR CONTRIBUTIONS

IF and RL conceived the study. IF collated the data, fitted the models, analyzed the results, and wrote the manuscript. AS-I helped conceive the study, provided information on the vector control measures, and contributed to discussion of the study results. RS provided insight on vector-borne diseases and surveillance in El Oro. GC-E helped with the environmental data sources and extraction. MS, EB-A, TO, and JA provided the malaria case data and intervention data for analysis. FS provided insight on the malaria situation in Ecuador, including treatments, and diagnostics. KJ provided input on model formulation. KJ and CD provided input on interpretation of model results. RL and AS-I edited the manuscript. All authors critically reviewed and approved the final version of the manuscript. Special recognition of MS, posthumously (1959–2020), a leader in the field of medical entomology and tireless advocate for community public health in El Oro. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Zero-Valent Iron Sand Filtration Can Reduce Human and Plant Pathogenic Bacteria While Increasing Plant Growth Promoting Bacteria in Reclaimed Water

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The increasing use of reclaimed water for irrigation in areas lacking access to advanced wastewater treatment and reclaimed water distribution systems calls for an examination of irrigation-site-based treatment technologies that can improve the quality of this alternative water source. To address this need, we investigated the impact of zero-valent iron (ZVI)-sand filtration on the bacterial community structure and functional potential of conventionally treated reclaimed water utilized in downstream applications. Over a 2-month period, reclaimed water was collected from a tertiary wastewater treatment plant in the Mid-Atlantic, U.S. and trucked to our greenhouse facility. The water was stored in rain barrels and then filtered through one ZVI-sand filter every 5 days. Filtrate was then subjected to enumeration, phylotyping, shiga toxin screening, and antimicrobial susceptibility testing of *Escherichia coli*. Aliquots of filtrate were also DNA extracted, and purified DNA was subjected to 16S rRNA gene sequencing and metagenomic shotgun sequencing. The genera *Dechloromonas*, *Desulfotomaculum*, *Leptonema*, and *Thermomonas*, which contain denitrifying and sulfate reducing species, commonly used in bioremediation, and known to increase the inherent reactivity of ZVI, were significantly more relatively abundant in ZVI-sand filtered reclaimed water compared to reclaimed water. The concentration of *E. coli* in ZVI-sand filtered reclaimed water was significantly lower compared to that of reclaimed water, and cefoxitin- and tetracycline-resistant *E. coli* were undetectable after ZVI-sand filtration. ZVI-sand filtration reduced the occurrence of human as well as plant pathogenic genera (*Aeromonas*, *Mycobacterium*, *Shewanella*, *Acidovorax*, *Agrobacterium*, and *Clavibacter*) but increased the proportion of *Azospira*, a nitrogen fixing bacterial genera, in the microbial community. Our exploratory functional

analysis showed a modest non-significant increase in the proportion of open reading frames for genes associated with iron uptake, oxidative stress, as well as defense and repair mechanisms after ZVI-sand filtration. These data indicate an iron rich environment in the filter causing an oxidative stress response by the bacterial community present in the reclaimed water. Our findings demonstrate that ZVI-sand filtration effectively filters conventionally treated reclaimed water. Longer-term, field-based studies are needed to evaluate the effectiveness of the filter in agricultural settings and inform the development of future agricultural water reuse regulations.

Keywords: zero-valent iron, reclaimed water, point-of-use treatment, small-scale agriculture, metagenomics, 16S rRNA sequence analysis

INTRODUCTION

Reclaimed water (treated municipal wastewater) has emerged as one of the most commonly used alternative sources of irrigation water in the United States (U.S.) (U.S. Environmental Protection Agency (EPA), 2012). Historically drought-prone states have been early adopters of reclaimed water, and have developed stringent treatment requirements (U.S. Environmental Protection Agency (EPA), 2012). For example, California requires oxidation, coagulation, filtration and disinfection of reclaimed water before use for irrigation [California Department of Public Health (CA DPH), 2009]. Climate change is beginning to compromise the quality and availability of groundwater and other freshwater resources in areas of the U.S. previously considered water-rich (e.g., the Mid-Atlantic region; U.S. Environmental Protection Agency (EPA), 2012; U.S. Global Change Research Program, 2015), and proactive water management, including the use of reclaimed water, is emerging in these areas (U.S. Environmental Protection Agency (EPA), 2012). However, the type of advanced level treatment performed in California is not typical of conventional wastewater treatment across the U.S., and in these emerging-use areas, the infrastructure is not in place to perform such advanced treatment (U.S. Environmental Protection Agency (EPA), 2012).

In areas with long-established use of reclaimed water for irrigation, advanced treatment is often performed at a central location and then reclaimed water is distributed, for use in irrigation, to areas with predominantly agricultural land-use (Monterey Regional Water Pollution Control Agency (MRWPCA), 2013; American Farmland Trust, 2017a). In contrast, emerging-use areas tend to be closer to municipal wastewater treatment plants (Thebo et al., 2017), which often perform more conventional wastewater treatment, and usually lack the infrastructure required for the type of centralized advanced treatment and distribution seen in established use regions (U.S. Environmental Protection Agency (EPA), 2012). Moreover, land use in emerging-use areas is more often a mixture of agricultural and residential applications, with agricultural irrigation performed on a much smaller scale compared to areas of established reclaimed water use, such as California (Monterey Regional Water Pollution Control Agency (MRWPCA), 2013; American Farmland Trust, 2017a,b).

Bacterial, viral, and protozoal pathogens have been shown to persist in reclaimed water after conventional wastewater treatment (Rose et al., 1996; Harwood et al., 2005; Brissaud et al., 2008; Jjemba et al., 2010; Rosenberg Goldstein et al., 2012, 2014). Specifically, indicator organisms and opportunistic pathogens have been detected in reclaimed water distribution systems after being non-detectable or present in low concentrations in conventionally treated wastewater (Jjemba et al., 2010). In addition, secondary treated, chlorinated, and dechlorinated reclaimed water can be a reservoir of antibiotic resistance genes (Fahrenfeld et al., 2013). Therefore, to facilitate the safe adoption of conventionally treated reclaimed water, it may be necessary to examine point-of-use treatment solutions that work within the existing infrastructure and scale of irrigation in emerging-use areas where centralized advanced treatment and dispersal may not be feasible.

A potential candidate for on-site treatment is zero-valent iron (ZVI) sand filtration. ZVI has been predominantly used for the remediation of groundwater contaminated with chlorinated compounds, but previous studies have shown it to be effective in the removal of viruses and bacteria as well (You et al., 2005; Ingram et al., 2012; Shi et al., 2012; Chiu, 2013; Shearer and Knier, 2018; Marik et al., 2019). Furthermore, since ZVI does not generate potentially harmful by-products (United States Environmental Protection Agency (EPA), 2015), applications such as drinking water treatment and wastewater treatment are currently being explored (You et al., 2005; Ingram et al., 2012; Chiu, 2013).

Nevertheless, very few studies have been conducted on the effectiveness of ZVI treatment of reclaimed water and its potential as an on-site filtration system for conventionally treated reclaimed water use on small-scale farms. Therefore, the goal of this study was to examine the influence of ZVI-sand filtration on the bacterial community structure and functional potential of conventionally treated reclaimed water utilized in downstream applications.

MATERIALS AND METHODS

Reclaimed Water Collection Site

Reclaimed water was collected from a tertiary wastewater treatment plant (WWTP) located in a rural town, in the Mid-Atlantic U.S., with land use including suburban developments

and farmland (Maryland Department of Commerce, 2016). The WWTP has a maximum daily capacity of 1,900 m³ and treats between 1,100 and 1,400 m³ of domestic wastewater per day. This WWTP performs conventional wastewater treatment—large debris and grit removal followed by activated sludge treatment, secondary clarification and chlorination. The chlorinated effluent is used for groundwater recharge by spray irrigation. Chlorinated effluent used for this experiment was collected from an open-air lagoon prior to land application.

ZVI-Sand Filter

The container (approximate total volume of 55.5 L) from a commercially available biosand filter (HydrAid® BioSand Water Filter, NativeEnergy, Burlington, VT, USA) was adapted for this experiment. Fine filtration sand (crushed quartzite (silica) provided with the filter) (Manz, 2000; Triple Quest LLC, 2010) and ZVI (Peerless Metal Powders and Abrasives Company, Detroit, MI, USA) were sieved to achieve a particle size range of 400–625 µm. A schematic of the cross section of the ZVI-sand filter (**Supplementary Figure 1**) can be found in the supplementary material. The empty filter was first filled with 20 L of ultrapure water, and equal parts by volume (~25.2 L) of ZVI and sand were mixed thoroughly to the filter in batches by displacing the ultrapure water to prevent the formation of air gaps and ensure complete compaction of the ZVI-sand mixture. Preferential flow through the filter was avoided by using a gravel filled diffuser plate to manually pour reclaimed water into the filter to achieve gravity filtration. The approximate porosity of the filter was 0.52 [Center for Affordable Water and Sanitation Technology (CAWST), 2015], the approximate average flow rate through the filter was 5.6 L/min, and the filtration rate was 18 L/min/m². The approximate contact time with the ZVI-sand filtration medium was 2.58 min, and was calculated using the following formula:

$$\frac{\text{Total Volume of Filter} \times \text{Porosity} \times \text{ZVI Content}}{\text{Volumetric Flow Rate}}$$

Collection of Chlorinated Effluent

The experiment was designed to simulate reuse site conditions in which reclaimed water would be delivered to the reuse site and stored until filtration and irrigation (**Figure 1**). Every fortnight, 240 L of chlorinated effluent was collected from the WWTP, driven to the reuse site (University of Maryland Research Greenhouse Complex, College Park, MD), divided equally (80 L each) into three 189 L rain barrels (Cat # 81313 Algreen Products Inc., Ontario, Canada) and stored until needed for filtration.

ZVI-Sand Filtration

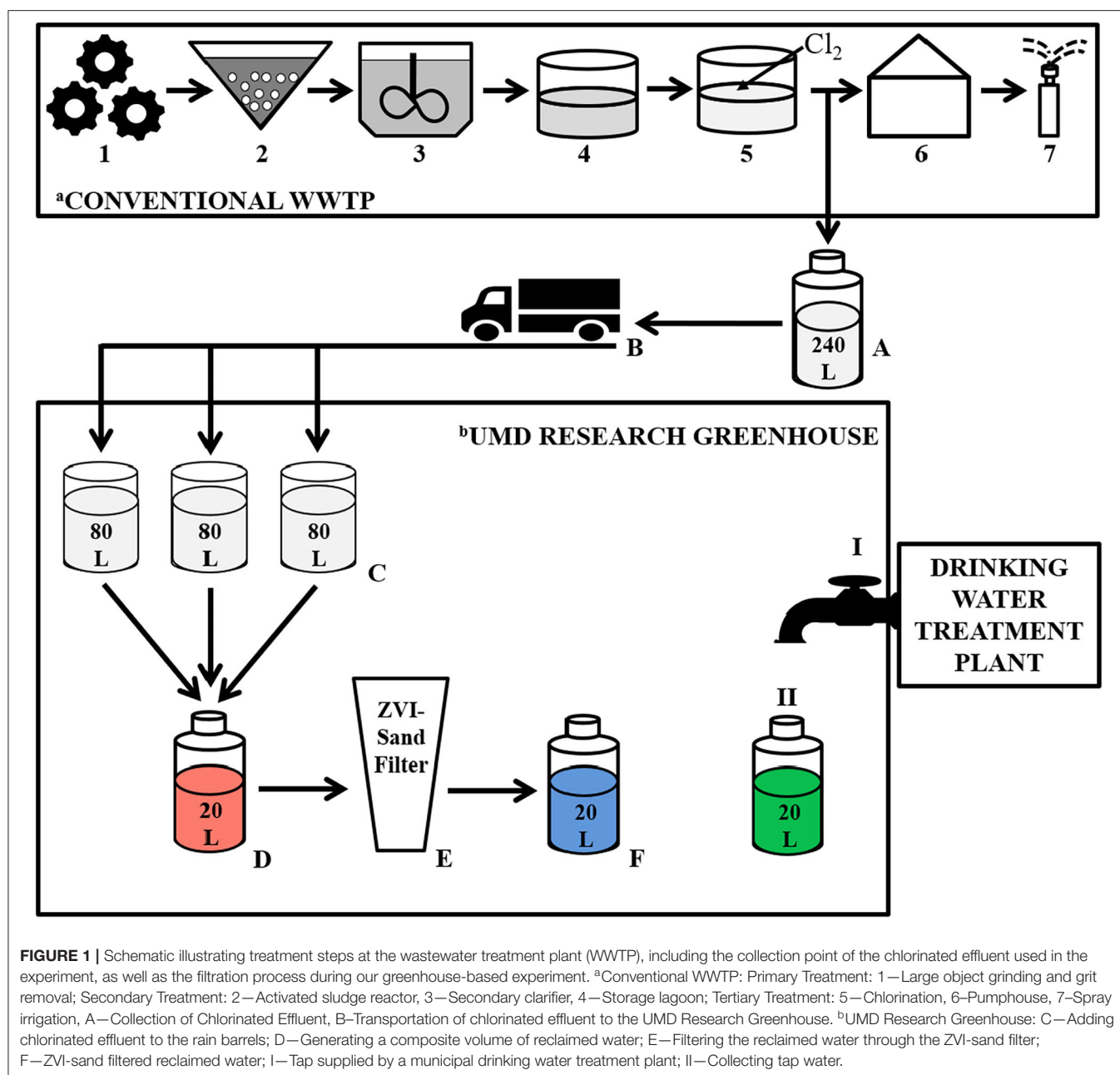
ZVI-sand filtration took place at the University of Maryland Research Greenhouse Complex in the same room in which the chlorinated effluent was stored in the rain barrels (**Figure 1**). Reclaimed water (RW) was gravity filtered, every 5 days, by pouring it through the ZVI-sand filter. Specifically, equal volumes of chlorinated effluent stored in each of the three rain barrels were combined to generate a 20 L composite of reclaimed water. The ZVI-sand filter was kept submerged in reclaimed

water between filtration events, and just prior to filtration, the 5-day old water held in the ZVI-sand filter was displaced, and thus completely flushed out, by pouring the aforementioned 20 L composite of reclaimed water through the filter. This displaced 5-day-old water was discarded. A new 20 L composite of reclaimed water was then generated, as described above, and poured through the ZVI-sand filter and the resulting 20 L filtrate (ZVI-sand filtered reclaimed water—“ZW”) was collected for analysis. One liter of tap water (TW) supplied to the greenhouse from a drinking water treatment plant was also collected at each filtration event. The tap water samples were used as a representation of safe, high quality irrigation water, to which the reclaimed water and ZVI-sand filtered reclaimed water could be compared. All the samples were filtered every 5 days, starting on 7/15/2016 and ending on 8/24/2016. However, a test sample that was filtered on 6/21/2016 was also included in the data analysis.

“In total there were 10 filtration events resulting in $n = 10$ 20 L reclaimed water samples and $n = 10$ 20 L ZVI-filtered water samples. Every time a filtration event occurred, a one-liter tap water sample was also collected as a control, as noted above. Prior to filtration, two 500 mL aliquots were taken from each 20 L reclaimed water sample. After filtration, two 500 mL aliquots were taken from each 20 L ZVI-filtered water sample. Two 500 mL aliquots were also taken from the one-liter tap water sample. These aliquots were collected for DNA extraction and enumeration of *Escherichia coli*. Immediately after collection, each 500 mL aliquot was taken to the laboratory on ice and held at 4°C and processed within 24 h of collection.”

DNA Extraction

Within 24 h of collection, each 500 mL aliquot was vacuum filtered through a 0.2 µm, 47 mm hydrophilic polyethersulfone (PES) filter (Pall Corporation, Port Washington, NY, USA). Total genomic DNA was extracted from the filters utilizing enzymatic and mechanical lyses using previously published procedures (Zupancic et al., 2012; Jackson et al., 2014). After each filter was aseptically placed in a sample lysis tube (Lysing Matrix B) (MP Biomedicals, Solon, OH, USA), ice-cold molecular biology grade 1X Phosphate Buffered Saline (PBS) (Gibco-Life Technologies, Grand Island, NY, USA), lysozyme from chicken egg white (10 mg/mL, Sigma-Aldrich, St. Louis, MO, USA), lysostaphin from *Staphylococcus staphylolyticus* (5 mg/mL Sigma-Aldrich, St. Louis, MO, USA), and mutanolysin from *Streptomyces globisporus* ATCC 21553 (1 mg/mL Sigma-Aldrich, St. Louis, MO, USA) were added, followed by incubation at 37°C for 30 min. A second enzymatic lysis step was conducted using Proteinase K (20 mg/mL, Invitrogen-Life Technologies, Grand Island, NY, USA) and 10% (w/v) sodium dodecyl sulfate (SDS) (BioRad, Hercules, CA, USA) followed by incubation at 55 °C for 45 min. Mechanical lysis (6.0 m/s for 40 s) was then performed using the FastPrep®-24 benchtop homogenizer (MP Biomedicals, Irvine, CA, USA). Further DNA purification was performed using the QIAmp DSP DNA mini kit 50, v2 (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. DNA quality was measured using a NanoDrop® spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and gel electrophoresis.



16S rRNA Gene Amplification and Sequencing

Previously published procedures were used to perform polymerase chain reaction (PCR) amplification of the V3-V4 hypervariable region of the 16S rRNA gene using universal primers 319F and 806R (Caporaso et al., 2012; Sellitto et al., 2012; Fadrosch et al., 2014). To allow for multiplexing samples in a single Illumina MiSeq (Illumina, San Diego, CA, USA) run, unique 12 base pair (bp) sequence tags were included with the 806R primer to barcode for each sample (Fadrosch et al., 2014). Phusion High-Fidelity DNA polymerase and mastermix (Thermo Fisher Scientific, Waltham, MA, USA) with 20 mg/mL

additional bovine serum albumin (BSA) (to overcome PCR inhibition) (Sigma-Aldrich St. Louis, MO, USA) was used to perform PCR amplification in a DNA Engine Tetrad 2 thermal cycler (Bio-Rad, Hercules, CA, USA). The cycling parameters were: 30 s at 98°C, followed by 30 cycles of 10 s at 98°C, 15 s at 66°C, 15 s at 72°C, and 5 min at 72°C. For each primer pair, negative controls excluding templates were also processed. Gel electrophoresis was used to confirm amplicon presence, with quantification performed using a KAPA library quantification kit (KAPA Biosystems, Wilmington, MA, USA). From each sample, equimolar (25 ng) PCR amplicons, were mixed in a single tube and amplification primers and reaction buffers were removed

using the AMPure kit (Agencourt Biosciences, Beverly, MA, USA). Amplicons were pooled and sequenced according to the manufacturer's protocol using the Illumina MiSeq (Illumina, San Diego, CA, USA).

16S rRNA Sequencing Analysis Pipeline and Data Normalization

Multiplexed 16S rRNA reads were screened for the removal of low-quality base calls and insufficient raw read lengths. PANDAseq (Masella et al., 2012) was used for assembly resulting in high-quality consensus sequences, which then underwent demultiplexing followed by trimming of barcodes and 5' and 3' primer regions. UCHIME (Edgar et al., 2011) was used in *de novo* mode to assess for chimeras. Chloroplast and eukaryotic DNA were filtered out to reduce interference. The resulting dataset was analyzed for taxonomic and diversity analysis using the CloVR-16S pipeline (White et al., 2013) which uses two parallel protocols (a QIIME-based analysis and a Mothur/RDP-based analysis). The number of observed sequences compared to the estimated coverage can be seen in **Supplementary Figure 2**. The estimated coverage was determined using the Good's coverage metric (Hsieh et al., 2016). Sufficient sequencing depth was obtained and samples containing fewer than 100 sequences were excluded from downstream analysis. Data were normalized with cumulative sum scaling using metagenomeSeq (Paulson et al., 2013).

Shotgun Metagenomic Sequencing Analysis Pipeline

A subset of RW ($n = 3$) and ZW ($n = 3$) samples were selected for shotgun metagenomic sequencing which was performed by CosmosID (Rockville, MD, USA). An HS DNA Qubit fluorescent concentration assay was used to quantify each DNA sample. For each sample, all of the DNA was used in the tagmentation reaction. This was followed by 13 cycles of PCR amplification using Nextera i7 & i5 index primers & 2X KAPA master mix according to the modified Nextera XT protocol. The KAPA SYBR FAST qPCR kit was used to quantify the final libraries, with concentrations ranging from (0.1 to 212) ng/uL. Library concentrations were measured using KAPA qPCR prior to pooling. The pooled libraries were then loaded onto a high sensitivity (HS) chip run on the Caliper LabChipGX. The base pair size reported was in the range of 254–895 bp. Each pool of 84 samples was run across 8 lanes of an Illumina HiSeq 4000 flow cell targeting 100 bp paired end reads per sample. The CosmosID cloud bioinformatics platform (CosmosID Inc., Rockville, MD) was used to analyze unassembled metagenomic sequencing reads for identification at the species, subspecies, and/or strain level as well as for the quantification of relative abundance using previously described methods (Hasan et al., 2014; Lax et al., 2014; Ottesen et al., 2016; Ponnusamy et al., 2016).

Functional Annotation

Metagenomic reads were assembled into contigs. Initial quality filtering was performed using Trimmomatic (Bolger et al., 2014), paired end reads were merged using FLASH (Magoč and Salzberg, 2011), and merged reads were assembled using Spades

(Bankevich et al., 2012). Individual assemblies were generated for each sample. Open reading frames (ORFs) were predicted from the assembled contigs using Metagen (Beauparlant et al., 2017). Functional annotation of ORFs was performed using the evolutionary genealogy of genes: non-supervised orthologous groups (eggNOG) database (Huerta-Cepas et al., 2016). ORFs were mapped to eggNOG using emapper-0.99.2-3-g41823b2 (Huerta-Cepas et al., 2017) and sequence searches using DIAMOND (Buchfink et al., 2014). Relative abundance values represent the number of ORFs assigned to a gene of interest and normalized by total sum scaling (TSS), dividing abundance values by total number of assigned ORFs.

Enumeration, Phylotyping, Shiga Toxin Screening, and Antimicrobial Susceptibility Testing of *E. coli*

Viable organisms were enumerated by using the most probable number (MPN) determination assay. One hundred milliliters aliquots were generated from each of the aforementioned 500 mL aliquots of reclaimed water and ZVI-sand-filtered reclaimed water. These 100 mL aliquots underwent serial dilution in Tryptic Soy Broth (TSB, Accumedia, Lansing, MI), followed by incubation at 42°C for 24 h, and isolation streaking on Tryptone Bile X-glucuronide (TBX, Accumedia, Lansing, MI) agar with incubation at 37°C for 24 h. MPN values of *E. coli* in the samples were calculated using an online MPN calculator (Curiale, 2016) and PCR confirmation of the presence of the *uidA* gene in presumptive *E. coli* was performed (Jefferson et al., 1986; Bej et al., 1991). PCR confirmed *E. coli* isolates were stored at –80°C until antibiotic susceptibility testing, phylotyping, and the detection of Shiga toxin-producing *E. coli* (STEC) were performed. Antibiotic susceptibility testing was performed based on methods determined by the United States Centers for Disease Control and Prevention (CDC) National Antimicrobial Resistance Monitoring System (NARMS) for *E. coli* (US Food Drug Administration, 2016). Isolates were tested on a panel of 14 antibiotics (CMV3AGNF) using a Sensititre automated microdilution system (Thermo Scientific, Waltham, MA, USA). Minimum inhibitory concentrations (MIC) were based on resistance breakpoints published by the Clinical and Laboratory Standards Institute (CLSI, 2018). *E. coli* phylo-typing was performed using the Clermont 2000 PCR method (Clermont et al., 2013). The detection of STEC was performed using an 11-gene multiplex polymerase chain reaction (mPCR) for detection of the presence of seven major serotypes (O26, O45, O103, O111, O121, O145, and O157) of enterohemorrhagic *E. coli* (EHEC), a subset of STEC, along with four major virulence factors (*stx1*, *stx2*, *ehxA*, and *eae*) (Bai et al., 2012).

Statistical Analysis

Normalized data were used to estimate the Shannon Index (Shannon and Weaver, 1948) and Simpson's Diversity Index (Simpson, 1949) using R statistical software, version 3.3.0 (R Core Team, 2017) using packages phyloseq, version 1.16.2 (McMurdie and Holmes, 2013) and vegan, version 2.3.5 (Dixon, 2003). The Kruskal-Wallis test was used to evaluate differences

TABLE 1 | Number of contigs, mean contig length, % reads recruited, open reading frames (ORFs), and complete ORFs per sample by collection date.

Sample type	Collection date	Contigs	Mean contig length	% Reads recruited (%)	ORFs	Complete ORFs
RW	07 – 25 – 2016	298,545	955	54	516,860	150,579
RW	08 – 04 – 2016	249,850	744	57	395,559	88,278
RW	08 – 14 – 2016	274,561	1,012	53	484,149	147,692
ZW	07 – 25 – 2016	277,473	1,099	75	535,207	188,523
ZW	08 – 04 – 2016	301,611	791	77	479,729	107,868
ZW	08 – 14 – 2016	261,297	1,123	78	513,639	183,925
TW	07 – 25 – 2016	112,224	1,503	76	249,780	110,967
TW	08 – 04 – 2016	81,989	966	87	139,802	38,915
TW	08 – 14 – 2016	77,381	1,330	79	159,300	64,889

RW, Reclaimed Water; ZW, ZVI-sand filtered reclaimed water; TW, Tap water.

in alpha-diversity estimates, while the breakaway betta model (Willis et al., 2017) was used to test for differences in richness (number of bacterial species) in samples collected before and after ZVI filtration. Beta diversity was estimated using Bray-Curtis dissimilarity (Bray and Curtis, 1957) and compared using analysis of similarities (ANOSIM) on the normalized data with 999 permutations; the pairwise differences were calculated using Tukey's test. Differential relative abundance of operational taxonomic units (OTUs) across samples was estimated using metagenomeSeq, version 1.14.2 (Paulson et al., 2013). For the comparison of differential relative abundance, OTUs present in fewer than half of the samples with counts at least equal to 1 were excluded from the analysis to reduce potential biases in the statistical test due to sparsity (high frequency of unobserved OTUs). In the case of comparison of differential relative abundance specifically across treatment processes, OTUs present in less than half the samples were excluded from the analysis. MetagenomeSeq was used to compare the log-fold changes in ORF relative abundances between reclaimed water and ZVI-filtered reclaimed water samples. MPN estimates of *E. coli* in reclaimed water and ZVI-filtered reclaimed water were compared using the Wilcox test. In all analyses, differences were considered statistically significant at $p < 0.05$. When comparing reclaimed water and ZVI-filtered reclaimed water samples, the paired nature of the samples was accounted for during significance testing. All visualizations were performed using ggplot2, version 2.1.0 (Wickham, 2009).

RESULTS

Ten reclaimed water, 10 ZVI-sand filtered reclaimed water and nine tap water samples were sequenced. 573,152, 191,137, and 3,927 16S rRNA gene sequences were obtained for reclaimed water, ZVI-sand filtered reclaimed water and tap water samples, respectively. The estimated coverage using the Good's coverage metric (Hsieh et al., 2016) is illustrated in **Supplementary Figure 2**.

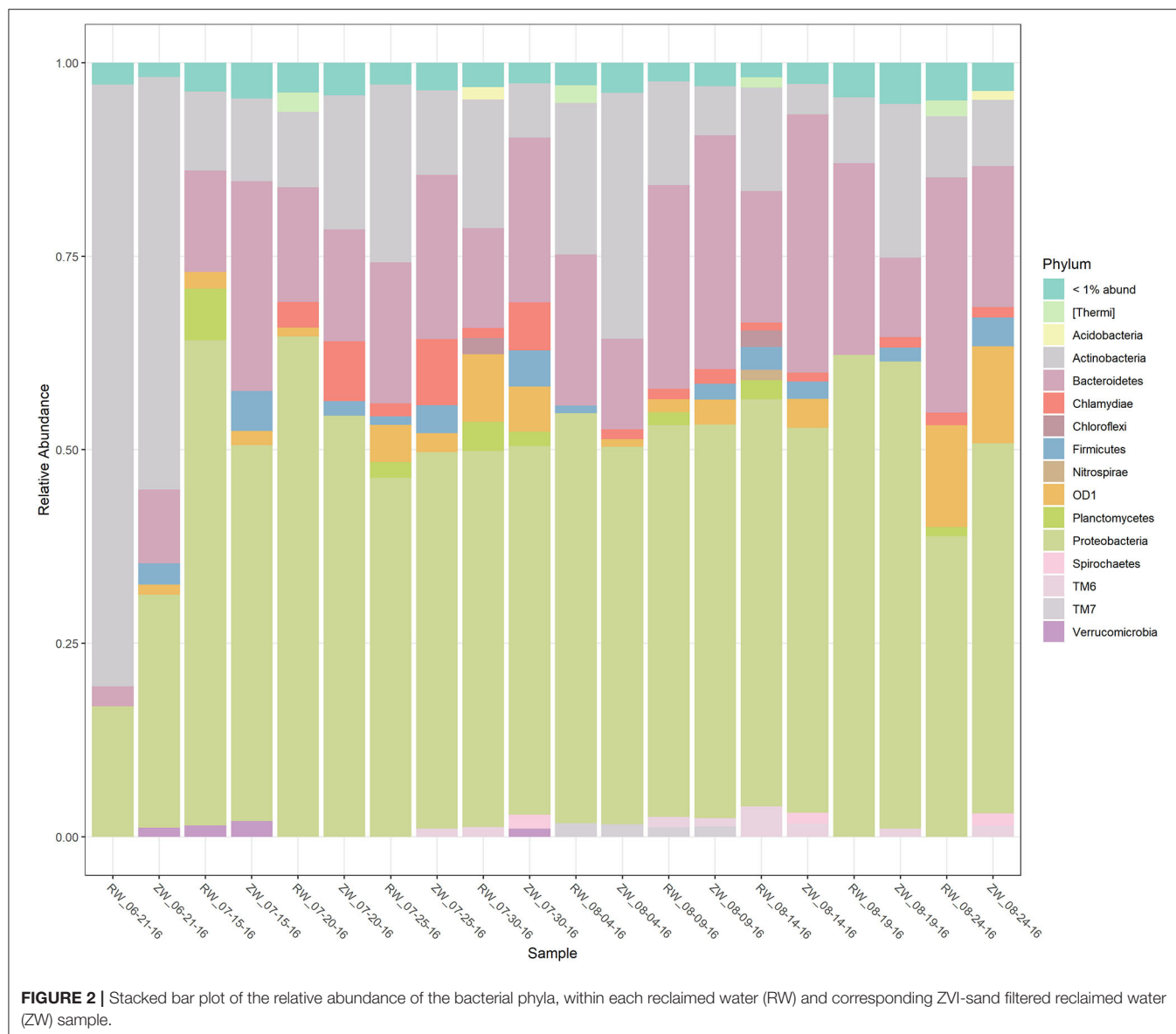
The total number of assembled contigs, mean contig length, percentage of reads recruited, ORFs and complete ORFs for the three reclaimed water, three ZVI-sand filtered reclaimed water and three tap water samples that were shotgun sequenced are listed in **Table 1**.

Bacterial Community Composition Differences Between Reclaimed Water and ZVI-Sand Filtered Reclaimed Water

Figure 2 illustrates the relative abundance, at the phylum level, within each reclaimed water and corresponding ZVI-sand filtered reclaimed water sample collected during the experiment. *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* were the most relatively abundant phyla across all samples. A heatmap based on the relative abundance of cumulative sum scaling (CSS) normalized counts, at the genus level, within each reclaimed water and corresponding ZVI-sand filtered reclaimed water sample collected during the experiment is shown in **Figure 3**. Differences in richness and estimates of Simpson's and Shannon's alpha diversity between reclaimed water samples before and after ZVI-sand filtration were not significant (**Figures 4, 5**). When the reclaimed water, ZVI-sand-filtered reclaimed water and tap water samples were analyzed collectively, tap water clustered away from reclaimed water and ZVI-sand-filtered reclaimed water samples (**Figure 6**; ANOSIM statistic $R: 0.6941$, $p < 0.01$). Further analysis indicated that the differences between the tap water and the reclaimed water clusters and the differences between the tap water and the ZVI-sand-filtered reclaimed water clusters were significant ($p < 0.05$). The top five differentially relatively abundant genera between reclaimed water and ZVI-sand-filtered reclaimed water are displayed in **Figure 7**. The most dominant genus that was significantly ($p < 0.01$) higher in reclaimed water samples compared to ZVI-sand-filtered reclaimed water samples was *Mycobacterium*. Bacteria belonging to the genera *Dechloromonas*, *Desulfotomaculum*, *Leptonema*, and *Thermomonas* were significantly ($p < 0.01$) more relatively abundant in samples after ZVI-sand filtration compared to before.

Differences in the Concentration and Antimicrobial Susceptibility of *E. coli* Between Reclaimed Water and ZVI-Sand Filtered Reclaimed Water

MPN estimates of *E. coli* were significantly ($p < 0.05$) lower in all samples after ZVI-sand filtration (**Figure 8**). The logMPN estimates of *E. coli* of reclaimed water and ZVI-sand filtered reclaimed water are listed in **Supplementary Table 7**. Among

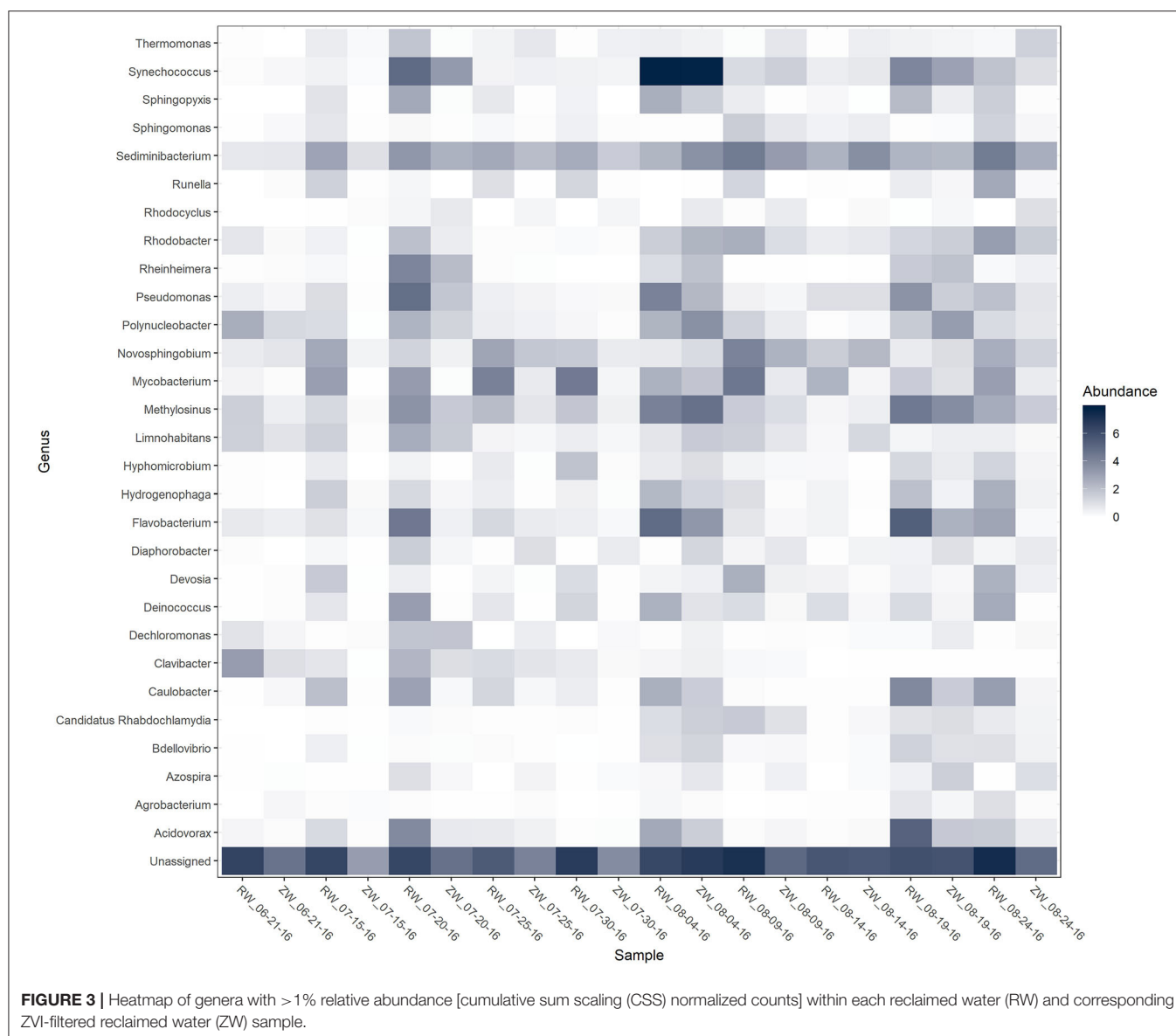


all PCR confirmed *E. coli* isolates analyzed, no STEC were detected in either reclaimed water or ZVI-filtered reclaimed water samples. All PCR confirmed *E. coli* isolates analyzed were classified as belonging to phylogroup B1 and only two of all of the 20 *E. coli* isolates analyzed were found to be resistant to antibiotics. Both resistant isolates were recovered from reclaimed water samples—one collected on July 15th was found to be resistant to cefoxitin ($\text{MIC} \geq 32 \mu\text{g/ml}$) and one collected on August 4th was found to be resistant to tetracycline ($\text{MIC} \geq 16 \mu\text{g/ml}$) (CLSI, 2018). None of the *E. coli* isolates analyzed from corresponding ZVI-sand-filtered reclaimed water samples were found to be resistant to any of the antibiotics included in the panel, including isolates from ZVI-sand filtered samples corresponding to reclaimed water samples filtered on July 15th and August 4th.

Differences in Functional Potential of the Bacterial Community of Reclaimed Water Before and After ZVI-Sand Filtration

The log-fold changes in expression of ORFs for genes after ZVI-sand filtration (classified by function) are listed in **Supplementary Table 1** through **Supplementary Table 6** provided in the Supplementary Material.

A modest non-significant decrease in log-fold expression levels of ORFs for genes encoding siderophores including aerobactin, enterobactin, and mycobactin was observed in ZVI-sand-filtered reclaimed water (**Supplementary Tables 1–6**). Siderophores are ferric ion specific chelating agents synthesized and secreted by microorganisms under conditions of stress caused by low iron (Neilands, 1995). However, log-fold expression levels of ORFs for genes encoding membrane proteins



responsible for binding and uptake of various iron compounds including ferrous iron ions and iron dicitrate, heme binding, and ferric uptake regulation were slightly, but non-significantly, higher after ZVI-sand-filtration (Supplementary Tables 1–6). The log-fold expression levels of ORFs for genes associated with detoxification (hydrogen peroxide and superoxide removal and redox homeostasis) were also higher but non-significantly in ZVI-sand-filtered reclaimed water compared to reclaimed water (Supplementary Tables 1–6). A non-significant, increase in the log-fold expression levels of ORFs for genes associated with general stress responses (heat shock, cold shock, pH imbalance, starvation, radiation, and osmoregulation response) was observed after ZVI-sand filtration (Supplementary Tables 1–6). ORFs for genes associated with other defense mechanisms (biofilm formation, cell adhesion, dormancy, cell wall biogenesis, and peptidoglycan biosynthesis) were non-significantly higher in

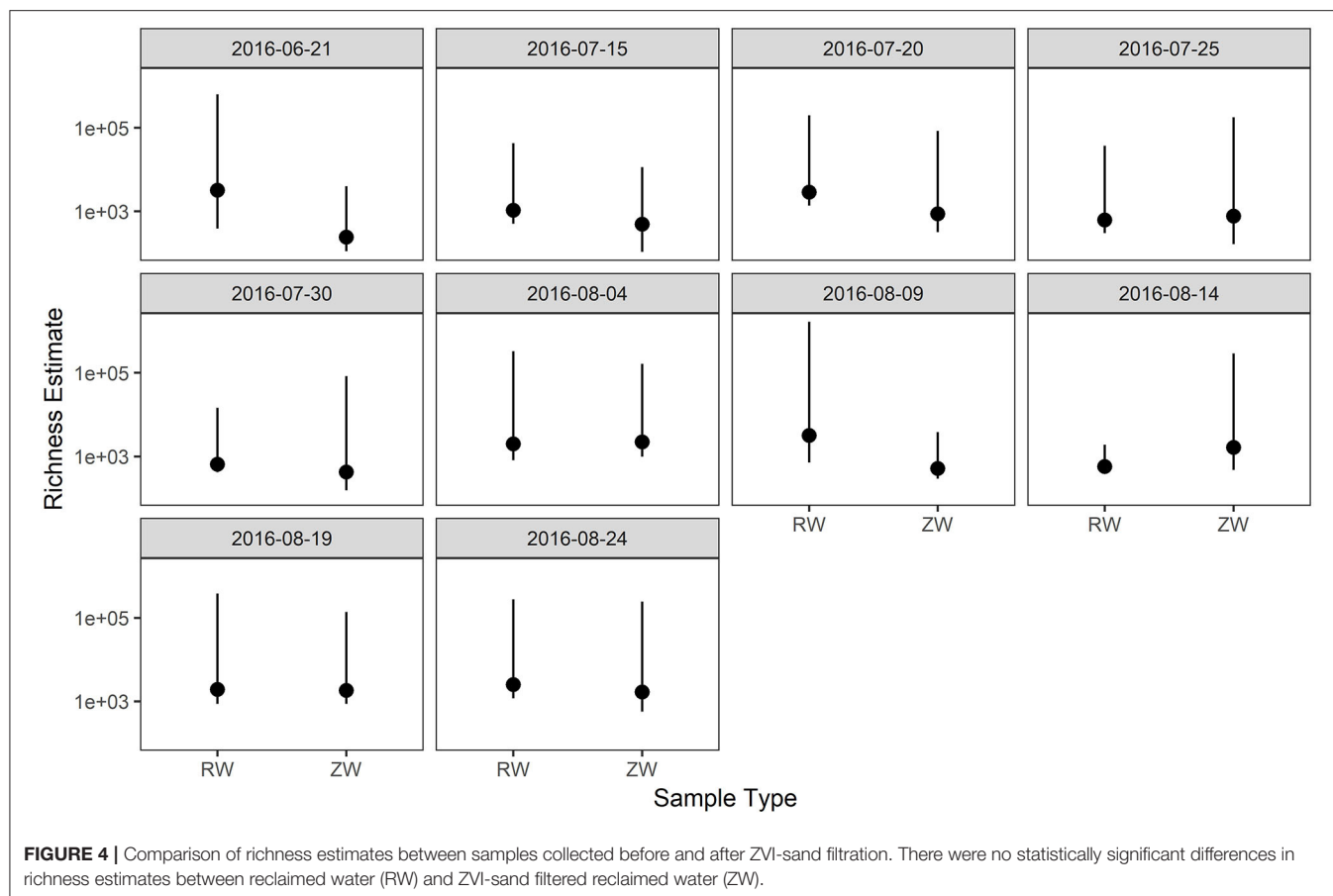
ZVI-sand-filtered reclaimed water compared to reclaimed water (Supplementary Tables 1–6).

Data Deposition—Datasets Are in a Publicly Accessible Repository

Sequence data generated and analyzed in this study were deposited with GenBank and linked to BioProject number PRJNA522745 in the NCBI BioProject database <https://www.ncbi.nlm.nih.gov/bioproject/>.

DISCUSSION

Our analysis revealed that the dominant bacterial communities detected within the ZVI-sand filtered reclaimed water were denitrifying and sulfate-reducing bacteria, commonly used in bioremediation applications, and known to increase the inherent



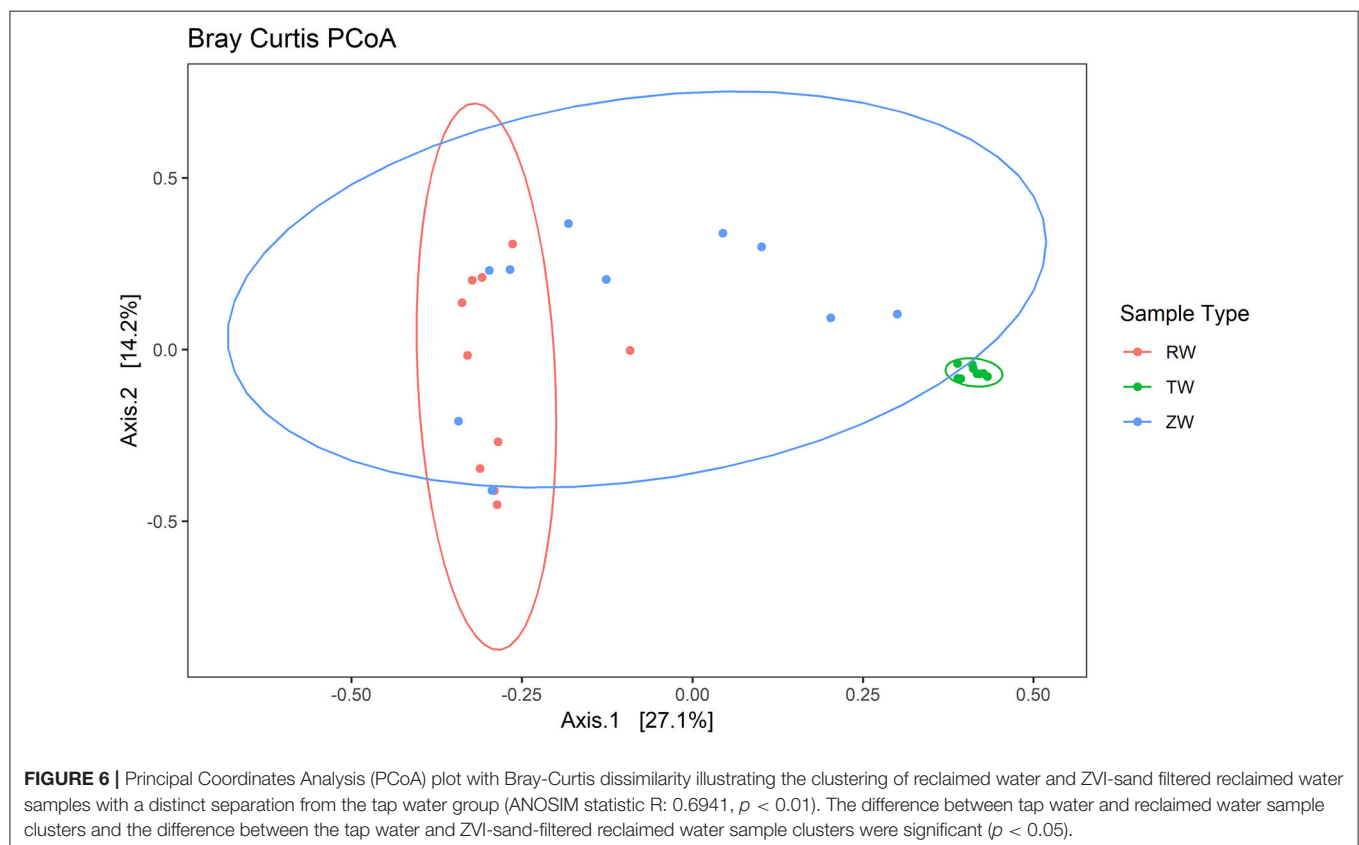
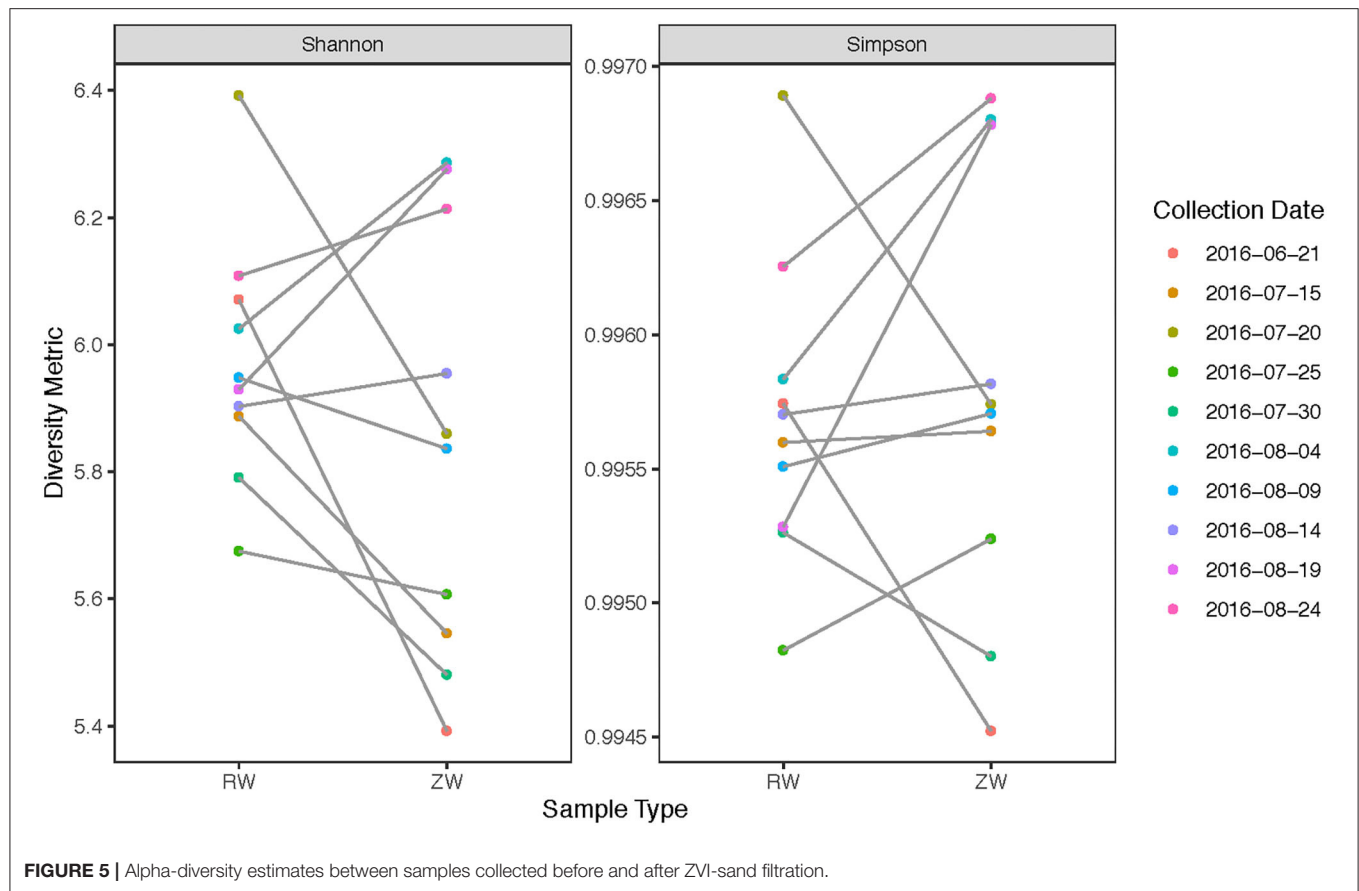
reactivity of ZVI (van Nooten et al., 2008, 2010). Our functional analysis revealed that a potential abundance of iron ions in the extracellular environment within the ZVI-sand filter may have resulted in a state of oxidative stress within the bacterial cells in the reclaimed water, resulting in several stress response genes being expressed by the reclaimed water bacterial community. ZVI-sand filtration was able to reduce the *E. coli* concentration in reclaimed water and eliminate antibiotic-resistant *E. coli* detected in reclaimed water.

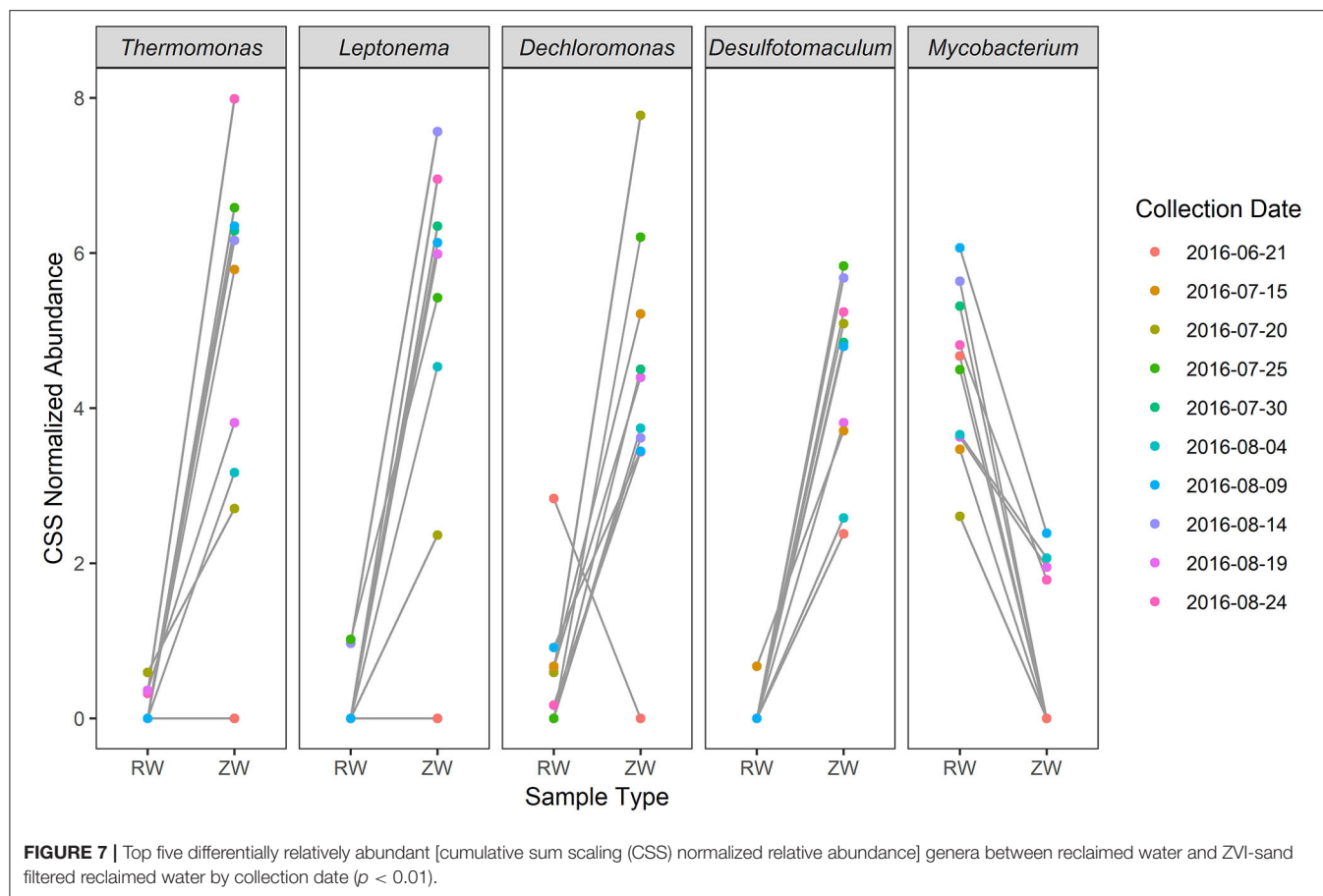
Total Bacterial Community Composition of ZVI-Sand Filtered Reclaimed Water

Aerobic as well as anaerobic corrosion reactions within the ZVI filter give rise to highly-reducing, oxygen-diminished and hydrogen gas-rich conditions (Rowland, 2003), which are ideal for both sulfate-reducing (Rowland, 2003) and denitrifying (van Nooten et al., 2010) bacteria. These conditions may have resulted in *Dechloromonas*, *Desulfotomaculum*, *Leptonema*, and *Thermomonas* being the dominant genera in the ZVI-sand filtered reclaimed water. Conversely, the most relatively abundant bacteria in reclaimed water (pre-treatment) belonged to *Mycobacterium*, a genus of aerobic organisms (Grange, 1996), prolific in aquatic environments, and previously detected in chlorinated reclaimed water (Grange, 1996; Jjemba et al., 2010).

Thermomonas spp., *Dechloromonas* spp., and *Leptonema* spp. have all been isolated from an H_2 -dependent (hydrogenotrophic) denitrification reactor used for groundwater remediation (Long-Bohanon, 2010). *Dechloromonas* and *Thermomonas* are gram-negative, non-fermenting, non-spore forming, heterotrophic, facultative anaerobic denitrifying bacteria (Achenbach et al., 2001; Busse et al., 2002; Mcilroy et al., 2016). *Thermomonas* spp. perform phenol and nitrate reduction under low-oxygen conditions (Baek et al., 2003) and *Dechloromonas* spp. perform chlorate or perchlorate reduction (Bruce et al., 1999; Chakraborty et al., 2005), can use sulfide as an electron donor (Bruce et al., 1999) and are utilized for phosphate removal in enhanced biological phosphorus removal processes (Mcilroy et al., 2016). The genus *Desulfotomaculum*, is comprised of gram-positive spore-forming sulfate-reducing obligate anaerobic autotrophic bacteria which oxidize molecular hydrogen in anaerobic conditions (Liamleam and Annachhatre, 2007; Aüllo et al., 2013).

Sulfate-reducing and denitrifying bacteria are both known to increase ZVI reactivity by the generation of ferrous sulfides, which can be more reactive than iron, and by converting NO_3^- , which competes with ZVI for reactive sites, into N_2O and N_2 (van Nooten et al., 2008). Moreover, van Nooten et al. (2008) showed that ZVI reactivity, and subsequently, contaminant reduction, may be achieved, not only through physical-chemical processes,

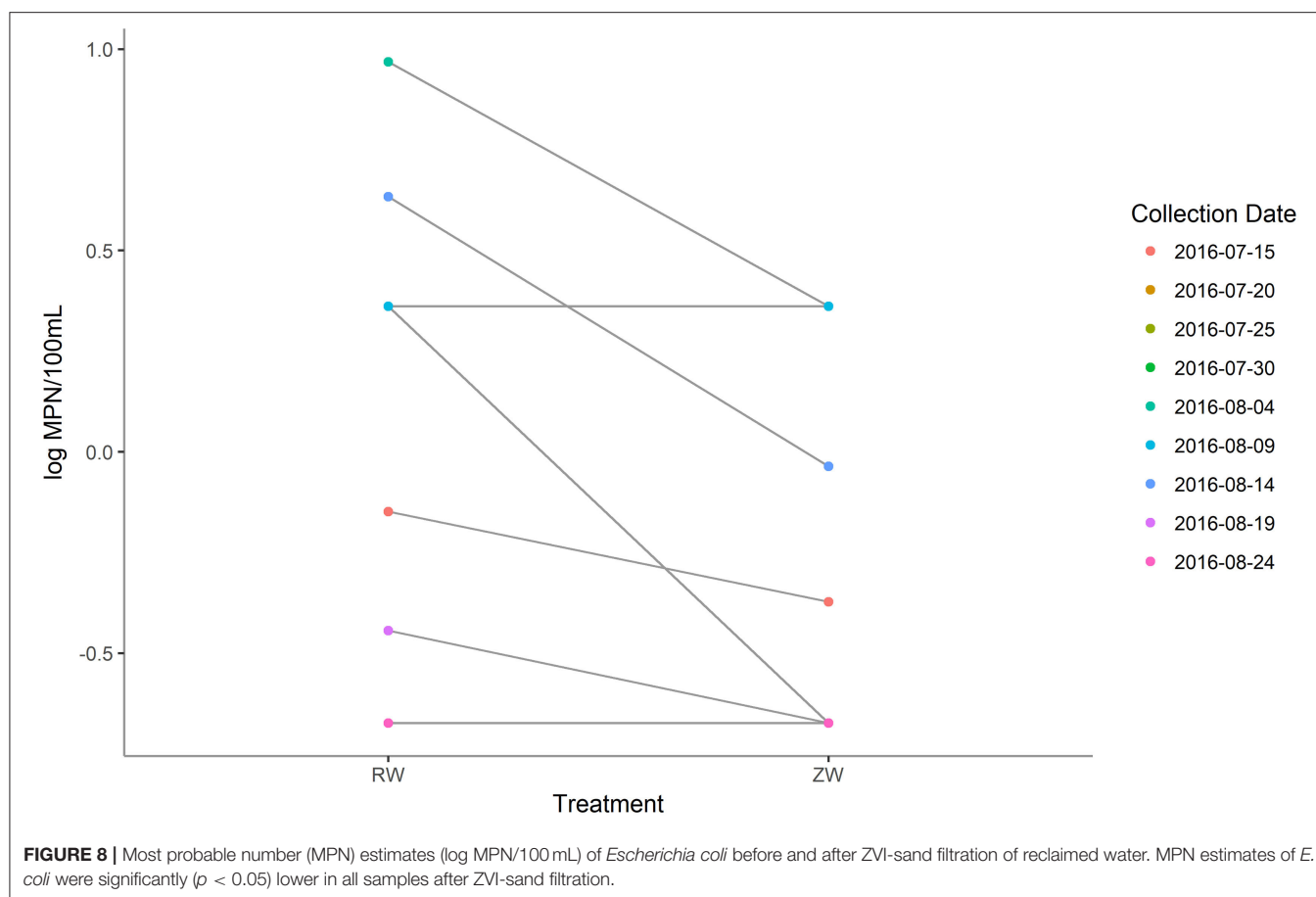




but also through the contributions of the specialized microbial community generated within ZVI columns (van Nooten et al., 2008). Both denitrifying and sulfate-reducing bacteria have been used for the anaerobic bioremediation of potentially harmful inorganic as well as organic compounds (Casella and Payne, 1996; Anderson and Lovley, 2000; Hussain et al., 2016), including those that have been detected in conventionally treated reclaimed water (National Research Council, 1996, 1998; Miège et al., 2008; U.S. Environmental Protection Agency (EPA), 2012). The ZVI-sand filter examined in this study was able to reduce both potentially harmful inorganic contaminants (Kulkarni et al., 2019) as well as potentially pathogenic bacterial species detected in reclaimed water.

In a companion study, the concentrations of azithromycin, ciprofloxacin, oxolinic acid, penicillin G, sulfamethoxazole, linezolid, piperidic acid, vancomycin, nitrate (NO_3^- -N) and nitrite (NO_2^-) found in conventionally treated reclaimed water were significantly reduced after ZVI-sand filtration (Kulkarni et al., 2019). Azithromycin, the antimicrobial with the highest median concentration (320 ng/L), was reduced to below the limit of detection after ZVI-sand filtration (Kulkarni et al., 2019). Although not statistically significant, our ZVI-sand filtration system also achieved reductions in concentrations of antimony, cadmium, lead, and selenium found in reclaimed water (Kulkarni et al., 2019).

ZVI-sand filtration was able to reduce the relative abundance of bacterial genera, containing several potential human pathogenic species, detected in our reclaimed water samples. Specifically, genera containing species which were implicated in foodborne and enteric infections (*Aeromonas*, *Arcobacter*, *Comamonas*, and *Vibrio*) (Collado and Figueras, 2011; Igbinosa et al., 2012; Farooq et al., 2017; Department of Health Human Services, 2019) respiratory infections (*Achromobacter*, *Cupriavidus*, *Delftia*, *Klebsiella*, *Legionella*, *Mycobacterium*, and *Sphingobacterium*) (Bagley, 1985; World Health Organization, 2007; Kalka-Moll et al., 2009; Lambiase et al., 2009; Neonakis et al., 2010; Bilgin et al., 2015; Swenson and Sadikot, 2015; Al Hamal et al., 2016), sepsis and bacteremia (*Gordonia*, *Lysinibacillus*, *Myroides*, *Shewanella*, and *Sphingomonas*) (Ryan and Adley, 2010; Sharma and Kalawat, 2010; Ramanan et al., 2013; Wenzler et al., 2015; Beharrysingh, 2017), opportunistic infections (*Citrobacter*, *Chryseobacterium*, *Morganella*, and *Stenotrophomonas*) (Ranjan and Ranjan, 2013; Liu et al., 2016; Imataki and Uemura, 2017; National Institutes of Health, 2018), and genera containing several antibiotic-resistant species (*Pedobacter*) (Viana et al., 2018) were reduced after ZVI-sand filtration. At the species level, *Aeromonas hydrophila*, *Arcobacter cryaerophilus*, *Bacillus cereus*, and *Plesiomonas shigelloides*, which cause diarrhea, vomiting, and gastroenteritis (Janda and Abbott, 1998; Janda et al., 2016; Barboza et al.,



2017; United States Department of Health Human Services, 2019), *Mycobacterium arupense* responsible for pulmonary infections (Neonakis et al., 2010; Al Hamal et al., 2016), *Eggerthella lenta* and *Elizabethkingia meningoseptica* which cause bacteremia (Gardiner et al., 2015; Shinha and Ahuja, 2015), and pathogens causing other severe infections—*Brevundimonas diminuta* (antibiotic-resistant opportunistic infections) (Han and Andrade, 2005), *Clostridium bifermentans* (necrotizing endometritis and empyema) (Edagiz et al., 2015; Hale et al., 2016), *Propionibacterium acnes* (opportunistic infections of the bones and joints, mouth, eye, and brain) (Perry and Lambert, 2011), and *Pseudomonas alcaligenes* (endocarditis and bloodstream infections) (Valenstein et al., 1983)—were detected at lower levels in ZVI-sand filtered reclaimed water samples compared to reclaimed water samples.

ZVI-sand filtration was also able to reduce the relative abundance of bacterial genera containing phytopathogenic species detected in our reclaimed water samples. Specifically, *Acidovorax* (bacterial fruit blotch on cucurbits) (Shetty et al., 2005), *Agrobacterium* (crown gall disease) (Moore et al., 1997), *Clavibacter* (bacterial wilt and canker in tomatoes and potatoes) (Gartemann et al., 2003), and *Erwinia* (fire blight on apples and pears, bacterial wilt in cucurbits, and wound infections in pea plants) (Huang et al., 2004) were reduced after ZVI-sand filtration.

A higher relative abundance of plant growth promoting bacteria were detected in ZVI-sand filtered reclaimed water compared to reclaimed water. Specifically, the nitrogen-fixing species *Azospirillum massiliensis* and *Pseudomonas stutzeri* (Lalucat et al., 2006; Cassán et al., 2015), and phosphate solubilizing species *Acinetobacter rhizosphaerae* (Gulati et al., 2009) were detected after ZVI-sand filtration. Bacterial belonging to the genus *Azospira*, consisting of nitrogen fixing species of bacteria (Bae et al., 2007), were much more relatively abundant in ZVI-sand filtered water samples. Furthermore, *Rhodospirillaceae*, purple non-sulfur bacteria that contain species (belonging to the genus *Azospirillum*) which have the potential to promote plant growth (Baldani et al., 2014), were detected in both reclaimed water and ZVI-sand-filtered reclaimed water samples. Therefore, the retention of, and in some cases, the increase in relative abundance of, plant growth-promoting bacteria after ZVI-sand filtration showed that ZVI may not necessarily reduce any potentially positive impacts that reclaimed water might have on plant growth. However, further analysis of the impact of ZVI-sand filtration on plant beneficial bacteria is required.

Concentration of *E. coli* in ZVI-Sand Filtered Reclaimed Water

The reduction of *E. coli* levels after ZVI-sand filtration were consistent with the findings of other studies of bacterial reduction

by ZVI (Lee et al., 2008; Diao and Yao, 2009; Mudd et al., 2011; Ingram et al., 2012; Marik et al., 2019). Micro-scale ZVI filtration achieved up to 4–5 log CFU *E. coli* O157:H7 inactivation in inoculated water (Mudd et al., 2011), and a significantly higher (6 log CFU/100 mL) reduction in the concentration of *E. coli* O157:H7 compared to sand filtration (0.49 CFU/100 mL) (Ingram et al., 2012).

Functional Potential of Bacterial Community in ZVI-Sand Filtered Reclaimed Water

ZVI compromises bacterial cell membrane permeability allowing Fe^{2+} to enter the bacterial cell and react with intracellular H_2O_2 resulting in the formation of highly reactive oxygen species (ROS) generating oxidative stress and denaturing macromolecules resulting in cell death (Lee et al., 2008). Bacterial cells can defend against ZVI toxicity by global (DNA repair, maintenance of metabolic robustness etc.) and oxidative stress response (cellular detoxification and iron homeostasis etc.) mechanisms and by reducing contact with ZVI through sporulation and biofilm formation (Lefevre et al., 2015; Seo et al., 2015). All of these mechanisms may have taken place within our ZVI-sand filter as described below.

In spite of the decrease observed in the expression of ORFs for genes associated with several siderophores and iron uptake proteins (Caspi et al., 2016; Bateman et al., 2017), the overexpression of ORFs for the *fur* gene (involved in iron-sensing transcription regulation), and genes associated with ferrous iron binding and transport (*feoB*, *galT*, *mntH*) (Caspi et al., 2016), was also observed after ZVI-sand filtration (Supplementary Tables 1, 2). Therefore, the potential abundance of iron ions may have resulted in their incorporation into the intracellular environment leading to the production of ROS within bacterial cells.

The potential uptake of iron into the intracellular environment may have resulted in the generation of H_2O_2 and superoxides (Supplementary Table 3). The log-fold expression of ORFs for *oxyR* (H_2O_2 sensor) and H_2O_2 -inducible genes (*ahpC*, *ahpF*, *dps*, *fur*, *grxA*, *katG*) (Seo et al., 2015; Caspi et al., 2016) was higher after ZVI-sand filtration. ORFs for genes encoding thioredoxins and glutathiones (*grxA*, *trxA*, *trxB*, *trxC*), endogenous H_2O_2 scavengers (*ahpC*, *ahpF*), peroxidases (*ccp*, *katG*, *yfcg*), oxidoreductases (*fpr*, *glpA*, *glpB*, *glpC*, *wrbA*), hydrogenases (*hyaA*, *hyaB*, *hyaC*, *hyfC*, *hyfG*, *lpd*), ubiquinol oxidases (*cydA*, *cydB*, *cydC*), and ORFs for superoxide dismutase (*sodB*), superoxide response (*fumC*, *ribA*, *yggE*) and superoxide and H_2O_2 removal genes (*ccmA*, *ccmB*, *ccmC*, *ccmD*, *ccmE*, *ccmF*, *ccmG*) (Caspi et al., 2016; Bateman et al., 2017) were overexpressed in ZVI-sand filtered reclaimed water.

Contact with ZVI may have resulted in altered environmental conditions, resulting in the activation of the global stress response system (Supplementary Table 4). The log-fold expression levels of ORFs for genes encoding proteins involved in general stress response regulation (*rpoS*), temperature shock (*cspA*, *cspD*, *rbfA*, *clpS*, *dnaK*, *grpE*, *oppA*, *pncB*, *pnp*, *rpoH*, *rseC*), osmoregulation

(*envZ*, *fixX*, *gshA*, *hchA*, *mglA*, *osmY*, *proV*, *proX*, *yciE*, *yiaO*), pH imbalance (*adiA*, *apaH*, *gadC*, *hdeA*, *nhaA*), and starvation (*cstA*, *yhhY*) (Caspi et al., 2016; Bateman et al., 2017) increased after ZVI-sand filtration.

An overexpression of mechanisms involving repair and metabolic response was also observed after ZVI-sand contact (Supplementary Table 5). Specifically, ORFs for genes involved with DNA repair (*casI*, *dam*, *dsdA*, *mutM*, *mutS*, *nfo*, *nth*, *phr*, *polA*, *recA*, *uvrA*, *uvrB*, *uvrC*, *xthA*), DNA synthesis and replication (*priB*, *purA*, *nrdB*, *nrdF*, *nrdH*), and metabolic response (*glgA*, *glpK*, *malE*, *malF*, *malG*, *pfkB*, *puuP*) (Caspi et al., 2016; Gamma-Castro et al., 2016; Bateman et al., 2017) were overexpressed after ZVI-sand filtration. The expression levels of genes which allow for H_2O_2 stress adaptability, namely, *mazF* (blocks protein synthesis), *dps* and *yaaA* (sequester iron to protect against DNA damage), *dmsB* and *dmsC*, (reverse oxidative stress damage), and *erpA*, *napB*, *napC*, *napF*, *napG*, *pgi*, *uxuA* (increases growth and metabolic processes) were higher after ZVI-sand filtration (Caspi et al., 2016; Gamma-Castro et al., 2016; Bateman et al., 2017).

Finally, ORFs for genes involved in processes that reduce ZVI contact (Supplementary Table 6) were also overexpressed in ZVI-sand filtered reclaimed water, namely, cell adhesion and biofilm formation (*ackA*, *bolA*, *csgD*, *csgE*, *csgF*, *csgG*, *fimA*, *glnA*, *htrE*, *nlpE*, *znuA*), dormancy process (*fau*), and cell wall synthesis (*ampD*, *blc*, *envC*, *fadD*, *lpxC*, *manA*, *mltB*, *mltF*, *mpl*, *ygeA*) (Gamma-Castro et al., 2016).

Study Summary, Limitations, and Future Research

Our findings demonstrate the ZVI-sand filter to be a potentially effective on-site treatment for conventionally treated reclaimed water for use in small-scale agricultural irrigation. The organic and inorganic contaminant remediation achieved by the ZVI-sand filter may have been due to a combined impact of chemical reduction and adsorption due to ZVI, the reactive oxygen species generated upon contact of bacterial cells with ZVI as well as the specialized community of denitrifying and sulfate reducing bacteria that may have developed within the ZVI-sand filter. Our ZVI-sand filter reduced or eliminated potentially pathogenic bacterial species in reclaimed water. *E. coli* populations were significantly reduced after ZVI-sand filtration and ZVI-sand filtration was successful in filtering cefoxitin- and tetracycline-resistant *E. coli* isolates from reclaimed water. The scope of our bacterial community analysis was fairly narrow due to the small sample size and the absence of a sand-only filter comparison. Our findings can serve as a basis toward more in-depth investigations that could address the longevity of ZVI-based filters as well as the effects of filtration on human and plant pathogenic bacteria in treated water, to name a few. Long-term, field-based studies that evaluate the effectiveness of the filter given fluctuating levels of contaminants over time should also be conducted before ZVI-sand filters can be adopted to provide point-of-use filtration of conventionally treated reclaimed water for agricultural use.

DATA AVAILABILITY STATEMENT

Sequence data generated and analyzed in this study were deposited with GenBank and linked to BioProject number PRJNA522745 in the NCBI BioProject database <https://www.ncbi.nlm.nih.gov/bioproject/>.

AUTHOR CONTRIBUTIONS

PK, AS, and MS conceived and planned the experiments. PK, AB, RB, EH, CE, and LD performed the experiments. PK, NO, and JP analyzed the data. EM, LH, JG, and DN assisted with data analysis. PK wrote the manuscript. AS and MS supervised the project. NO, AB, RB, LD, LH, EH, JP, JG, DN, CE, JVK, KK, PC, EM, MS, and AS provided critical feedback. All authors have approved the final article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2020.541921/full#supplementary-material>

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Species Sensitivity to Toxic Substances: Evolution, Ecology and Applications

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Because it is only possible to test chemicals for effects on a restricted range of species and exposure scenarios, ecotoxicologists are faced with a significant challenge of how to translate the measurements in model species into predictions of impacts for the wider range of species in ecosystems. Because of this challenge, within ecotoxicology there is no more fundamental aspect than to understand the nature of the traits that determine sensitivity. To account for the uncertainties of species extrapolations in risk assessment, “safety factors” or species sensitivity distributions are commonly used. While valuable as pragmatic tools, these approaches have no mechanistic grounding. Here we highlight how mechanistic information that is increasingly available for a range of traits can be used to understand and potentially predict species sensitivity to chemicals. We review current knowledge on how toxicokinetic, toxicodynamic, physiological, and ecological traits contribute to differences in sensitivity. We go on to discuss how this information is being used to make predictions of sensitivity using correlative and trait-based approaches, including comparisons of target receptor orthologs. Finally, we discuss how the emerging knowledge and associated tools can be used to enhance theoretical and applied ecotoxicological research through improvements in mechanistic modeling, predictive ecotoxicology, species sensitivity distribution development, mixture toxicity assessment, chemical design, biotechnology application and mechanistically informed monitoring.

Keywords: ecotoxicology, toxicokinetics, toxicodynamics, mechanism of action, predictive ecotoxicology

INTRODUCTION

Chemicals are the business of some of the largest industrial sectors. Whether as raw materials from mining, oil and gas, as agrochemicals, textile, plastics, cosmetics, personal care, cleaning or through use in the pharmaceutical industry, chemicals pervade every aspect of modern life. When in widespread use, it becomes almost inevitable that chemicals will find their way into the environment (Rockstrom et al., 2009). Routes may be through direct application and run-off of agrochemicals (Kohler and Triebkorn, 2013; Elias et al., 2018), intended or accidental industrial releases (Batty and Hallberg, 2010), or via waste and wastewater infrastructure, such as for down the drain products passing through wastewater treatment (Munoz et al., 2008; Gardner et al., 2012), or in leachates from landfills (Masoner et al., 2014; Kummerer et al., 2019). As well as their recognized health effects (Landrigan et al., 2018), pollutant chemicals can also affect wildlife

species and ecological communities (Hayes et al., 2018; Johnson et al., 2020), placing real costs on ecosystem functions and services (Wang et al., 2019). For example, Pretty et al. (2000) identified external costs of £120 M and £16 M for pesticide and fertilizer applications to the United Kingdom drinking water supply chain. Demonstrated, expected and even perceived impacts on ecosystem widely shape public opinion on chemical safety and use, even in those cases where no impacts may actually exist (i.e., where a source-pathway-receptor link cannot be established). Given the scale, diversity and potential costs, effective management of chemicals through risk assessment is critical for environmental management (Jenck et al., 2004).

To tackle issues in risk assessment, science-based chemical management faces the fundamental problem of how to extrapolate data produced for a small number of “standard” test species to predict impacts for a large number of species from different phyla present in an ecosystem that may differ in their physiology, diet and life-history traits. This challenge is relevant both in cases of prospective assessments to assess the likelihood of future hazard and risk (e.g., during the registration of a new substance, such as a pesticide) or for retrospective assessments of the impact of known chemical releases (e.g., through industrial emissions or an accidental release) on ecosystem structure and function (Faber et al., 2019). With >80,000 chemicals produced at >1 ton volume in Europe, testing even for a “representative” subset of “standard” species cannot be achieved in any sensible timescale. Defining “safe” contaminant concentrations for ecosystems, therefore, often relies on the use of an arbitrary “safety factor” (usually division of a toxicity metric, such as an LC₅₀, EC₅₀ or NOEC by 100 or 1,000) placed on data available often for only a small number of species (e.g., in aquatic risk assessment often only *Daphnia*, fish, algae) or at best species sensitivity distributions (SSDs) constructed with at most 10s of species (Posthuma et al., 2019).

This assessment factor approach is a pragmatic solution to address the uncertainties relating to lack of knowledge on the basis and range of species sensitivity. The approach has notable technical flaws, not just in that the size of assessment factor used is largely arbitrary, but also that the derivation of any value for regulatory application (e.g., a predicted no-effect concentration) is driven by the most sensitive species. As a consequence, hazard values derived using generic safety factors may sometime be over-precautionary, but in others may fail to protect vulnerable species (Lau et al., 2014). Failures of protection from the application of assessment factors have previously been found to occur in cases where a given chemical has an impact on a specific physiological trait, such as behavior, immuno-competence, genotoxicity and physiology that is not commonly measured in standard regulatory toxicity tests. Such unforeseen effects account for some of the most well-known impacts of chemicals on populations, such as tributyltin on marine mollusks (Matthiessen and Law, 2002); neonicotinoid pesticides for bees (Jin et al., 2015; Woodcock et al., 2016; Douglas et al., 2020); DDT on birds of prey (Newton and Wyllie, 1992); and the anti-inflammatory drug diclofenac on vulture populations (Oaks et al., 2004).

For the chemicals of highest concern, such as some trace metals, high volume use pesticides, selected pharmaceuticals and more recently some nanomaterials, specific research has resulted in the development of a richer ecotoxicological effects database covering a greater range of species (Posthuma et al., 2001; Semenzin et al., 2015). The additional data available for these well-studied chemicals almost always comprises results from short-term laboratory toxicity tests for an extended range of species. Even for the most investigated chemicals, the number of species for which toxicity has been assessed remains relatively small. For example, Mebane (2010) identified a data-set of 27 species within 21 genera for cadmium; while Zhao and Chen (2016) extracted sensitivity data for 207 freshwater and saltwater species for the organophosphate insecticide chlorpyrifos in what is probably the largest ecotoxicological effect database currently available for any substance. In those relatively few cases where the ecotoxicological effects literature is large and taxonomically broad, then the distribution of toxicity metrics (e.g., LC₅₀s, EC₅₀s, NOECs etc.) can be modeled as a statistical distribution to assess the pattern and order of species sensitivities. Such analyses have become the basis for sensitivity distribution (SSD) approaches in ecotoxicology (Posthuma et al., 2001).

The initial conceptualization of species sensitivity distributions as descriptive tools to summarize ecotoxicological data was founded on principles that species sensitivity varies in a symmetrical distribution on a log scale (Kooijman, 1987). This concept was later formulated into an approach for deriving ecosystem protection goals for chemical exposure by selecting a protection goal for accepted effect on a given percentage of species (Van Straalen and Denneman, 1989). Conventionally the threshold for effects routinely used with SSDs has been to protect 95% of species and, thereby, to accept an adverse effect on 5%. This protection goal is known as the hazardous concentration for 5% of species (the “HC5”). By using systematically collated toxicity data from multiple studies to gain a more complete estimation of response than achieved by a single study, SSD represents an early example of the application of meta-analysis approaches to environmental management problems. In the thirty years since their initial development, SSDs have passed into routine use in chemical policy and management.

In the period immediately after SSD conceptualization and integration into policy, a series of concerns with their technical and ecological bases were raised. An initial methodological issue concerned the shape of the distribution of sensitivity between species. Doubts were expressed on the choice of the log-logistic distribution as the best model to describe the pattern of rank order for sensitivity, even by Kooijman (1987) in the founding model paper. A comparison of SSD model fits has suggested that a model based on a Burr III distribution may provide a better fit to data than either log-normal or log-logistic fits (He et al., 2014). However, models using a Burr III distribution have computation drawbacks in their processing complexity, meaning that the log-logistic fit remains the dominant model form used in SSD studies (Xu et al., 2015).

A more fundamental issue with the use of SSDs for setting environmental protection goals concerns the selection of acceptable thresholds and the potential for effects on keystone

species (Hopkin, 1993). The framework for assessment assumes that the range of sensitivity of the tested species used to generate the SSD matches those of the species in the focus ecosystem. This is not a justified assumption, based on the fact that the majority of test data comes from laboratory species presenting similar traits of short generational time, rapid growth and high fecundity that are not replicated in many species in natural communities (Beaudouin and Pery, 2013). Evidence that some types of chemicals may strongly affect species in specific groups that undertake key ecosystem processes (e.g., herbicide effects on primary producers) have resulted in the development of species sensitivity distribution for only species from those taxa (King et al., 2015; Wang et al., 2015; Baillard et al., 2020). This selection, which can be based on taxonomy or function, avoids setting protection goals that disproportionately leave key functional species among the affected 5%, even when the protection goals for 95% of species is met (Hopkin, 1993; Forbes and Calow, 2002).

A further concern raised on the value of SSDs is whether the sensitivity of organisms tested in the laboratory matches that found among field species. Again aware of this issue, Kooijman (1987) identified disagreement between biologists on whether the species selected for testing were likely to be on average hardier or more susceptible than field species given their amenability for laboratory culturing and experimental use. To make this comparison, SSDs derived from laboratory toxicity data have been compared to community impacts in the field. Results have been found to both corroborate SSD predictions (e.g., Posthuma and DeZwart, 2006) and also to suggest that SSDs may underestimate the effect (e.g., Smetanova et al., 2014). Because of this remaining uncertainty, current risk assessment schemes, such as that for pesticides in the EU, do not use the unamended HC5 derived from an SSD as the protection goal (Brock et al., 2006). Instead, assessment factors scaled according to the amount of data used for SSD development are placed on the HC5. The need to include an additional assessment factor as a blunt tool to support precautionary analysis indicates that, while undoubtedly useful, descriptive SSD models provide only a partial solution to the pan-species problem in chemical risk assessment.

UNDERSTANDING THE BASIS OF DIFFERENTIAL SENSITIVITY

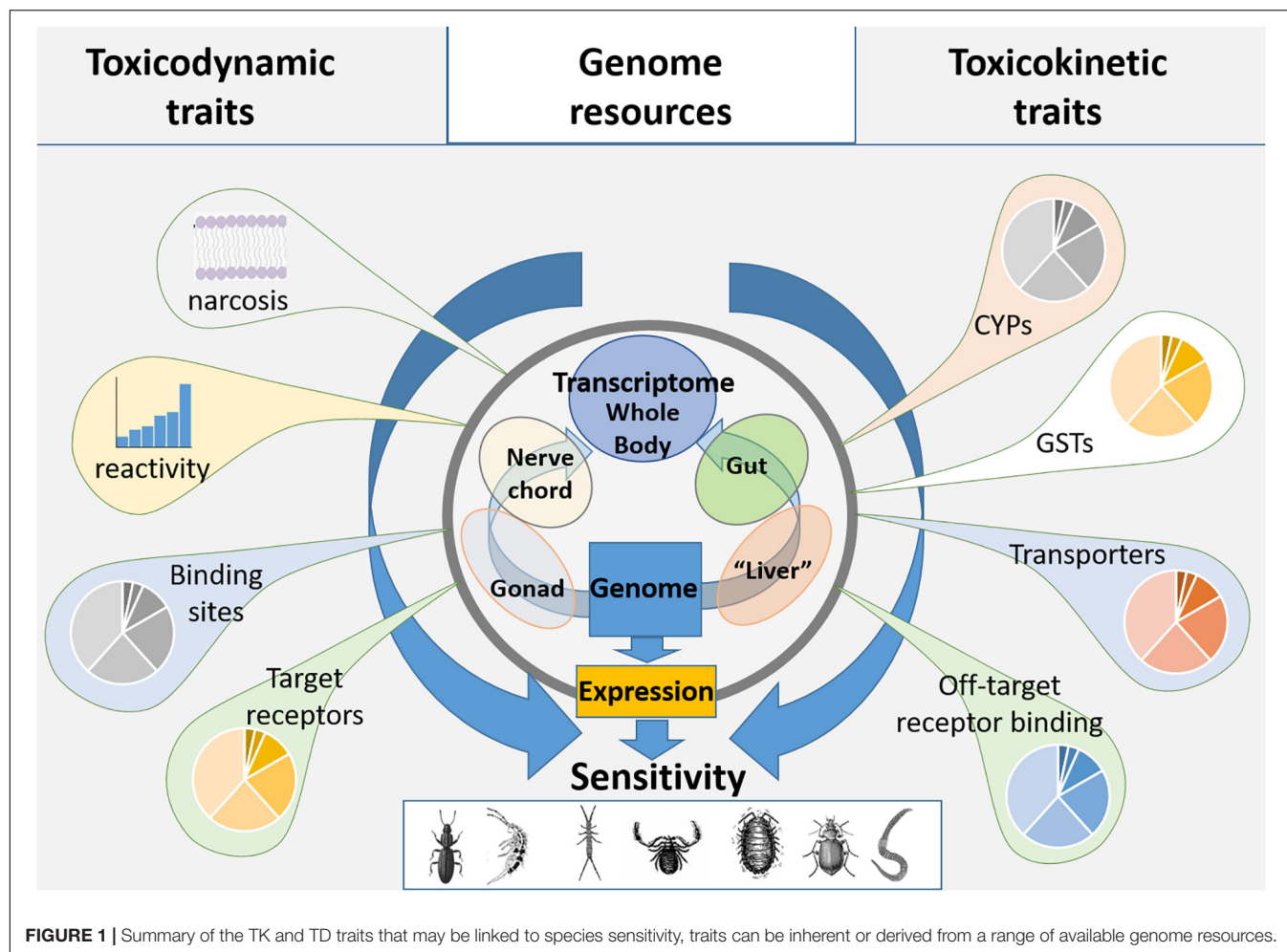
The basis of species sensitivity is that chemical exposure at a sufficient concentration imparts negative impacts on the physiology, behavior and ultimately life-history and population dynamics of an exposed species. This interaction of the chemical with the physiology of species has been recognized through the development of the adverse outcome pathways (AOPs) concept, an approach that combines existing knowledge of xenobiotic interactions with a specific biomolecule as the “molecular initiating event” (MIE) to apical endpoints across different levels of biological organization (Ankley et al., 2010). The AOP concept provides an approach to support more mechanistically driven toxicology including by the identification and analysis of conserved toxicokinetics (TK) and toxicodynamics (TD) pathways. Toxicokinetic pathways underlie

the Adsorption-Distribution-Metabolism-Excretion (ADME) traits that determine chemical concentration in organisms, and ultimately reaching target sites (McCarty and Mackay, 1993; Nyman et al., 2012). Toxicodynamics covers the processes of interaction between the chemical and the target and the resulting cascade of events leading to phenotypic change within the AOP (McCarty and Mackay, 1993; Nyman et al., 2012). An understanding of species TK and TD traits have a significant potential to contribute to support species read-across (Rivetti et al., 2020). Consequently, an understanding of the range of biochemical pathways that contribute to TK and TD traits can be informative for understanding the nature and severity of species response to chemical exposures (see **Figure 1** for trait organization and integration).

AOPs currently exist in a range of forms, from putative and partially characterized to well-described and fully validated (Coady et al., 2019). They range from qualitative to semi-quantitative and quantitative AOP (qAOP) models (Spinu et al., 2020). A fully developed qAOP can help guide the interpretation of comparative effects between species, as the link between exposure level and the degree of response of the adverse outcome is established. However, for the purposes of predicting species sensitivity, the application of qAOPs may be challenged by the very differences that they seek to investigate. In some cases, comparative analyses may be able to reliably attribute a mechanism and a scale of toxicant effects across species based on binding to the highly conserved MIE target. For example, a qAOP for an organophosphate could be plausibly transferred across species, due to the conservation of nervous system and of the target acetylcholinesterase receptors (Brockmeier et al., 2017), although even in this context variation may remain depending on species specific processes such as the relative irreversibility of acetylcholinesterase inhibition (Wallace and Herzberg, 1988). However, generalizing a qAOP to understand chemical sensitivity for species outside of the taxonomic range for which it has been formulated needs to be considered within different evolutionary and physiological contexts (Gunnarsson et al., 2008; Verbruggen et al., 2018). For example, for compounds that mimic estrogen, the target estrogen receptor sequences show more variance and may not even be present in some taxa (e.g., for *Daphnia*). Thus, qAOPs for mechanisms of effect applied to species with a different receptor structure may lead to highly erroneous sensitivity prediction for a chemical working through this pathway. Understanding the contribution of evolutionary processes and traits to sensitivity is, thus, needed to understand the contexts into which qAOPs can or cannot be applied.

THE CONTRIBUTION OF EVOLUTIONARY PROCESS TO SPECIES SENSITIVITY

Microevolutionary or macroevolutionary changes that impact on TK and TD traits would be anticipated to modify sensitivity by changing either target site exposure or chemical-target site interactions (Klerks et al., 2011). There are some well-established examples of known microevolutionary changes that



can modify sensitivity. The development of antibiotic resistance in microbial populations is such a case. Antibiotic resistance can arise from exposure to specifically designed antimicrobials or through cross tolerance to other contaminants (Bell et al., 2014; Xie et al., 2018). Indeed there is growing evidence of a co-evolutionary process of antimicrobial resistance arising from environmental exposure to organic pollutants and even metals leading to the development of clinically relevant resistance (Pal et al., 2015). Because of its obvious therapeutic relevance, antibiotic resistance is one of the most widely understood microevolutionary processes for any group of toxicants. The many mechanisms identified leading to resistance include changes in both TK traits, such as acquisition or modification of cellular chemical transport systems that remove chemicals from cells, and also TD processes such as modifications to the structural confirmation and functional characteristics of cellular targets (Davies and Davies, 2010). These widely observed and well understood trajectories establish a dominant paradigm of TK and TD change as causes of differences in population sensitivity to toxicants.

Understanding the microevolution for TK and TD traits also provides a rationale for characterizing pesticide resistance. The

development of insecticide resistance was initially documented for inorganic pesticides even during their early use (Melandar, 1914). In the intervening years, the diversification of pesticides has been associated with expansion in the range of species exhibiting resistance. Studies that have addressed the causes of resistance identified two major non-competing mechanisms: increased metabolic detoxification and decreased sensitivity of the target-site that represent, respectively, TK and TD changes (Van Leeuwen et al., 2010; Liu, 2015; Siegwart et al., 2017). Increased metabolic detoxification arises following mutational changes that increase the expression or efficiency of one or more enzymes involved in endogenous pesticide metabolism or excretion. Typically esterases are the main enzymes involved, although resistance has also been linked to changes in glutathione-s-transferase expression and activity (Hemingway et al., 2004). Target site insensitivity also has multiple mechanistic bases, including point mutations that change structural confirmation and, thereby, ligand-receptor affinity (Hemingway et al., 2004). There is obvious value in identifying the dominant mechanisms of resistance as this can support mitigation. However, as TK associated changes to metabolism and TD associated changes to target site interactions

are effectively independent, many cases of insecticide resistance show characteristics of both types of response (Reyes et al., 2007; Siegwart et al., 2017).

Microevolutionary processes leading to the development of tolerance have also been shown to shape population responses to inorganic chemicals, e.g., trace metal exposures. There is a significant body of evidence for the development of population tolerances to metals in long-term exposed invertebrates, plants and microbial populations (see review of Posthuma and Van Straalen, 1993; Meharg, 2005; Singh et al., 2016; Chandrangsu et al., 2017). Tolerance to metal exposure in animals has been associated with microevolutionary processes that change the expression of metallothioneins and in plants to changes in phytochelatin synthases that together alter the TK processes of metal uptake, transport and detoxification in the adapted species (Dallinger and Hockner, 2013; Weng and Wang, 2014; Zanella et al., 2016; Shaw et al., 2019; Lwalaba et al., 2020; Mekawy et al., 2020). For example, in populations of the Collembola *O. cinctus*, Costa et al. (2012) showed that a metal-tolerant phenotype was mainly influenced by *cis*-genetic factors acting on metallothionein expression, with specific promoter genotypes found dominantly in cadmium tolerant populations. Observations in a further springtail species *Folsomia candida* also supported a role of constitutive metallothionein expression in cadmium tolerance, influenced by both *trans* and *cis* regulatory variation (Costa et al., 2012).

While mechanisms of adaptation can become hard wired into the genome, more transient changes in the epigenome have also been associated with the capacity of organisms to respond to chemicals. Such epigenetic changes appear to be associated with downstream modification of both TK and TD processes (Bruce et al., 2007; Mirbahai and Chipman, 2014; Brander et al., 2017; Horemans et al., 2019). Components of the epigenome, such as DNA methylation, are emerging as the interface between the static genome and the dynamic and changing environment in which species live and persist (Marczylo et al., 2016; Horemans et al., 2019). Evidence that epigenetic marks induced by adverse conditions encountered by the parents, such as stressor exposure can be partly stable across generations is beginning to build in plants, vertebrates and more recently in invertebrates (against prior expectations) leading to transgenerational heritable changes in offspring (Greer et al., 2011; Pecinka and Scheid, 2012; Uller et al., 2015; Klosin et al., 2017; Stassen et al., 2018). Examples include the observation of the transfer of stress tolerance associated with gene expression changes linked with altered trimethylation of histone H3 lysine 9, identified as a mechanisms for transgenerational inheritance of the maintenance of stressor responses in *C. elegans* (Klosin et al., 2017) and heritable changes in a DNA methylation in *Arabidopsis* in response to nickel exposure (Li et al., 2015). A role of epigenetic mechanisms in heritable effects has also been identified in *Daphnia magna* in response to ionizing radiation (Jeremias et al., 2018; Trijau et al., 2018), silver nanoparticles (Ellis et al., 2020), uranium (Dias et al., 2008; Massarin et al., 2011; Dutilleul et al., 2014; Goussen et al., 2015) and a DNA methylation inhibitor (Lindeman et al.,

2019). How these epigenetic mechanisms are linked to sensitivity is currently unknown. However, evidence of altered DNA methylation in an arsenic tolerant populations of the earthworm *L. rubellus* (Kille et al., 2013) that also express high levels of phytochelatin (Liebeke et al., 2013) suggests a role in regulating detoxification mechanisms.

PHYSIOLOGICAL TRAITS AS DETERMINANTS OF SPECIES SENSITIVITY

Multiple studies have identified how species biological traits can lead to differences in responses to chemical exposure. An understanding of the evolutionary background of species and especially their shared and distinct TK and TD traits can, thus, contribute greatly to our understanding of their role as drivers of sensitivity. The role of TK/TD and AOP traits in toxicity can be seen as orthogonal (**Figure 2**). TK traits related to adsorption distribution, metabolism and excretion determine the concentration of the chemical entering into the organisms, as well as the distribution to and removal from the target site at which the MIE occurs. Examples for traits that may affect TK rates leading to changes in exposure include species behavior (e.g., feeding traits), anatomical traits (e.g., surface area-to-body mass scaling relationship), metabolic capacities (e.g., direct via CYP divergence or indirect via the AH-receptor that activates CYP expression), stress-response capacities (e.g., Metallothionein/phytochelatin expression patterns) and microbiome differences (e.g., presence of gut bacteria known to degrade xenobiotic chemicals). The specific nature of the TD interactions cover mechanisms through which chemicals interact at target sites to result, triggering a MIE that leads to the biochemical, cellular, tissue organism that together comprise the toxicant AOP (**Figure 2**). Examples of traits that may affect TK rates include lipid content variations affecting the nature of narcotic interactions, presence/absence of target receptors to determine whether species experience toxicity through specific trigger AOPs, gene duplication and loss varying receptor ortholog compliment and target receptor sequence leading to binding site differences that affect ligand-receptor interaction strengths. Evidence relating to the importance of such changes affecting sensitivity are detailed below.

Toxicokinetic Traits and Species Sensitivity

Species traits can govern the extent to which exposure may occur. At the simplest level, the movement of species through the landscape and dietary choices can influence exposure. For example, Woodcock et al. (2016) identified that bee species foraging on oilseed rape treated with systemic neonicotinoids showed greater population declines than non-oilseed rape feeding species not similarly subject to insecticide exposure. In this case there was no indication that different species population trajectories were driven by inherent sensitivity, but rather by feeding traits that governed exposure. A somewhat analogous

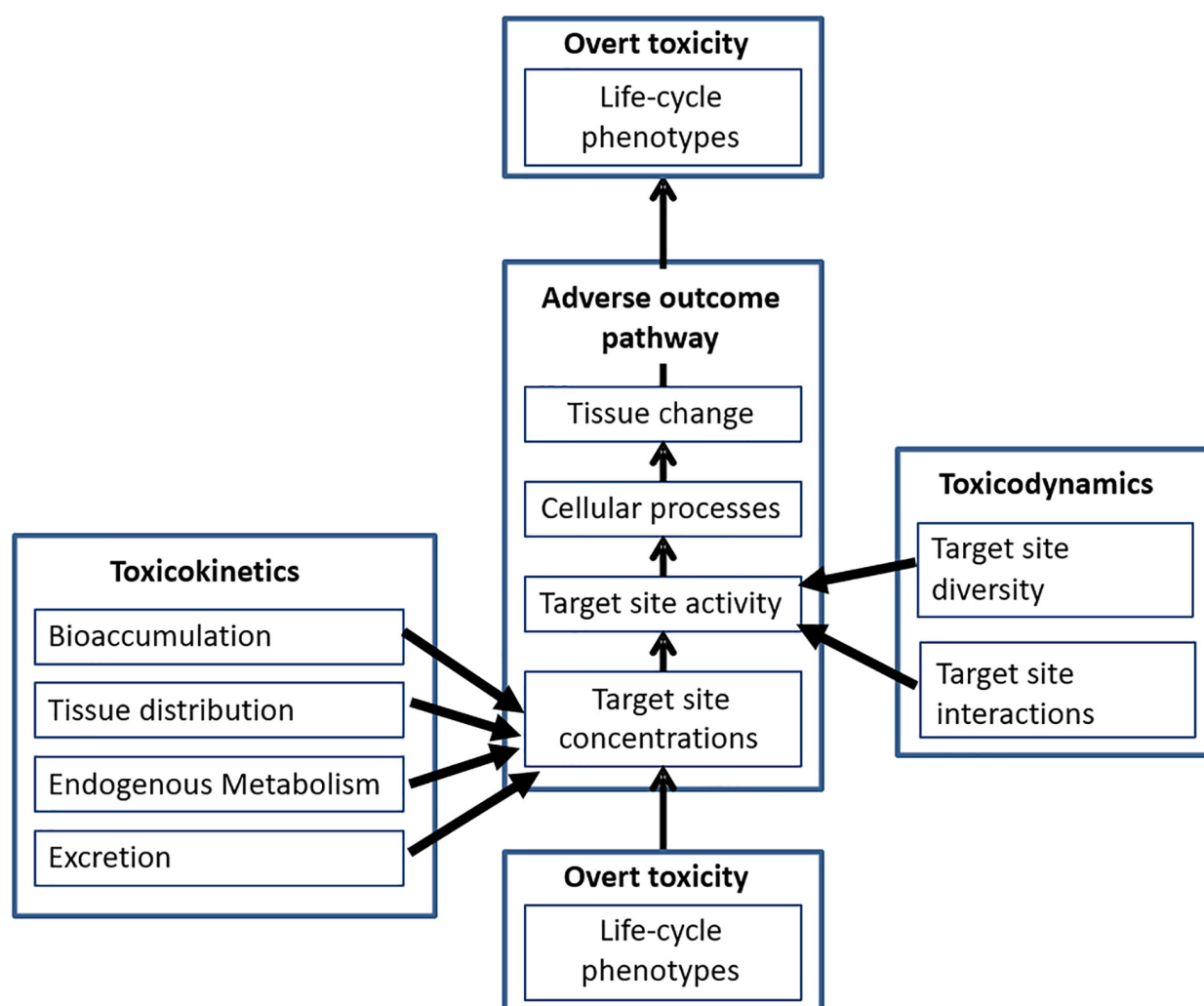


FIGURE 2 | The integration of TK and TD traits within the AOP framework, TK traits determine the concentration of the chemical reaching the site, TD traits determine the nature of the target site and influence downstream AOP processes leading ultimately to overt toxicity.

situation exists for other established cases of species vulnerability, such as top predator (e.g., predatory birds) exposure to persistent, bioaccumulative and toxic chemicals through food chain biomagnification. However, within the highly exposed species groups, differences in vulnerability may still occur as the product of physiological traits leading to differences in inherent sensitivity (Farmahin et al., 2013; Heard et al., 2017).

A number of studies have attempted to link variations in species anatomical or physiological traits to chemical sensitivity (Baird and Van den Brink, 2007; Rubach et al., 2012, 2010a; Qiu et al., 2014; Gergs et al., 2015; Rico and Van den Brink, 2015; Van den Berg et al., 2019; Dalhoff et al., 2020). Most commonly the traits available and used have been either taxonomic, anatomical (e.g., body size and weight, presence of external respiratory surfaces), life-history (e.g., life-span, reproductive strategy) or ecological (life-stage specific habitat, feeding guild), although Rubach et al. (2012) and Van den Berg et al. (2019) both included measured TK traits and Dalhoff et al. (2020) included TK and TD

parameters derived from modeling. Relationships that link both single and also multiple traits to sensitivity have been identified. For example, Gergs et al. (2015) found a relationship between sensitivity and body size, which has been linked to possible effects on TK traits for Triphenyltin hydroxide through a surface area to body mass scaling relationship (Rubach et al., 2010a). Dalhoff et al. (2020) also found a strong correlation between surface area/volume and TK parameters (sorption and uptake rate constants and the resulting BCF) for a pyrethroid insecticide. However, in this case, there was no correlation between any TK parameter and sensitivity. Instead sensitivity was correlated with “killing rate,” which is the major TD parameter derived from the General Unified Theory Reduced Stochastic Death (GUTS-RED-SD) TK-TD model. This finding suggests that species variations at the target site, or associated with different AOP components, may act as the major drivers of arthropod pyrethroid sensitivity rather than any ADME parameter. Despite some progress, not all trait-based assessments have succeeded in identifying links between

specific species characteristics and sensitivity. The absence of such simple relationships highlights the complexity of potential predictive model development due to factors such as multiple trait contributions (Qiu et al., 2014), lack of relevant trait data for key intrinsic characteristics (Rubach et al., 2010a; Van den Berg et al., 2019), and the need to better consider TK (and TD) traits within models (Gergs et al., 2015).

Although trends across a wide range of taxa have not been established, more focussed species specific comparisons have identified cases in which TK traits have been shown to contribute to sensitivity difference. Such an example is the exposure of two cryptic species of *Capitella* to the PAH, fluoranthene (Selck et al., 2003; Li et al., 2004). In this polychaete annelid, higher fluoranthene biotransformation capability was found in *Capitella* sp. I than in its closely related sibling species *Capitella* sp. S. (Selck et al., 2003). Between the two polychaetes, Li et al. (2004) identified two novel cytochrome p450s present in *Capitella* sp. I (CYP331A1 and CYP4AT1), both potentially capable of PAH biotransformation, that were not found in *Capitella* sp. S. These two enzymes supported a higher rate of fluoranthene biotransformation in *Capitella* sp. I that could in turn explain the lower sensitivity of the species for fluoranthene compared to *Capitella* sp. S. (Bach et al., 2005). For lead, Andre et al. (2010) identified differences in the potential for two cryptic species of the earthworm *L. rubellus* to persist in polluted soils could be linked to species difference in calcium metabolism. Specifically, sequencing of the SERCA Ca-transport gene in the two cryptic clades revealed mutations in the protein cytosolic domain likely to contribute to differences in lead accumulation and internal sequestration, differences expected to influence sensitivity under different soil pH conditions.

A further set of examples of the role of TK trait differences for sensitivity comes from studying the responses of bird species to dioxin exposure. The initial mechanisms of dioxin congener interactions leading to toxicity are comparatively well known. Initially dioxins act as a ligand and bind to the aryl-hydrocarbon receptor (AH-receptor). This results in co-chaperone dissociation and heterodimerization with the AhR nuclear translocator (ARNT) which acts as a transcription factor, most notably for biotransformation enzymes such as cytochrome P450 1A1 (Denison et al., 2011). Gene expression changes resulting from AH-receptor mediated transcriptional activation in birds has been convincingly related to a range of pathologies linked to DNA damage, changes in hormone cycling, teratogenicity, neurotoxicity and carcinogenesis (Mandal, 2005). Binding studies have indicated that ligands with a low inhibitory constant induce cytochrome P450 1A1 expression at lower concentrations and this expression and activity is associated with higher potency for these compounds through the production of reactive metabolites (Hestermann et al., 2000). Based on this mechanism, expectations are that high ligand binding to species specific AH-receptor sequences result in higher cytochrome P450 1A1 expression and activity, that would result in greater toxicity through metabolite production and downstream effects.

The link between AH-receptor sequence variations and dioxin sensitivity has been convincingly illustrated in comparative

studies with bird species. Observations that some species of piscivorous birds (e.g., the common tern *Sterna hirundo*) are highly exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related halogenated compounds but show limited effects have been linked to their lower sensitivity (up to 250-fold) to these chemicals compared to chicken (*Gallus gallus*), a species commonly used for toxicity testing. The expressed AH-receptor from *Sterna hirundo* was found to show lower TCDD binding affinity and exhibits a reduced ability to support TCDD-dependent transactivation as compared to chicken AH-receptors (Farmahin et al., 2013). A further analysis of avian AH-receptors revealed particular amino acid sequence polymorphisms in the ligand-binding domain at sites 324 and 380 that were strongly linked to the reduced activation potential (Farmahin et al., 2013). This model of AH-receptor sequence mediated variation in sensitivity was further tested through sequence analysis and *in ovo* exposure using a luciferase reporter gene assay to measure cytochrome P4501A induction (Farmahin et al., 2013). Although further polymorphisms between species were found for different AH-receptor residues, only those at the two sites identified from the initial study (324 and 380) affected luciferase activation as linked to toxicity (Farmahin et al., 2013). Indications of a relationship between AH-receptor mediated gene expression and dioxin and halogenated organic toxicity in fish species suggests the link between binding domain sequence variation and sensitivity may extend beyond bird species (Doering et al., 2018).

The potential vulnerability of bee species to xenobiotic exposure, as illustrated by the recent cases of neonicotinoid impacts (Rundlof et al., 2015; Woodcock et al., 2017; Main et al., 2020), has been linked to the constrained metabolic potential of bee species for these insecticides. Relative to most other insects, sequenced bee genomes have a deficit of detoxification genes from the Phase I, II, and III gene families (Berenbaum and Johnson, 2015). For example, the honey bee *Apis mellifera* has 46 identified cytochrome P450 genes and the bumblebee *Bombus huntii* 44 compared to 52–92 for a range of other insect species (e.g., 85 for *Drosophila melanogaster*). The reasons for the reduced complement of metabolic enzymes in honey bees is not established. However, it is suggested that detoxification in eusocial bees may be supplemented by behaviors that produce a “social detoxification system.” These include forager discrimination, dilution by pollen mixing, and food processing through microbial fermentation, all of which might lower the number or quantity of ingested chemicals that require detoxification (Berenbaum and Johnson, 2015). The reduced enzymatic potential for biotransformation in bees has been proposed as a key trait that may engender higher sensitivity to some individual xenobiotics (Berenbaum and Johnson, 2015). Such comparative sensitivity of bees compared to other insects is supported by meta-analysis showing the sensitivity for honey bees across commonly tested insecticide classes (Hardstone and Scott, 2010).

The potential role of specific cytochrome P450s in bee sensitivity to xenobiotics has been shown in comparative toxicity studies for the neonicotinoids imidacloprid and thiacloprid for *Apis mellifera* and *Bombus terrestris*. Of the two insecticides, thiacloprid has the lowest toxicity to both species. In *Apis*

mellifera, this compound is more rapidly metabolized by the single Cytochrome P450 (CYP9Q3), identified as the major Phase I metabolic enzymes for this class of insecticide (Manjon et al., 2018). Mining of an available *Bombus terrestris* genome identified five orthologs of *Apis mellifera* CYP9Q3, of which two (CYP9Q4, CYP9Q5) were found to metabolize thiacloprid, but not imidacloprid (Manjon et al., 2018). This was consistent with a lower toxicity of thiacloprid than imidacloprid in bumblebees. The affinity of the family of cytochrome P450 enzymes for the varoicides tau-fluvalinate and coumaphos has also been linked to the low sensitivity of *Apis mellifera* to these compounds compared to their mite targets (Mao et al., 2011). Further, recent work has identified functionally conserved metabolic enzymes in the solitary bee *Osmia bicornis* that play a critical role in the biotransformation of pesticides including neonicotinoids, which in turn determines the sensitivity of this species to exposure (Beadle et al., 2019).

The functional role of TK traits in chlorpyrifos sensitivity was investigated across a range of 15 taxonomically varied freshwater arthropod species (Rubach et al., 2010b). Differences in TK traits influencing both uptake and elimination rates were found. Those TK traits relating to uptake, elimination and bioconcentration were compared to sensitivity (expressed as log EC₅₀). Significant negative relationships were found for the kinetic parameters, but not for bioconcentration factor values. Perhaps most surprising was the significant negative relationship of elimination rate and sensitivity. Expectation may have been that higher biotransformation leading to elimination would be linked to lower sensitivity, as for insecticides and adapted *Capitella* populations exposed to PAHs (Selck et al., 2003; Bach et al., 2005). However, key to understanding this relationship may be the requirement for chlorpyrifos to be metabolized to chlorpyrifos-oxon, a more potent acetylcholinesterase inhibitor, to initiate toxicity. Hence, species with high metabolism may increase internal chlorpyrifos-oxon levels leading to greater inhibition and toxicity. Separately, uptake and elimination traits accounted for 38 and 28% of the differences in species sensitivity indicating that, although important, the predictive power of such TK trait measurements was not high (Rubach et al., 2012). The limited value of TK traits for sensitivity prediction has also been demonstrated in a study for cypermethrin (Dalhoff et al., 2020), further questioning the importance of their role.

Metallothioneins, other metal binding proteins and small molecular weight metabolites (e.g., phytochelatins and glutathione) can all play a role as determinants of TK traits for metal exposures. The induction of metallothionein and other metal chaperones following exposure has been widely demonstrated (Scott-Fordsmand and Weeks, 1998; Weeks et al., 2004; Amiard et al., 2006), and as discussed above, basal metallothionein expression has also been linked to metal tolerance (Deeds and Klerks, 1999; Costa et al., 2012). Hence, there is expectation that basal metallothionein expression levels and induction potential could be linked to sensitivity (Shaw et al., 2019). Although systematic studies are lacking, small scale studies have highlighted a potential role for metal binding systems in species response to exposure. For example, a role for metal binding proteins to sequester accumulated metals in a quasi-inert

fraction has been suggested to play a role in earthworm species sensitivity to cadmium (Fourie et al., 2007). Further, studies in three marine species demonstrated a higher sensitivity to copper for the common limpet (*Patella vulgata*) could be explained by lower metallothionein expression as compared to the relatively insensitive shore crab (*Carcinus maenas*) and blue mussel (*Mytilus edulis*) (Brown et al., 2004). Differences in the extent of production, oligomer length and granular binding of phytochelatins has also been linked to sensitivity in two algal species (*Chlamydomonas reinhardtii* and *Pseudokirchneriella subcapitata*) to cadmium (Lavoie et al., 2009). In contrast to these positive results, studying the promoter region of fish species, Olsson and Kille (1997) found that metallothionein locus induction was not quantitatively linked to sensitivity, indicating that this is not a ubiquitous trend.

A final emerging area of research relevant to understanding the contribution of TK traits to sensitivity is the potential contribution of the host associated microbiome. There is a growing understanding that a multiplicity of interactions linking host organisms to their microbiomes (McFall-Ngai et al., 2013) through consortia involvement in a range of physiological processes (Lee and Hase, 2014). Bacteria in the microbiome have been associated with a number of processes that influence xenobiotic TK fate and exposure for the host. For example, perturbation of the mammalian gut microbiome has been shown to alter the concentrations of inorganic arsenic metabolites present in urine in laboratory studies (Lu et al., 2013). These changes in arsenic speciation in individuals with altered gut microbiomes have been linked to an increased sensitivity to arsenic toxicity (Chi et al., 2019). At present relatively little is known of the role of the gut microbiome in modifying arsenic speciation in natural populations. However, observations of the presence of methylated and organoarsenic forms in a range of aquatic and terrestrial species points to a role of bacteria, perhaps in conjunction with host metabolic capacity, in arsenic handling (Langdon et al., 2002; Rahman et al., 2012; Liebeke et al., 2013).

The host associated microbiome has also been shown to be involved in the degradation of assimilated organic chemicals. There is growing evidence that in some cases, removal of the host microbiome through treatment with antibiotics can change biotransformation, potentially resulting in significant changes in host pesticide susceptibility (Daisley et al., 2018; Fernandez et al., 2019). Bacterial species isolated from the gut of the diamondback moth *Plutella xylostella* have been shown to degrade a range of xenobiotic chemicals (Ramya et al., 2016), suggesting mechanisms through which species and populations may vary in sensitivity based on their potential to harbor such degradative isolates. Further, atrazine exposure has been found to result in heritable changes in the wasp gut microbiome that result in increased rates of metabolism (Wang G. H. et al., 2020). The potential for populations to develop tolerance to pesticide exposure, has been linked to an increased metabolic capacity of the gut flora, findings further indicating how possession of a specific microbiome may alter susceptibility (Kikuchi et al., 2012; Cheng et al., 2017). This is an emerging area and given the critical role of bacteria species in chemical biotransformation in environmental media, it seems likely that the capacity of species

to harbor different symbionts may emerge as key determinants of TK traits (Gressel, 2018). Hence the functioning of the gut microbiome may remain an important area for ecotoxicological research (Evariste et al., 2019).

Toxicodynamic Traits and Species Sensitivity

A second set of physiological components that can contribute to species sensitivity are related to TD traits. As the first critical interface of the chemical with the AOP, the nature and strength of the MIE has the potential to act as a key determinant of a species response to exposure, with the caveat that the receptors involved may not be relevant for all species and cases. For chemicals with non-specific mechanisms of action, toxicity may result from chemical interactions with a range of targets, such as multiple protein thiol binding sites for metals or more generalized membrane interactions for non-polar narcotics. Conversely, for some specifically acting substances (e.g., pesticides, pharmaceuticals) the MIE may be associated with more specific interactions identifiable from knowledge of the substance's known mechanism of action. These more specific TD interactions may have a greater potential to vary between species.

Non-polar organic chemicals are proposed to act through a narcotic mechanism based on interactions with lipid bilayers leading to changes in the cell membrane or its associated proteins (Escher et al., 2008). Since cell lipid bilayers have a conserved structure, such interactions are potentially generalized across species. The mechanism of action of narcotics through lipid membrane interactions underpins phase partitioning based theories such as the target lipid model. The approach is based on the assumption that toxicity occurs when the chemical concentration in target lipids in biological membranes reach a threshold concentration (Di Toro et al., 2000). A key model assumption is that this concentration is species specific, rather than universal. Traits based studies have established differences in species responses based on lipid content, with low lipid levels linked to higher sensitivity, such as for petroleum hydrocarbons in soil invertebrates (Gainer et al., 2018) and the organophosphate fenitrothion in freshwater phytoplankton species, in which this pesticide likely exhibits narcotic toxicity (Kent and Currie, 1995). However, lipid composition currently provides only a partial explanation for the sensitivity indicated by the range of species-specific critical target lipid concentrations (Di Toro et al., 2000). Further, traits such as the presence of lipid reserves may further complicate such assessments, because these can act as a reservoir for organic chemical accumulation that may reduce membrane concentrations and also provide protection from narcosis. As such, the interactions of the chemical with the other lipid pools may act as a TK mechanism to reduce exposure of the chemical to the membrane lipid that acts as the primary TD site of action for the narcotic effect.

For chemicals such as pesticides and pharmaceuticals that have a known mechanism of action, toxicity results from interactions with a target receptor to elicit downstream responses. For many, but far from all, pesticides, biocides and human and veterinary medicines, the nature of these interactions, at least

for the target species (e.g., humans for medical pharmaceuticals, insect pests for insecticides etc.) is often known. As increasing genome resources become available¹, it has become possible to screen non-target species for chemical receptor orthologs (Gunnarsson et al., 2008; LaLone et al., 2016; Santos et al., 2017; Verbruggen et al., 2018). The principal is that the toxicity of chemicals will be greater in those species possessing a receptor ortholog relevant to the mechanism of action compared to those species that do not. This provides a concept of response equivalence in different species resulting from a common receptor complement that can be used for designing monitoring and testing programs based on *in vitro* methods for hazard identification (Bowes et al., 2012; LaLone et al., 2018; Blackwell et al., 2019).

For human pharmaceuticals, the taxonomic conservation of receptor orthologs in species has been explicitly linked to relative sensitivity. For example, for the statin drug target HMG-CoA reductase, orthologs were predicted in almost all eukaryotic species, archaea, and in certain true bacteria, consistent with inhibitory effects of statins on fungal species growth and on HMG-CoA reductase activity across different plant and invertebrate species (see Gunnarsson et al., 2008). For the sodium dependent serotonin transporter, orthologs are found in vertebrate and arthropod species consistent with specific responses of zebrafish, *Drosophila* and a flatworm to exposure to serotonin reuptake inhibitors, such as fluoxetine, that target this receptor (Gunnarsson et al., 2008). This contrasts with the apparent insensitivity of plants and bacteria to such chemicals, in which orthologs of this receptor are missing (see Gunnarsson et al., 2008).

The potential application of the ortholog approach can be extended beyond human pharmaceuticals to chemicals such as pesticides and biocides that also have known mechanisms of action targeting specific receptors. As an example, it has been shown that the nematode *C. elegans* has low sensitivity to pyrethroid insecticides (Svendsen et al., 2010). Analysis for this species indicates that, despite the presence of most major ion channel families, there are no predicted voltage-activated sodium channel orthologs (the known target for pyrethroids) in the *C. elegans* genome (Bargmann, 1998). Going beyond ortholog presence, the analysis of sequence can also highlight potential drivers of sensitivity. For example within species, comparative analysis of resistant strains can highlight key mutational changes in receptors leading to differences in population sensitivity increasing our knowledge about traits underlying differential sensitivity for between species sensitivity prediction.

As examples of ortholog and sequence analyses, sensitivity to organotin as an endocrine disruptor has been linked to the emergence of multiple peroxisome proliferation activation receptors (PPAR) orthologs early in the invertebrate lineage (named PPAR α , β , and γ). Specifically, the duplicated PPAR genes coincide with the acquisition of TBT sensitivity. In contrast, the single unduplicated PPAR copy in the invertebrate chordate amphioxus is irresponsive to TBT. This simple picture is slightly complicated by TBT insensitivity in some teleosts, but this is

¹<https://www.sanger.ac.uk/science/programmes/tree-of-life>

thought to be a consequence of ligand binding pocket mutations in specific species (Capitao et al., 2018). Similarly, comparative assessment of the potential for aromatase inhibition related to sequence variations has also been used to identify the range of potential sensitivities of fish species to endocrine disrupting chemicals (Doering et al., 2019a,b). Changes to the ligand binding domain of the nAChR have also been convincingly associated with altered sensitivity for species to neonicotinoid exposure. Specifically, homology modeling suggests that replacement of an arginine (associated with high binding) in a critical region of the ligand binding domain in various tick species may contribute to their lower imidacloprid sensitivity (Erdmanis et al., 2012). Furthermore, mutation of the equivalent arginine to threonine (associated with lower binding) is thought to underlie increased neonicotinoid resistance in a strain of the aphid *Myzus persicae* (Bass et al., 2011).

A complexity that may arise when using the presence of gene family representatives as simple determinants of sensitivity include cases where orthologs retain sequence homology but have evolved divergent roles outside the target function or tissue. As an example, earthworms have comparatively lower sensitivities to the organophosphate chlorpyrifos (Ma and Bodt, 1993) than soil arthropod species such as springtails (Broerse and van Gestel, 2010; Jegede et al., 2017; Kamoun et al., 2018). Earthworms do not lack the target acetylcholinesterases in the nervous system. Indeed, there is evidence that the gene family has expanded, with multiple orthologs present that show ligand binding domains conserved with those in susceptible insects. However, the chlorpyrifos-oxon binds and inhibits the activity of a range of esterases, including other cholinesterases (ChEs) and carboxylesterases (CbEs). Tissue specific analysis of earthworms has shown high ChE and CbE activity in a range of non-neural tissues, with notably high ChE and CbE activities in the pharynx and gut, respectively (Vejares et al., 2010). The activity of these non-neural CbE/ChEs are inhibited by organophosphates indicating their capacity to bind the chlorpyrifos-oxon (Sanchez-Hernandez, 2010; Vejares et al., 2010; Sanchez-Hernandez et al., 2018). This has led to the suggestion that the binding of the chlorpyrifos-oxon by these gut CbEs may prevent the molecule interacting with neural targets (Sanchez-Hernandez, 2010; Vejares et al., 2010). In this way, the gut system may act as a buffer against nervous system exposure, leading to lower sensitivity. As these non-neural receptors are thought to influence the extent of chlorpyrifos-oxon reaching the critical neural target, they could technically be classified as a TK, rather than a TD, trait. It is also worth noting that although the toxicity associated with non-neural CbE/ChE inhibition is lower than that associated with the neuronal receptor, inhibition of non-neural receptors still has non-trivial effects on the function of these non-neural tissues (Sanchez-Hernandez et al., 2018). Such characteristics mean that these gut esterase enzymes may bind chlorpyrifos and in doing so prevent the molecule interacting with neural targets. In this way, the gut system may act as a buffer against nervous system exposure, leading to lower sensitivity. Ultimately, through such mechanisms, what may be identified as a receptor involved in TD process, may also take a role in TK processes

linked to chemical distribution that affects concentration at true target sites.

The expression of apparent receptors outside of the target tissue providing protection through binding the chemical away from primary target sites, as highlighted above for organophosphates in earthworms, may also have mirrors among other toxicants. Studies have shown clear differences in the sensitivity to neonicotinoids of relatively susceptible insects and tolerant arachnids. Although both these taxa possess the classical nicotinic acetylcholine receptor (nAChR) target, these two taxa have differences in off-target receptors, specifically the presence of acetylcholine binding proteins (AChBPs) in arachnids (Torkkeli et al., 2015; Bao et al., 2017). Unlike nAChRs, AChBPs are truncated and comprise the ligand binding domain but not the transmembrane domain of the target (Shahsavari et al., 2016) and have been found in molluscs (Banks et al., 2009; Saur et al., 2012), polychaetes (McCormack et al., 2010) and earthworms (Short et al., unpublished). AChBPs have been shown to bind neonicotinoid insecticides *in vitro* (Tomizawa et al., 2007) and could act as additional receptors for neonicotinoid binding at locations away from the critical synaptic nAChRs, thereby alleviating the toxic effects of neonicotinoid insecticides. Indeed, Short et al. (unpublished) have suggested the presence of specific amino acids in the AChBP ligand binding domains expressed by different earthworm species are linked to altered magnitudes of off-target stoichiometric binding and may underlie observed differences in sensitivity. Off-target binding has also been proposed as a mechanism to explain the development of resistance to pyrethroids in malarial mosquitoes via increased expression of sensory appendage protein SAP2 (Ingham et al., 2020). These common findings suggest that the degree of off-target binding may make an important, but currently poorly understood, contribution to species sensitivity differences.

USING OUR KNOWLEDGE TO MOVE BEYOND DESCRIPTION TO SENSITIVITY PREDICTION

As knowledge has developed on the range of drivers that underlie species sensitivity, there has been an increasing interest in the development of predictive approaches. Critical to this has been both the expansion of the available ecotoxicological databases from which to identify differences and also the development of the AOP concept (Ankley et al., 2010). The assembly of the ecotoxicological data into searchable databases provides researchers the opportunity to look at sensitivity relationships between closely and distantly related species to develop evolutionary and correlative models. The AOP concept provides a structure within which to assess how the degree of conservation or divergence of species traits or toxicologically relevant biochemical pathways contribute to sensitivity (Rivetti et al., 2020). This includes through trait-based approaches and the further development of the ortholog concept by assessment of the gain or loss of molecular targets

(Gunnarsson et al., 2008; Verbruggen et al., 2018) and their structural or functional variations (Celander et al., 2011; LaLone et al., 2013). These developments have supported the growth of two dominant types of approach for sensitivity prediction designed to address the current challenges for chemical risk assessment, challenges that arise from the need to use short-term bioassay data for a few species to predict long-term chemical risks for complex communities. The first type of approach is based primarily on the phylogenetic comparison of species and the use of this information to predict sensitivity. Methods following this approach include phylogenetic model development, interspecies correlation estimation (ICE) models for species pairs and trait-based predictions. The second major type of approach is based on the development of target ortholog assessments often based on the use of genomic, transcriptomic and/or metabolomic data. Approaches of this type include metabolic pathway assessments for TK analysis, receptor ortholog cataloging, receptor expression and functional determination and target receptor binding prediction analysis. The methods applicable within each of these approaches are described below.

Phylogenetic and Species Correlations Approaches for Predictive Ecotoxicology

As highlighted above, the genes and gene pathways contributing to TK (e.g., detoxification) traits, such as monooxygenases, glutathione-S-transferases, metallothioneins and phytochelatin synthases, and TD (AOP) traits, such as target receptors and repair mechanisms are commonly observed to underlie differential sensitivity both within and between species. Hence, knowledge of the evolution of these gene families can contribute to an understanding of species responses. Phylogenetic analyses conducted on genes associated with both TK and TD traits have identified taxon relevant phylogenetic structure. Seren et al. (2014) analyzed the metallothionein sequences available for vertebrate species. Distinct major metallothionein clades were found within the assembled sequence trees for fish, eutherians, amphibian/reptile/bird and amniotes, indicating that functional divergence and duplication has created a distinct metallothionein in the vertebrate lineage. A phylogenetic analysis of microorganisms, yeasts, algae and plant phytochelatin synthases also indicated that plant variants had notably diverged from those of other organisms (Kolahi et al., 2018). For TD linked receptor targets, phylogenetic structure linked to taxonomy has been shown for acetylcholinesterase both across phyla (e.g., chordates arthropods and nematodes) and within phyla (e.g., between arthropods) (Bally et al., 2016). For the latter group, higher sequence similarity was found for ortholog sequences in more closely related species for each of the two known acetylcholinesterase genes (Kaur et al., 2015). The presence of a clear phylogenetic structure for such key genes increases the likely success of sensitivity predictions made using homology assessment.

The phylogenetic structure observed for genes in TK and TD pathways points to the likelihood of similar sensitivity in

related species that share closely related metabolic systems and target structures. This phylogenetic signature for toxicity has been investigated in a small number of studies. For endosulfan, Jones et al. (2009) found up to 100-fold differences in the sensitivities of nine amphibian species. Within this variation a phylogenetic signature was observed, with the Bufonidae being least susceptible, Hylidae moderately susceptible, and Ranidae most susceptible. A phylogenetic signature of amphibian sensitivity has also been found for copper after the co-varying effects of exposure temperature were accounted for (Chiari et al., 2015). Fish species sensitivity to nitrite has also been shown to have a phylogenetic basis, specifically through the taxon specific distribution of chloride channels through which NO_2^- can be actively taken up. Thus, species that actively assimilated Cl^- via gills, such as the channel catfish (*Ictalurus punctatus*), showed greater sensitivity to NO_2^- than species such as killifish (*Fundulus heteroclitus*), European eel (*Anguilla anguilla*) and bluegill (*Lepomis macrochirus*) that access Cl^- also through other (e.g., dietary) mechanisms (Brady et al., 2017). Further studies have also provided some support for the presence of a strong phylogenetic signature for some chemical classes, such as organophosphates for fish and other vertebrate species (Hylton et al., 2018; Moore et al., 2020), but only a weak signature for other classes such as organochlorines and metals (Hylton et al., 2018). In a study of energetic compound effects, the overlap of transcriptional network response showed a degree of conservation, but showed greater divergence in increasingly taxonomically distant species (Garcia-Reyero et al., 2011). This again indicates a phylogenetic basis in species responses to these chemical classes.

Building on the concept of phylogenetic conservation of critical pathways, Guenard et al. (2011) developed a predictive modeling approach that uses phylogeny to estimate sensitivity. These approaches use known molecular phylogenies to derive estimates of the taxonomic distance between species expressed as path length to the first common ancestor. This phylogenetic information was then regressed against known species sensitivities to individual pesticides in relation to overall chemical potency and species susceptibility to derive a predictive model. Within the analysis of Guenard et al. (2011), phylogeny explained up to 61–85% of among-species variations, with up to 83% of between species variation being phylogenetically structured. The high level of phylogenetic structure indicates a clear potential for the phylogenetic model to provide justifiable predictions of sensitivity for untested species (Guenard et al., 2014). Using the same approach, Malaj et al. (2016) developed models to explain species sensitivity for eight metals using combined phylogenetic and metal physicochemical properties. The models explained 70–80% of the variability in species sensitivity, with phylogeny, explaining >40% of variance, as the most important component.

The phylogenetic relationship between TK and TD pathway components, coupled with the potential for phylogeny alone to explain a significant proportion of sensitivity, indicates that species' responses to chemical stress are not random. Instead, sensitivity can be viewed as a trait founded in the evolutionary relationships between species. As quantitative characteristics are

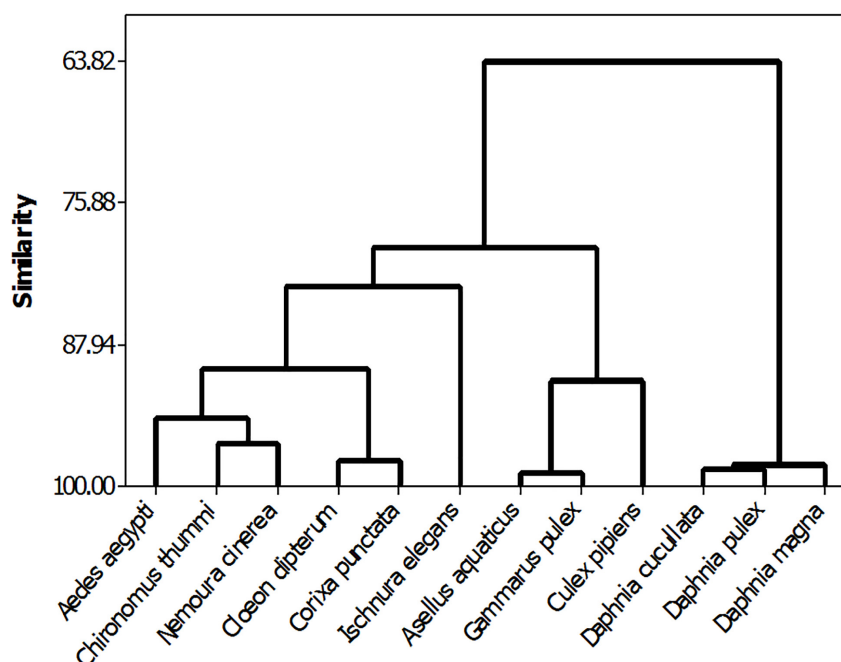


FIGURE 3 | Phylogeny of aquatic invertebrate species reconstructed from the set of toxicity data for 15 chemicals assembled by Baird and Van Den Brink (Baird and Van den Brink, 2007), note the resulting phylogeny closely reflects the conventionally assumed taxonomic relationship between the different species.

partly explainable in terms of species evolution, there is potential to use chemical sensitivity data as an additional set of traits for the construction of species phylogenies and to compare and contrast physiologies in closely related or cryptic species. The potential to use chemical data to establish phylogenies can be demonstrated using effect data for multiple species and chemicals. For example, Baird and Van den Brink (2007) assessed the relationship of chemical sensitivity to a range of species morphology, life history, physiology and feeding ecology traits. Four traits explained 71% of species sensitivity variation. Using the same data matrix as input for phylogenetic assessment, a tree structure was found that closely mirror the conventionally understood taxonomic relationships between the tested species. Thus, species that are closely related, such as the three cladoceran species (*Daphnia cucullata*, *Daphnia pulex*, *Daphnia magna*) are closely grouped indicating a high similarity in their responses to the 15 chemicals tested. This is also the case for the two crustacean species (*Gammarus pulex*, *Asellus aquaticus*). The seven insect species included show a lower degree of relatedness based on sensitivity to the 15 chemicals, reflecting their lower taxonomic association to class level only (Figure 3). The capacity for reconstructing phylogenies based on toxicity information provides further evidence of the phylogenetic conservation of toxicity mediated through common pathways.

The phylogenetic signature for toxicological responses has been used to justify the development of predictive correlative models designed to fill gaps in our knowledge of species sensitivity. The most commonly developed and widely used of such approaches are ICE models. These methods use the available toxicity data (e.g., as ECx or LCx values) for chemicals tested in

two species to generate a log-linear least-squares regression-based ICE model specific to that species pair. Using species pair ICE models, toxicity measured for further chemicals in just a single taxon can be used to predict toxicity for the same chemical in the second untested taxon (Leblanc, 1984; Raimondo et al., 2010).

Theoretical ICE models can be developed for any species/taxa pair for which there is sufficient toxicological data available either across all chemicals or for a specific chemical class (Bejarano, 2019; Raimondo and Barron, 2020; Wang X. N. et al., 2020). In a quantitative comparison of 780 ICE models derived for 550 chemicals in 77 aquatic species, predictive power was found to be strongly governed by relatedness (Raimondo et al., 2010). For models derived from data for two species related at family level, ICE predicted sensitivities were within a factor of 5 of the measured value in 94% of cases and only 1% of predictions differed >50-fold from measured values. In contrast, for species pairs from different phyla, only 61% of model predictions were within a fivefold factor and 8% differed >50-fold (Raimondo et al., 2010). On the basis of their high predictive power, especially for closely related species pairs, ICE models have been proposed for the generation of *in silico* toxicity data for untested chemicals for SSD derivation in closely related species (Awkerman et al., 2014; Bejarano et al., 2017), for predicting hazards to untested endangered species (Willming et al., 2016) and for use in QSAR development (Raimondo and Barron, 2020).

Even though there is a clear signature of phylogenetic conservation, a close relationship alone cannot be taken as an absolute guarantee of similar sensitivities. For example, in an analysis of ICE models, Raimondo et al. (2010) found that species related at genus level still differed in sensitivity

by >fivefold in 6% of cases and >10-fold in 4%. Further, studies of closely related “sibling” species, including known cases of species crypsis have reported different responses following exposure, including cases for copepods exposed to trace metals (Rocha-Olivares et al., 2004); different crustacean species exposed to nickel, a fungicide and an insecticide (Feckler et al., 2012); nematodes exposed to copper and lead (Monteiro et al., 2018); aquatic oligochaetes exposed to cadmium (Sturmbauer et al., 1999) cadmium, chromium and copper (Reynoldson et al., 1996); terrestrial oligochaetes to lead (Andre et al., 2010) and polychaetes exposed to different PAHs (Linke-Gamenick et al., 2000; Bach et al., 2005).

Trait-Based and Ortholog Approaches for Predictive Ecotoxicology

As sensitivity data for many species, including rare or difficult to keep organisms is challenging to obtain, trait-based and ortholog approaches have been proposed as a means to allow predictive assessment (Baird and Van den Brink, 2007). Traits potentially relevant for sensitivity prediction may occur at multiple levels of biological organization within the AOP, from the nature and dynamics of the energy budget to life-cycle characteristics and species-species interactions. Within any proposed trait-based approach, an obvious consideration is the nature of the traits to include for predictive assessment. Within the literature, a dichotomy has largely existed between mechanistic trait assessments based on AOPs and approaches that use higher tier physiological or ecological characteristics. For initial AOP development, the dominant paradigm is based on a bottom up approach centered on identifying the AOPs from gene regulation networks, often using high dimensional gene, protein or metabolite expression data (Perkins et al., 2019). This approach is established in human toxicology (Kimber et al., 2014; Abdul Hameed et al., 2019) and is transferable to ecotoxicological cases (Murphy et al., 2018; Gomes et al., 2019; Kim et al., 2020; Song et al., 2020). In contrast, ecological trait-based approaches are based on building correlative models that link physiological features or ecological habit (e.g., size, respiratory anatomy, trophic level, diet, reproductive strategy, habitat preference, generalized metabolic traits) (Baas et al., 2015; Berger et al., 2018; Van den Berg et al., 2019). Such approaches underpin techniques such as Species at Risk (SPEAR) assessment. This predictive model framework uses trait information on (i) sensitivity (e.g., to insecticidal compounds), (ii) generation time, (iii) presence of aquatic stages, and (iv) ability to migrate and recolonize; to provide and index of the severity of chemical exposure effects (Liess and Von der Ohe, 2005).

Full conceptual AOP development can provide a framework for sensitivity assessment that brings together the mechanistic approach within a wider trait concept. Such a merger can occur when the critical driver of toxicity is identified; the variation for these key processes quantified and their implications for sensitivity defined. This is not a simple process because at each stage of the AOP there is the potential for interspecific variation. Within the AOP, a particularly critical interaction is that between the chemical and its primary target that acts as the

MIE. Differences in the nature and strength of this interaction has the potential to radically change sensitivity in a manner that is easier to conceptualize and which is likely to be more specific, and hence variable, than that for more downstream responses such as tissue, organ physiological, behavioral or life-cycle changes. Hence, to understand and predict sensitivity there has been a specific focus on critical receptors that represent MIEs. Such assessments have used an ortholog approach that covers both the receptor ortholog complement and sequence variation. Linkage of TK/TD approaches with resource allocation models have potential for understanding the development of ecotoxicological responses across multiple species (Ashauer and Jager, 2018; Baas et al., 2018).

The receptor complement hypothesis of sensitivity in ecotoxicology was initially proposed by Gunnarsson et al. (2008), who analyzed the sequenced genomes of 16 species for 1,318 human drug targets and linked ortholog complements to comparative species sensitivity. The work of Gunnarsson et al. (2008) has been expanded to cover a receptor analysis for 640 eukaryotic genomes (Verbruggen et al., 2018). These analyses have been assembled into the “ECOdrug” database which details the connection of orthologs to phylogeny for 1,194 active pharmaceuticals targeting 663 drug targets. The case studies presented by Verbruggen et al. (2018) highlight a range of potential predictive applications for ECOdrug. Examples include the fore selection of appropriate test species, such as for testing protein pump inhibitors in fish species based on the presence of the H^+/K^+ -ATPase ortholog target in some, but not all teleosts (Castro et al., 2014); the selection of higher plants rather than algae for assessing the effects of cholesterol lowering drugs (e.g., statins), due to the presence of 3-hydroxy-3-methylglutaryl Coenzyme A Reductase (Verbruggen et al., 2018); and the identification of relevant model species for testing toxicity based on the presence of thyroid hormone receptors in some invertebrate species, but not in *Daphnia* the most commonly tested model species (Verbruggen et al., 2018).

Further building on the concept of known target conservation as an indicator of sensitivity, LaLone et al. (2016) developed the web-based Sequence Alignment to Predict Across Species Susceptibility tool (SeqAPASS)². SeqAPASS provides a platform for assessing not just ortholog presence, but also gene structure against the known target to identify similarities. SeqAPASS analysis can be conducted at three levels to identify orthologs and compare their overall sequence similarity at the amino acid level between a species with a known sensitivity and a species under investigation. Thus, beyond ortholog identification, gene structures can be compared to identify homology in key motifs in ligand-binding domains or for individual amino acid residues at important positions for protein conformation and/or ligand-receptor interactions.

LaLone et al. (2016) used SeqAPASS analysis to investigate the differences in insect species responses to molt accelerating diacylhydrazines. Ortholog analysis showed the presence of target receptor orthologs of the ecdysteroid receptor with conserved ligand binding domains for insects and a range of other taxa,

²<https://seqapass.epa.gov/seqapass/>

such as Merostomata, Malacostraca, Arachnida, Branchiopoda, Chilopoda, Maxillopoda, and Priapulidae. Ligand binding studies with the natural steroid ligand of the ecdysteroid receptor in the Lepidopteran *Heliothis virescens* indicated that sensitivity was strongly associated with the presence of a valine at residue 384. This amino acid was conserved among lepidopteran species which show sensitivity to this class of insecticide. Ecdysteroid receptor sequences in Hemiptera insensitive to diacylhydrazines were found to possess an isoleucine at residue 55 that prevents ligand interactions through steric hindrance. Furthermore, a lysine at 48, phenylalanine at 222, and glutamine at 227 also restricted ligand-receptor interactions in the Hemiptera (Amor et al., 2012). Hence the sensitivity of these two insect groups to diacylhydrazines could be explained by amino acid differences at key binding residues, rather than by the presence or absence of the ortholog alone.

In a further example of the use of SeqAPASS, LaLone et al. (2016) focused on differences for neonicotinoids among arthropod groups showing high (insects including honey bees) and low (arachnids including ticks) sensitivity. This example was more difficult to fully resolve than the ecdysteroid receptor case. As such, this assessment highlighted some advantages and limitations of the ortholog and homology approach. The synaptic nAChR target of neonicotinoids is known to comprise of multiple α - and β -subunits. Between these two types, differences in the percent similarity of all sequences from insects to arachnids for the $\beta 1$ subunit provided a possible explanation for sensitivity. However, this observation alone may not be enough to resolve all aspects. The nicotinic acetylcholine receptor family is large, comprising multiple α - and β -subunit sequences (Jones et al., 2006; Jones and Sattelle, 2010). This evolutionary divergence across nAChR subunit gene families makes it difficult to resolve functional homology (Pedersen et al., 2019). Further, the total numbers of α - and β -subunit gene orthologs can vary significantly from lowers numbers in some taxa (e.g., 10 *D. melanogaster*, 11 *A. mellifera*, 16 human, 29 *C. elegans*) to a greatly expanded number (>100) in some Lophotrochozoan taxa (Jiao et al., 2019; Short et al., unpublished). Cases of multi-subunit receptors, in which subunits are encoded by large and divergent gene families, makes it difficult to identify which of the subunit orthologs should be analyzed for critical motif modifications and amino acid substitutions, a challenge that does not exist in cases where there is a single receptor target encoded by a single gene (LaLone et al., 2013).

Further limitations arise for ortholog (Verbruggen et al., 2018) and ligand binding analysis (LaLone et al., 2017) in cases where gene family expansion allows the functional divergence of members. Gunnarsson et al. (2008) already identified that “the presence of a drug target ortholog in a species does not guarantee that a functional interaction with the drug can occur” and that “Vice versa, functional interactions between a drug and other non-orthologous proteins are also possible.” The example given was of the molluscan estrogen receptors that, while expressed in reproductive tissues (e.g., in oyster *Saccostrea glomerata*), has been shown not to bind estrogen *in vitro* (Tran et al., 2016). The examples above detailing expression of the cholinesterase and carboxylesterase targets for organophosphates

in non-neural earthworm tissues and of AChBPs that interact with neonicotinoids via their ligand binding domain, but possess no ion channel functionality, provide comparable examples. In such cases, ortholog analysis would identify an orthologous gene and sequence analysis a functional ligand binding domain containing the critical motifs and residues. However, any ligand-receptor interaction will take place spatially removed from the target site (e.g., at the synapse in the case of AChEs and nAChRs). This spatial separation means that any downstream links in the neurotoxic AOP will not be initiated. Therefore, there remains a need for the further development of approaches to include not just the presence and homology of orthologs, but also evidence that downstream AOP linkage will occur, through methods such as tissue specific expression analysis and target function assessments. Inclusion of these additional aspects would advance predictive sequence based approaches to their range of application and predictive capability.

APPLICATIONS OF PREDICTIVE ECOTOXICOLOGY IN CHEMICAL RESEARCH AND MANAGEMENT

The current and future development of approaches for sensitivity assessment and prediction can support a number of emerging applications in theoretical and applied ecotoxicological research. These range from the refinement of modeling approaches to the development of alternative animal models, species orientated assessment and structure testing and assessment programs.

Integrating Mechanistic Insights Into TK and TD Models

The recognized importance of TK and TD in toxicity have underpinned the development of models to capture these aspects of internal exposure and accrued damage (Ashauer and Escher, 2010; Jager and Ashauer, 2018). By linking the TK and TD characteristics to resource allocation, it is possible to link exposure and damage to changes in energy fluxes (Kooijman, 1993).

Chemical exposure at target sites in TK-TD models for invertebrates are most commonly predicted using one compartment TK models (Jager and Ashauer, 2018), while for more physiologically complex organisms there is the potential to develop more complex TK models that include physiological based pharmacokinetic process that integrate a more complete set of physiological processes into a more integrated exposure modeling framework (Stadnicka et al., 2012; Brinkmann et al., 2016; Grech et al., 2017). These TK models can be coupled to physiological effect models to develop coupled TK-TD models, including those linked to dynamic energy budget approaches (Cropp et al., 2014). To provide insights into internal chemical fate, the characterization of genes critical to xenobiotic metabolism, including the phase I, II, and III pathways and metal binding protein pathways, all have the potential to ascribe physiological traits relating to kinetic rates to improve the basis for TK modeling.

The TD component of dynamic energy budget based models link internal exposure to effects on vital rates through five putative energetic or damage associated mechanisms, termed “physiological modes of action” (Kooijman and Bedaux, 1996; Alda Alvarez et al., 2006; Billoir et al., 2008; Swain et al., 2010; Ashauer and Jager, 2018). These five are: (1) Assimilation from food; (2) somatic and maturity maintenance; (3) costs for structure and maturation; (4) overhead costs for making an egg; and (5) Hazard during oogenesis (Jager and Zimmer, 2012; Ashauer and Jager, 2018). Measurement of traits relating to energy production (metabolic rate, mitochondrial activity, fatty acid metabolism, insulin signaling), cellular maintenance (protein synthesis and turnover, nervous system function), developmental change and reproduction (e.g., nuclear hormone receptor expression and cell death (e.g., caspases) have the potential to provide empirical support for these physiological impacts.

The development of quantitative relationships between physiological changes, TK and TD and parameters and sensitivity has the potential to support the development of more mechanistically informed models that can better ascribe sensitivity to key traits. For example, Baas et al. (2015) linked somatic maintenance, as a measure of metabolic rate, to the sensitivity of species to four pesticides, two requiring metabolic activation (chlorpyrifos and malathion), and two not (carbofuran and carbaryl). High sensitivity among smaller species was not linked simply to body size, but rather to the presence of high somatic maintenance costs in species linked to waste-to-hurry strategies under boom and bust population dynamics. This observation predicts high resource allocation to tissue cellular systems, measurable as a mechanistic trait that can be used to assess their contribution to sensitivity.

Predictive Ecotoxicology for the 3 Rs

As programs progress providing large-scale sequencing of animal, plant and microbial genomes, the potential to identify orthologs critical to sensitivity within and among species will increase. This growing availability of genome and other ‘omics (transcriptomics, proteomics, and metabolomics) data, when combined with other trait information, can support cross species assessment. Within toxicology there are significant ethical and regulatory drivers for the replacement of higher organisms as the main focus for testing. In the development of 3 Rs (Replacement, Reduction, Refinement) approaches, functional ‘omics techniques have already shown their potential value in screening for specific pathway effects (Laroche et al., 2018). Given knowledge of TK characteristics and an AOP, an assessment of phylogenetic conservation through comparative genomics could be used to evaluate the appropriateness of potential surrogates for use in assessing the effects of specifically acting chemicals targeting this pathway. Assessment of TK pathways can be used to determine effect development and dose specific responses (Creton et al., 2012). Non-targeted metabolomics analysis can be used to support the assessment and development of rapid biochemical profiles that can provide evidence of species equivalence, while orthogonal omics datasets from different species can be used to identify

components critical for conserved responses. In this manner it is possible to investigate the downstream response resulting from specific exposures in a replacement species, or even screen the potency of chemical effects through the specified AOP mechanism without the need to always proceed to higher vertebrate testing.

Endangered Species Protection

Just as there is potential to use mechanistic TK-TD information in predictive ecotoxicology for 3 Rs species replacement, it is also possible to use such information to predict risks for species of high conservation concern that cannot be directly tested (Ortiz-Santaliestra et al., 2018). For example in a given exposure scenario, resources may be better spent managing releases to water to protect a piscivorous bird possessing an AH-receptor with predicted high TCDD binding affinity, than on mitigating releases to land to protect insectivorous/vermivorous species possessing an AH-receptor with a predicted low TCDD affinity. For chemicals where bioaccumulation is a potential concern, knowledge of the specific capacity of metabolism or handling of different chemicals may help to identify trophic links within food chains that may lead to elevated exposure of predators. For example, earthworms are well known to accumulate cadmium due to the formation of detoxification granules (cadmosomes) (Stürzenbaum et al., 2004) and in doing so provide a route of exposure for worm eating species (Schipper et al., 2012). Past decisions on action for endangered species protection have been driven by observation of wide population declines (Newton and Wyllie, 1992; Woodcock et al., 2016). Combining analysis of the metabolic capacity of species and receptor orthologs (e.g., nAChRs and voltage-gated sodium channels in the case of neonicotinoids and pyrethroids) and competing off-target receptors (e.g., AChBPs and SAP2) distribution among species from “omic” analyses, with targeted biochemical pathway measurements has the potential to accurately predict the sensitivities among species. Such information can be used to identify potentially vulnerable species that can become a focus for future monitoring.

Risk Assessment—Better SSDs

While a goal may be to move toward a more predictive approach, for some time there is likely to remain species and chemicals for which reliable bottom up predictions of sensitivity will not be readily achievable. Cases where sensitivity can be linked to a few traits will be easiest to resolve. However, for species with multiple potential targets, full resolution to explain sensitivity may prove a daunting or even impossible task (LaLone et al., 2013). Protection of ecological communities is, therefore, likely to retain a statistical extrapolation component based on the use of species sensitivity distributions. Even though statistical models are likely to be retained as a key tool for ecological risk assessment, there is still the possibility to utilize mechanistic information to better inform such assessments. For example, it may be possible to evaluate the proportion of species present in an SSD that possesses one or more traits (e.g., absence of relevant detoxification pathway or presence of a receptor ortholog) linked to sensitivity. Soil SSDs are often dominated by earthworms. This

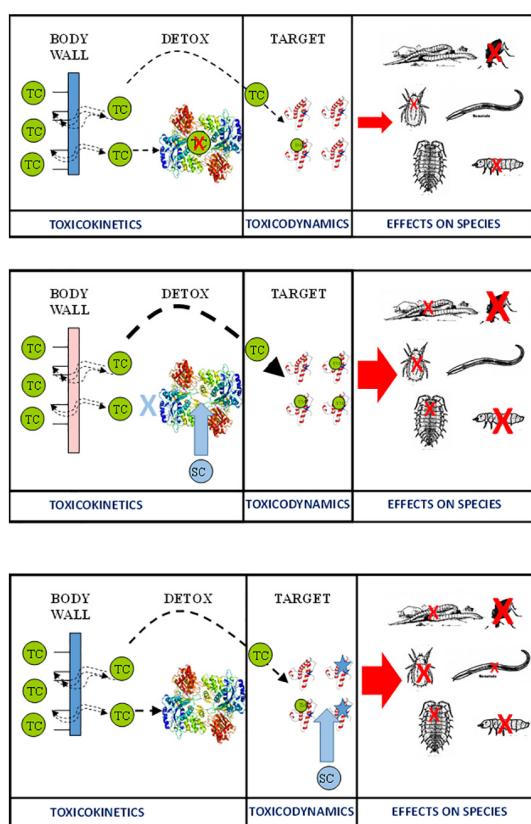
is the case because *Eisenia fetida* is the main standard soil test species, but its substitution by other species is readily possible meaning that results are often available for multiple earthworm species (Ma and Bodt, 1993; Hartnik et al., 2008; Kreutzweiser et al., 2008; Römbke et al., 2017; Silva et al., 2017). Similarly, cladocerans are often over represented in aquatic SSDs based on the common use of *Daphnia magna* and related species for testing. Taxonomic biases may cause issues if the dominant species shows particular sensitivity or insensitivity. For example; earthworms are highly sensitive to copper exposure (Lofts et al., 2005) and cladocerans insensitive to neonicotinoids (Raby et al., 2018). Hence distributions for these chemicals, that contain high frequencies of data from these commonly tested taxa are often erroneous, representing a greater sensitivity for copper and a lower sensitivity for neonicotinoids, respectively.

The issue of sensitive and insensitive species representation in SSDs has been identified as an issue for chemicals such as herbicides, fungicides and insecticides that are designed to target a specific taxon-relevant biochemical pathway. Thus, if a HC5 is derived from a taxonomically generalized SSD for these chemicals, then the species that fall in the 5% of the affected fraction would be overwhelmingly drawn from the known target species for these chemical classes (e.g., plants for herbicides etc.). Environmental

quality criteria set for such chemicals would, as a result, likely not protect against ecosystem level effects, since all impacted species will be drawn from a specific taxa, especially if that group makes a major contribution to key ecosystem processes. The derivation of separate SSDs for sensitive target and non-target groups can protect against the risk of inadequate protection. Extending beyond such simple taxa based segregation, it is possible to envisage ecological, TK-TD or specific molecular traits used to segregate the tested species cohort to allow separate assessments for vulnerable groups. For example, an SSD may be derived only for species likely to bioaccumulate a chemical, that possess a receptor ortholog with the relevant ligand binding domain, or which lack competing off-target receptors for the chemical ligand that may play a protective role.

Mixture Toxicology and Sensitivity

In the field, organisms are almost always exposed to measureable levels of more than one chemical. The mixture effects for the combinations of similarly and dissimilarly acting chemicals are classically explained by the models of concentrations addition and independent action (Altenburger et al., 2000; Backhaus et al., 2000; Faust et al., 2001, 2003). However, although applicable to many chemical combinations, not all mixture studies show



A Toxicity results from a combination of toxicokinetics (uptake) and toxicodynamics (target site interactions)

B Synergism can occur if one chemical changes the toxicokinetics of another chemical (e.g. block phase I, II and III metabolism so more gets to target site)

C Synergism can occur if one chemical changes the toxicodynamics of another (e.g. changes the structure of the target to allow better interaction)

FIGURE 4 | (A) Schematic representing the role of TK and TD traits in toxicity, following uptake the chemical may be detoxified by different enzymes or may escape detoxification to bind with the biological target, **(B)** interactive mixture effects (e.g., synergism can occur if one chemical changes the toxicokinetics of another chemical (e.g., block detoxification so more gets to target site) leading to greater effects on exposed species or **(C)** when one chemical changes the TD of another (e.g., changes the structure of the target to allow better interaction) again leading to greater effects.

additive effects. Indeed, patterns of response indicating synergism (greater than additive) or antagonism (less than additive) may occur in a small, but nonetheless significant proportion of cases (Jonker et al., 2005; Cedergreen, 2014). The role of mechanistic toxicology in identifying which additive mixture model to apply, based on comparing chemical mode of action, has already been highlighted (Heijne et al., 2005; Vandenbrouck et al., 2010). However, an evolutionary perspective of the traits underlying sensitivity could also play a role. For example, it has been demonstrated in a number of species that pyrethroid insecticides can be synergized on simultaneous exposure with azole fungicides (Johnson et al., 2013; Cedergreen, 2014). Similar evidence is also emerging for azole fungicides when exposed with other groups of insecticides such as the neonicotinoids (Robinson et al., 2017; Tsvetkov et al., 2017). Mechanisms leading to synergy may occur at different stages of intoxication (Spurgeon et al., 2010; **Figure 4**). One chemical may impact on soil or gut microbiomes resulting in changes in the degradation rates of a second (Cheng et al., 2017) or one may affect species activity or feeding altering another's bioavailability (Cedergreen et al., 2017). A major known TK based mechanism for synergism arises when chemicals alter Phase I, II, or III xenometabolism (Cedergreen, 2014; **Figure 4**). For example, changes to pesticide biotransformation are implicated as the cause of the synergism of insecticides by azoles. This occurs as the fungicide which targets CYP51 (lanosterol 14 α -demethylase) in the target taxa, also inhibits other cytochrome P450s in other species, leading to reduced insecticide metabolism resulting in higher internal concentration and, hence, greater toxicity (Chen and Ying, 2015). There is potential to apply TK models to assess mixture effects for similar and dissimilar mixture combinations (Timchalk and Poet, 2008; Tebby et al., 2019). However, the striking lack of knowledge of xenometabolism in invertebrates, especially beyond Phase I enzymes to the Phase II (e.g., glutathione-S-transferases) and Phase III (e.g., p-glycoproteins) systems means there could be numerous unknown targets for such interactions.

Changes to TD in responses in mixtures could result from interactions associated with the MIE or at different points along the AOP. The dynamics of target site interactions have been shown to cause different effects depending on the sequential order in which species are exposed to chemicals in time (Ashauer et al., 2017; **Figure 4**). Further, when different chemicals affect different receptors in the same organism (e.g., different specific receptor targets), there is also a potential for interactions. Identification of the TK and TD traits could, thus, indicate the potential for synergism and contribute to improved mixture risk assessment.

Design of Effective and Benign Chemicals for Applications

The development of pharmaceuticals has refined the practice of receptor-ligand binding analysis and the identification of off-target effects using sequence analysis, biochemistry, ligand binding studies and *in vitro* and *in vivo* testing. With the increasing availability of genetic resources (Verbruggen et al., 2018) and functional testing approaches for ecotoxicological species (Kumagai et al., 2017; Roth et al., 2019; Maki et al., 2020),

more options exist to extend such analyses to look at non-target chemical effects. Given the costs to develop new agrochemicals or biocides to market, a capacity to screen out molecules that may strongly affect a particular adverse outcome pathway in a species likely to be exposed is an appealing option. Tools such as EcoDrug and SeqAPASS may provide a basis for such screening, but will increasingly need updating as new information about the functional mediators of sensitivity become available, such as the reduction of exposure at classical target receptors due to the expression of alternative receptors associated with different functionality and/or expressed in non-target tissues.

Biotechnology Applications

Adaptation through evolution may result in predictable phenotypic traits conserved across species that determine sensitivity. For example, it has been commonly observed that insects exposed to pyrethroids evolve resistance through increased metabolic detoxification and decreased target sensitivity. The advent of methods for genetic editing using tools, such as CRISPR-Cas9, offer the possibility to change the metabolic capacity, target gene repertoire or expression of target sequences, with the potential to confer change in sensitivity. The concept of a gene drive for sterility have been advanced for the eradication of malarial mosquito populations (Gantz et al., 2015; Simoni et al., 2020). It is theoretically possible to imagine similar studies to introduce susceptible pests to eradicate an adapted population or the manipulation of the genes of vulnerable species to increase tolerance to a pervasive chemical threat.

Informed Species Monitoring

Assuming that we will not achieve sufficient understanding of all primary and secondary mechanisms of action to fully mitigate all chemical impacts, there is likely to remain a need for monitoring programs to track populations for any adverse chemical effects. This need underlies the concept of Pesticidovigilance (Milner and Boyd, 2017) as a future approach for post release impact assessment. For effects-based monitoring, any Pesticidovigilance framework will require the selection of focus species. Integration of data on ecological traits related to feeding preferences for certain pesticide-treated crops (Woodcock et al., 2016) or to particular TK or TD traits, relevant to the AOP for the chemical of concern, may be used to guide such a choice of species toward vulnerable taxa. Such approaches are complementary to currently used prospective risk-based frameworks for chemical management. As trait resources expand, the potential to make such decisions on focus species for retrospective chemical monitoring from a mechanistic viewpoint will increase in parallel.

AUTHOR CONTRIBUTIONS

DS, SS, and PK conceived the original structure of the review and developed the overall structure and layout. DS and SS prepared the manuscript. All authors contributed specific knowledge of relevant literature to particular areas covered and edited the manuscript.

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The Role of Membrane-Based Technologies in Environmental Treatment and Reuse of Produced Water

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Produced water (PW) generation has been increasing recently due to the expansion of fossil fuel extraction and the aging of oil wells worldwide, especially in the United States. The adverse health risks, seismicity, and environmental impacts associated with PW have become a challenging concern. Therefore, there is increased demand for improved PW treatment and reuse management options. There are multiple methods for treating PW; this article focuses on treatment through membrane filtration. Moreover, this mini review aims to summarize statistics on PW abundance and trends in PW generation over time, to briefly call attention to health-related issues, highlight some treatment challenges, and mention the potential purposes for reuse with an emphasis on the United States, the largest generator of PW worldwide.

Keywords: produced water, water management, wastewater reuse, membrane, review article, water pollution

INTRODUCTION

Global energy demand has led to robust and widespread oil and gas industry with exploration and production activities around the world (Halvorsen, 1977; Cleveland, 2005; Caulk and Tomac, 2017). The environmental concerns associated with the diverse oil and gas extraction processes include threats to human and ecological health and contamination of water, air, and soil (Mondal and Wickramasinghe, 2008; Fakhru'l-Razi et al., 2009; Cordes et al., 2016; McIntosh et al., 2018). Wastewater generated during the extraction of oil and gas is the largest waste stream in the oil and gas industry and is typically referred to as produced water (PW) (Veil, 2011; Dickhout et al., 2017; Al-Ghouti et al., 2019). PW generally contains substantial concentrations of petroleum components (such as phenols, polycyclic aromatic hydrocarbons, and volatile hydrocarbons), production chemicals (such as biocides and corrosion inhibitors), dissolved gases (such as CO₂ and H₂S), salts (such as sodium bicarbonate and sodium chloride), dissolved minerals including heavy metals (such as barium, zinc, lead, iron, etc.), and solids such as sand, silt, carbonates, and clays (Hansen and Davies, 1994; Duraisamy et al., 2013; Farag and Harper, 2014; Iggunnu and Chen, 2014; Al-Haddabi et al., 2015).

Produced water can be categorized according to the extraction activity, for example, oilfield PW, natural gas PW, and coal bed methane PW (Veil et al., 2004; Iggunnu and Chen, 2014; Al-Ghouti et al., 2019); of these three, oilfield PW contributes 60% of the total volume

(Fakhru'l-Razi et al., 2009; Igunnu and Chen, 2014). Oilfield PW generally results from two different processes (Al-Ghouti et al., 2019): (I) as a result of oil diffusion into the seawater during the oil extraction (Bader, 2007; Al-Ghouti et al., 2019); and (II) due to the water injection to the oil field in order to push the oil rise to the surface (Punternvold and Austad, 2008; Al-Ghouti et al., 2019). Similarly, PW from a natural gas processing field is mainly made up of the fracturing fluid. This fluid is a water solution containing multiple chemicals used to assist the hydraulic fracturing operation (Chapman et al., 2012). Also, this PW usually contains large volume of brines (Chapman et al., 2012; Orem et al., 2014). In coalbed methane wells, the hydrostatic pressure caused by water does not allow the gas to be released from the coal; therefore, the water should be removed in order to be able to collect gas, resulting in the coal bed methane PW (Veil et al., 2004; Orem et al., 2014).

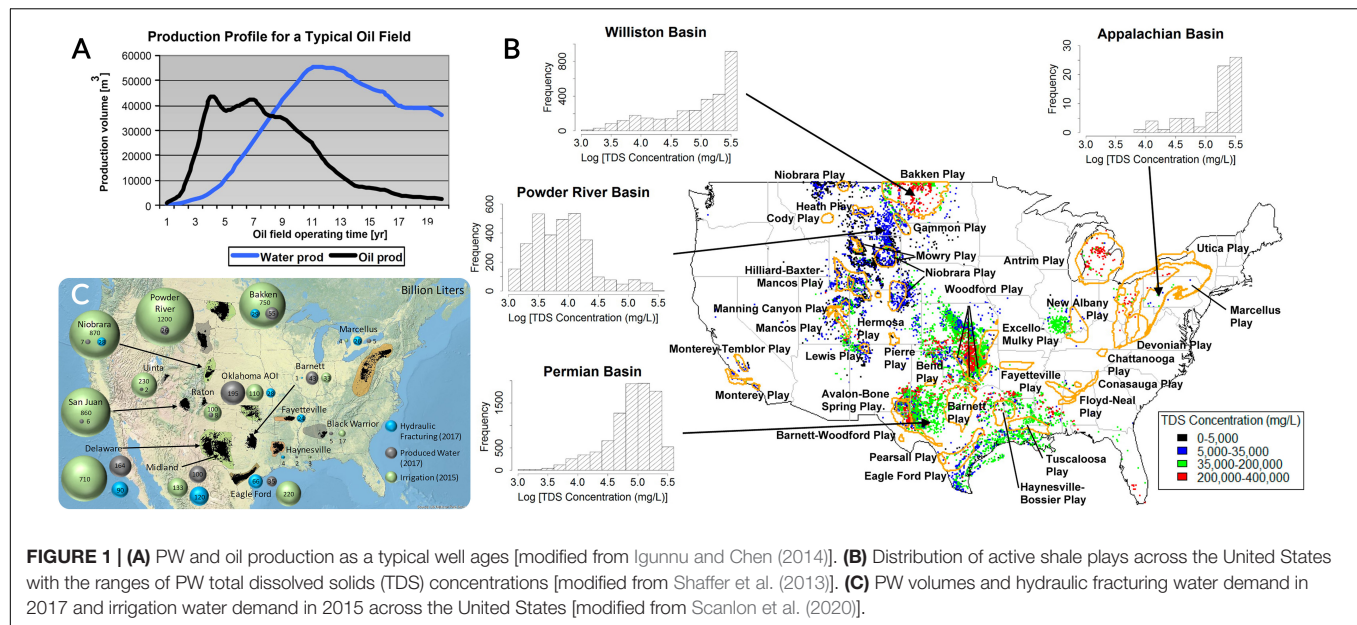
Improper management and control of PW can lead to the contamination of natural water resources as a result of oil spills or leaks (EPA, 2012; Esfahani et al., 2019). PW production is estimated to be more than 70 billion barrels annually (Al-Ghouti et al., 2019), and many of the associated contaminants are threats to marine life and human health (Neff, 2002; Chowdhury et al., 2004; Shakhawat et al., 2006; Mathieu et al., 2011; Beyer et al., 2012; Hosseini et al., 2012; Wilson and VanBriesen, 2012; Torres et al., 2016). High levels of organic and inorganic compounds have been reported in PW; these compounds are typically associated with shale gas wells (Torres et al., 2016). The level of these compounds varies based on the age of the reservoir, the type of produced hydrocarbon, and the local geology (Fakhru'l-Razi et al., 2009; Al-Ghouti et al., 2019). Dissolved organic carbon levels greater than 5.5 g/L have been reported for PW and include alkanes, carboxylic acids, aliphatic, and aromatic compounds with potential carcinogenic and other severe health effects (Torres et al., 2016). Animal studies indicate that PW-associated organic contaminants can cause respiratory complications in rats (Freeman et al., 2004; Torres et al., 2016), uterus complication in mice, and hemolytic anemia and adverse renal effects in animals (Vale and Meredith, 1981; ATSDR, 1999; Ismail et al., 2016; Alegbeleye et al., 2017), and respiratory complications and dysfunction of reproductive system in mice (Maguire-Boyle and Barron, 2014). Furthermore, volatile fatty acids (<53.7 mg/L) produced by bacteria in PW, cause unpleasant odors and have corrosive effects on skin with possible damage to eyes and nasal cavities (Maguire-Boyle and Barron, 2014; Orem et al., 2014). Furthermore, the inorganic contents of PW (**Supplementary Table 1**; Wilson and VanBriesen, 2012; Torres et al., 2016; Zhang et al., 2018) and their potential combination can cause health concerns (Warner et al., 2013). For example, if the bromide in PW is combined with disinfectant chlorine, it can yield brominated disinfection by-products that can cause brain, kidney, and liver damage (Brown, 2014; Vengosh et al., 2014). As another example, the interaction of the salt in the shale water with the surrounding rocks can mobilize radionuclides (Rich and Crosby, 2013). This results in a high concentration of radium-226 in some PW (Fisher, 1998; Osborn et al., 2011; Fan et al., 2016). Although water treatment approaches could reduce radioactive contamination by more than 90%

(Rich and Crosby, 2013; Brown, 2014), chronic exposure to the remaining radium-226 contamination in drinking water can pose serious health risks such as cancer, anemia, and dental fractures (Rich and Crosby, 2013; Warner et al., 2013; Brown, 2014). Therefore, more investigation is required to identify more effective treatment methods for radioactive contaminants in PW.

Moreover, underground disposal of PW, the most widespread PW management strategy in the United States, has resulted in additional concerns such as induced seismicity (Scanlon et al., 2019). The state of Oklahoma is a particular example of observed seismic activity associated with subsurface injection sites (Rajesh and Gupta, 2021). Seismicity in Oklahoma has been linked statistically to PW volumes and PW injection rates (Van der Baan and Calixto, 2017; Roach, 2018; Scanlon et al., 2019). PW injection into disposal wells contributes to seismicity mostly by reducing normal stress which causes movement along a pre-existing fault inducing earthquakes (Murray and Holland, 2014; Rubinstein and Mahani, 2015). However, to induce felt earthquakes by injection, a combination of several factors is necessary such as being near an active fault, fault size, stress size, and fluid pressure (Capper and Lee, 2017). The injection risk factors in addition to health hazards and water demand further motivate the treatment and reuse of PW.

The ratio of water to oil in the process of oil extraction is reported to be around three to one (Veil, 2011; Dickhout et al., 2017; Al-Ghouti et al., 2019). However, the amount of PW generation increases as the oil well ages (**Figure 1A**; Igunnu and Chen, 2014; Kusworo et al., 2018). Some studies (Global Water Intelligence, 2011; McCabe, 2020) predict that this ratio will be 12 to one on average by 2025 considering crude oil reservoirs. Even in 2004, an average of 9.5:1 ratio (water:oil volume) in the United States has been reported (Veil et al., 2004; Veil and Clark, 2011). The PW volume could even reach 98% of extracted product in fields that are close to being depleted (McCabe, 2020).

The United States is the largest generator of PW worldwide, reportedly producing about reported to be 24.4 billion barrels, ~30% of total PW in the world (Veil, 2015; Dickhout et al., 2017; Al-Ghouti et al., 2019; Veil, 2020). There are around one million oil/gas wells in the United States which are producing different amounts of PW (Veil and Clark, 2011; Veil, 2015). **Figure 1B** indicates how oil and gas wells are distributed across the United States with the ranges of PW total dissolved solids (TDS) concentrations (Breit and Otton, 2002; Shaffer et al., 2013). Moreover, wells in the United States remain in production longer than the global average well (Veil and Clark, 2011). A comprehensive 10-year study by Veil et al. in three different articles has shown that PW generation in the United States has been increased by 16.2% between 2007 and 2017, with a particularly sharp increase in recent years (15.2% between 2012 and 2017) (Clark and Veil, 2009; Veil, 2015, 2020). Generation of PW in the United States in 2017 was dominated by three states, Texas, California, and Oklahoma, which generated 41, 13, and 12%, respectively, of the total (Veil, 2020). Conclusively, as the leading PW generator in the world, the United States is urged to put more effort into PW treatment and reuse. However, to reduce the potential health risks of PW spills and/or discharge, and to consider further reuse for



different purposes, the aforementioned contaminations need to be investigated and lowered to acceptable levels (EPA, 2004). Here in this article, the aim is to discuss some reuse possibilities and examples, and review the application of membrane-based technologies for treating PW.

PRODUCED WATER REUSE

Reuse of treated PW has the potential to provide a useful water resource, particularly in water-scarce regions with increasing of oil and gas production (Echchel et al., 2020; Gray, 2020; Nadella et al., 2020). The proposed uses of treated PW are diverse and include irrigation, habitat watering, livestock consumption, dust control, power generation, oil and gas field operations, and as an extreme example, drinking water (Echchel et al., 2018; Al-Ghouti et al., 2019; Ginsberg et al., 2019; Scanlon et al., 2020). For instance, **Figure 1C** presents PW volumes and hydraulic fracturing water demand across the United States in 2017, in addition to water demand for irrigation in 2015 (Scanlon et al., 2020). Units are in billion gallons. This map includes Black Warrior, Powder River, San Juan, and Uinta basins as coal bed methane reservoirs, and Bakken, Barnett, Eagle Ford, Fayetteville, Haynesville, Marcellus, Niobrara, Oklahoma Area of Interest (AOI), and Permian (Delaware and Midland basins) as oil and gas reservoirs (Scanlon et al., 2020).

Depending on the intended application of treated water, different standards have been issued (Murray-Gulde et al., 2003; Uyttendaele et al., 2015). The United States Department of Agriculture (USDA) has provided such standards for irrigation and livestock consumption (Sirivedhin et al., 2004; Al-Ghouti et al., 2019). Additionally, regulations for treated water reuse as drinking water are generally provided by the United States Environmental Protection Agency (EPA) (Sirivedhin et al., 2004; Al-Ghouti et al., 2019). **Supplementary**

Table 2 demonstrates the standards recommended for reuse in irrigation, livestock consumption, and drinking (Hildenbrand et al., 2018; Al-Ghouti et al., 2019). Recommendations for drinking water are necessarily stricter and therefore it requires more substantial treatment of PW. In contrast, little treatment is needed for PW reuse in oil and gas industry and dust controlling operations (Al-Ghouti et al., 2019). Pondering different applications, a factor that determines obtainable options and plays an important role in considering reuse is expense efficiency (Bagheri et al., 2018), which is very site-specific (Echchel et al., 2018).

Reaching specified standards after the treatment, PW reuse for crop irrigation in arid and semi-arid areas has been recommended (Sirivedhin et al., 2004; Echchel et al., 2018), especially for crops that are salt-tolerant (Gray, 2020). It also helps to preserve freshwater resources in the region (Echchel et al., 2018, 2020). However, it is pivotal what crop type is determined to be irrigated by treated PW due to several challenges, largely due to the high content of TDS, including various salts and sodium (Drewes et al., 2009; Echchel et al., 2018). For instance, the impact of PW on biofuel crops has been investigated in a case study of treated PW reuse for irrigation in the United States (Pica et al., 2017). The water used in this study contained ~2 g/L total organic carbon (TOC) and ~20 g/L TDS and was taken from a central processing facility which supplies ~500 wells in the Denver–Julesburg Basin (Pica et al., 2017). The outcome of this study indicates that achieving 3.5 g/L TDS is necessary for the treated water to be considered for irrigation (Pica et al., 2017). Moreover, it is concluded that TOC concentration of <5 mg/l is needed to keep a viable biofuel crops production rate (Pica et al., 2017).

The water consumed by animals also requires some standards to avoid adverse impacts on their health (Embry et al., 1959; Faries and Loneragan, 2007). However, these standards are usually less strict compared to human ingestion standards, and

many livestock are more tolerant (Al-Ghouti et al., 2019). Salinity and TDS tolerance vary among different species (Alam et al., 2017; Arora and Dagar, 2019), but generally livestock can be watered with a source containing 1 g/L of TDS (Veil et al., 2004; Al-Ghouti et al., 2019). Moreover, moderately treated PW with confirmed harmlessness based on water quality regulations can be used as a source of drinking water for wildlife and also a habitat for water birds and fish species (Sirivedhin et al., 2004). In the United States state of Wyoming, PW with TDS concentration of <5 g/L can be discharged to surface waters in order to be used by livestock and wildlife (Ramirez, 2002; Gray, 2020). This PW with low salinity is primarily from coal bed methane operations, and the aforementioned discharge is subject to permit requirements (Ramirez, 2002). However, due to uncertainties in the water quality, some studies do not support PW reuse other than in the energy sector (Scanlon et al., 2020). The two most common applications for reusing PW in the energy sector are enhanced oil recovery (EOR) and makeup water used for hydraulic fracturing fluid (Khatib, 2007; Graham et al., 2015; Arora and Dagar, 2019); for these applications, high salt concentrations do not preclude use, and thus, only minimal PW treatment may be required for reuse (Webb and Zodrow, 2020). PW reuse for hydraulic fracturing requires efficient removal of solids and iron (≤ 10 mg/L) (Munirasu et al., 2016; Nadella et al., 2020); in addition, it is necessary to inactivate microorganisms to reduce the chance of well clogging and damage (Barnes et al., 2015). For EOR use, filtration may be implemented to remove further targeted particles (Shams Ashaghi et al., 2007; Buono et al., 2019).

PRODUCED WATER TREATMENT

As with other liquid waste streams, the options for PW include proper treatment and discharge, beneficial reuse, or some combination of these (Shaffer et al., 2013; McLaughlin et al., 2020). Because contaminants and concentrations in PW vary, physical, chemical, and biological treatment processes may be required (Fakhru'l-Razi et al., 2009). There are generally three core steps in PW treatment: pre-treatment, main treatment, and post-treatment (Al-Ghouti et al., 2019). It is essential to select a treatment approach based on efficiency, selectivity, and cost. Additionally, many PW treatment operations must take place in settings (e.g., offshore) with severe restrictions on space, weight of the equipment, durability, and ability to operate in harsh environments (Arthur et al., 2011; Dolan et al., 2018). Widely studied techniques for PW treatment include adsorption (Al-Ghouti et al., 2019), chemical precipitation (Blumenschein and Banerjee, 2005; Fakhru'l-Razi et al., 2009), and membrane filtration (Dickhout et al., 2017). Briefly, chemical precipitation is a method to separate a substance from a solution either by converting the substance into an insoluble form or by altering the solvent composition to decrease the solubility of the substance (Firouzjaei et al., 2020a; Peng and Guo, 2020). Adsorption is the adhesion of dissolved constituents to the surface of an insoluble interface, which leads to formation of a thin film creation on the adsorbent surface (Fathy et al.,

2018; Firouzjaei et al., 2020b). This article focuses on membrane treatment of PW.

Membrane filtration is a physical separation technique which selectively fractionates components from a flowing substance *via* pores in a continuous structure (Zirehpour et al., 2016; Firouzjaei et al., 2018a; Rahimpour et al., 2018; Mozafari et al., 2019). Membrane separations are categorized by the driving force and the pore size of applied materials (Xu et al., 2008; Pejman et al., 2020a). In addition to drinking water and wastewater treatment, there are many other applications associated with this technology in diverse industries including the petrochemical (Ravanchi et al., 2009), pharmaceutical (Firouzjaei et al., 2018b; Seyedpour et al., 2020a), and food industries (Pabby et al., 2015). There are different types of membrane materials and membrane processes applied to many applications, but the main goal is always the same: separation. The most common membrane materials are ceramic (Abadi et al., 2011), metal (Li et al., 2020), and polymer (Seyedpour et al., 2020b). Membranes can be classified based on the separation processes.

Microfiltration (MF) (Yang et al., 1998), ultrafiltration (UF) (Ma et al., 2017), nanofiltration (NF) (Anand et al., 2018), and reverse osmosis (RO) (Yang et al., 2019) are four main pressure-driven membrane processes (Van der Bruggen et al., 2003). They can also be categorized as porous (mainly MF and UF) and non-porous (mainly RO and NF) membranes (El-Samak et al., 2020; Zhao et al., 2020). In addition, forward osmosis (FO) is an osmotic pressure-driven membrane process (Pejman et al., 2020b). An ideal membrane for a specific separation purpose should have sufficient mechanical resistance, high flux, and high selectivity (Judd and Jefferson, 2003), with reasonable capital and operating costs. The mechanical resistance is dependent on material and the thickness (Elshorbagy and Chowdhury, 2013; Vajner et al., 2016). High flux can be achieved largely through high porosity and/or large pore sizes. In contrast, high selectivity can be achieved by having small pores with a narrow range of sizes. Therefore, there is usually a trade-off between flux and selectivity (Judd and Jefferson, 2003; Park et al., 2017; Moon et al., 2020). In addition, there is generally an inverse relationship between membrane thickness (number of layers) and flux (Shirazi et al., 2014) which suggests a general trade-off between mechanical resistance and flux. PW treatment using membranes is generally intended to remove contaminants with the size range of 1 nm–1 μ m from the feed (Ebrahimi et al., 2010; Alkhudhiri et al., 2013; Dickhout et al., 2017). Ceramic membranes have attracted major interest due to their extreme thermal stability, mechanical stability, and their compatibility with harsh cleaning methods (i.e., intensifying the cleaning regimes) (Fakhru'l-Razi et al., 2009; Aziz et al., 2019; Ebrahimi et al., 2020). Periodic cleanings are cycles of chemical soaks and backwashes to remove foulants from the membrane surface and are required in any membrane system. Ceramic membranes endurance to higher pressures results in more efficient backwashing (Freeman and Shorney-Darby, 2011). Furthermore, the combination of a wider tolerance of pressure and cleaning chemicals lead to yield more stable permeability in ceramic membranes. For instance, a sustainable flux of $700 \left[\frac{\text{L}}{\text{m}^2 \cdot \text{hr}} \right]$ with a permeability of $1,400 \left[\frac{\text{L}}{\text{m}^2 \cdot \text{hr} \cdot \text{bar}} \right]$ has been

reported by using silicon carbide MF ceramic membranes, fed by PW from an oilfield in the Persian Gulf (Zsirai et al., 2018). Moreover, more than 99% oil removal has been accomplished by using commercial α -alumina MF ceramic membranes (0.8 μ m pore size), fed by synthetic PW containing 250–1,000 ppm concentrations of heavy crude oil droplets of 1–10 micron diameter (Mueller et al., 1997; Al-Ghouti et al., 2019); in all these experiments, the permeate contained <2 ppm total hydrocarbons (Mueller et al., 1997). Polymeric and composite membranes are generally less expensive than ceramic membranes, and their performance has been studied in several research studies (Alzahrani and Mohammad, 2014; Lee et al., 2015; Dickhout et al., 2017). **Table 1** compares the membrane-based technologies utilized for the treatment of PW, highlighting their advantages and downsides (Iggunu and Chen, 2014; Al-Ghouti et al., 2019). Most PW contaminants can be removed efficiently with membrane treatment; for example, total suspended solids (TSS), oil and grease (O&G), and sulfate have been shown to be highly removed in PW treatment (Mondal and Wickramasinghe, 2008; Alzahrani and Mohammad, 2014; Al-Ghouti et al., 2019). For example, >99.9% removal of these three contaminants has been reported for NF followed by RO using commercially available polyamide thin film composite (PA-TFC) NF and low-pressure RO membranes, including NF270 (an NF membrane for salt rejection application) or BW30 (an RO membrane for brackish water treatment application) (Mondal and Wickramasinghe, 2008; Ozgun et al., 2013; Alzahrani and Mohammad, 2014; Al-Ghouti et al., 2019).

Applying non-pressure driven membrane processes such as membrane distillation (MD) is another separation approach (Wang and Chung, 2015). MD is a thermally driven membrane process and is based on the vapor pressure gradient and vapor transport across the membrane (Macedonio et al., 2014; Wang and Chung, 2015). There are many different MD techniques such as air gap MD (Meindersma et al., 2006), sweeping gas MD (Shirazi et al., 2014), and permeate gap MD (Shirazi et al., 2014). A process which has lately been considered for PW treatment is the direct contact membrane distillation (DCMD) (Ali et al., 2018; Anari et al., 2019; Zou et al., 2020). DCMD is a membrane process in which the aqueous feed and permeate are kept in different temperatures while in contact with a hydrophobic membrane which hinders the liquid permeation but allows the vapor to pass (Lawson and Lloyd, 1996; Ashoor et al., 2016); the temperature difference leads to a vapor pressure difference that drives the flux through the membrane from hot feed to the cold permeate (Lawson and Lloyd, 1996; Ashoor et al., 2016). Through DCMD, TDS can be removed by fabricated hollow fiber membranes with the ratio of >99% which sustains even for 100 h of operation (Ali et al., 2018; Zou et al., 2020).

It is also shown that pre-treatment techniques can improve membrane performance and reduce costs (Hilal et al., 2003; Padaki et al., 2015; Kusworo et al., 2018). For instance, the lifetime, flux, and anti-fouling properties of membranes can be improved by using adsorbents at the pre-treatment phase (Kusworo et al., 2018). Another example of pre-treatment is using bioreactors (Huang et al., 2020). For some classes of oil and gas industry wastewater including oil-gas field PW and

petrochemical wastewater, bioreactors can be a very efficient approach to reducing chemical oxygen demand (COD), with reported reductions of 80% for a PW with a COD range of 1,710–3,030 mg/L (Ozgun et al., 2013; Huang et al., 2020). Regarding COD reduction, bioreactors may provide superior performance as pretreatment for RO; pretreatment with MF and UF membranes has been reported to yield <25% reduction of COD (Ozgun et al., 2013; Al-Ghouti et al., 2019).

Membrane filtration is an efficient technique to remove TDS from PW (Çakmakce et al., 2008; Strong et al., 2017; Venkatesan and Wankat, 2017; Atoufi and Lampert, 2020) due to the following reasons: (1) the energy consumption and cost are relatively low [82–84], (2) rejection rates are relatively high (Webb and Zodrow, 2020), and (3) adding less chemicals is required through the process (Webb and Zodrow, 2020). Generally, MF and UF membranes remove a considerable fraction of solids and can serve as pretreatment for NF, RO, or FO techniques in order to reach desired TDS rejection (Atoufi and Lampert, 2020). For example, 95% removal of TDS from PW has been reported by implementing RO (Funston et al., 2002; Atoufi and Lampert, 2020). Specifically, membrane distillation and FO are reported to be very promising for treating PW with high TDS concentration due to the fact that these two techniques use temperature and concentration gradients, respectively, instead of pressure gradient (Coday et al., 2014; Duong et al., 2015; Webb and Zodrow, 2020). However, pressure-driven membranes processes like RO are impractical for treatment of very high salinity (e.g., >35 g/L) PW (Shaffer et al., 2013); because the hydraulic pressure needed to overcome the osmotic pressure of high-salinity waters can be over the allowable pressure of the RO membrane modules, desalination of high-salinity PW with TDS concentrations higher than ~35 g/L requires more energy intensive technologies (Greenlee et al., 2009; Shaffer et al., 2013). Unlike RO, FO is driven by osmotic pressure rather than hydraulic pressure, and is not restricted by the high-pressure operating limitation associated with TDS concentration as high as 70 g/L (Martinetti et al., 2009). Also, low-pressure NF has been practical for removing ions like sulfate and calcium (Fakhru'l-Razi et al., 2009; Webb and Zodrow, 2020). High heavy metal concentrations are an important concern for many PWs, as many heavy metals have major adverse health effects (**Supplementary Table 3**; Fakhru'l-Razi et al., 2009; Tchounwou et al., 2012; Atoufi and Lampert, 2020); NF and RO have been reported to be successful in treating feed water containing multiple heavy metals; reported results indicate an average removal efficiency of 97 and 99% for NF and RO, respectively (Qdais and Moussa, 2004).

In summary, membrane-based treatment of PW has shown success across many contaminants and applications. Although membrane filtration technologies are very effective, desalination of PW with high salinity (>70 g/L) still has room for improvement (Ahmad et al., 2020). Hybrid treating systems, i.e., combination of two or more membrane processes, show more efficacy for treating PW (Murray-Gulde et al., 2003; Ahmad et al., 2020). For example, a four-step method has recently been proposed by Atoufi and Lampert (2020) that is focused on removing salts from PW. The first step is called

TABLE 1 | The membrane technologies used for PW treatment and comparison of their properties [modified from Igunnu and Chen (2014)].

Technology	Feasibility	Chemical use	Pre/post-treatment	Life Cycle (years)	Advantages	Disadvantages
Ceramic MF/UF membrane	Applicable for the treatment of all PW types, especially oilfield PW, but could be problematic for PW with high concentration of salts and TDS.	- Coagulant agents used for precoagulation: aluminum sulfate, polyaluminum chloride, and ferric chloride. - Agents for cleaning process: acids, bases, and surfactants.	- Pre-treatment: cartridge filtration and coagulation. - Post-treatment: depending on the PW, polishing may be needed.	> 10	- Product water is completely free of suspended solids. - Operable in both dead-end and cross flow filtration modes - Product water has recovery range of 90–100%. - Longer lifetime compared to other membranes - Ceramic membranes are superior in thermal, mechanical, and chemical stability	- Periodic cleaning required for the membrane - Irreversible fouling may occur with large amount of iron in the feed water. - Recycling, disposal, or more treatment of the generated waste throughout cleaning and back-wash processes is needed.
Polymeric MF/UF membrane	Applicable for the treatment of PW with high concentration of TDS and salinity.	- Coagulant agents used for precoagulation: aluminum sulfate, polyaluminum chloride, and ferric chloride. - Agents for cleaning process: acids, bases, and surfactants.	- Pre-treatment: cartridge filtration and coagulation. - Post-treatment: depending on the PW, polishing may be needed.	≥ 7	- Product water is completely free of suspended solids. - Product water has recovery range of 85–100%.	- Periodic cleaning required for the membrane - Recycling, disposal, or more treatment of the generated waste throughout cleaning and back-wash processes is needed.
NF	Not recommended to be implemented alone for PW treatment and can be used for treating PW with 0.5–25 g/l of TDS.	- Fouling prevention occurs by caustic and scale inhibitors. - Agents for cleaning process: NaOH, HCl, H₂O₂, Na₂SO₄, and Na₄EDTA.	- Pre-treatment: extensively required for fouling inhibiting. - Post-treatment: may need remineralization in order to restore sodium adsorption ratio (SAR) values.	3–7	- High pH is tolerable. - Automatically operating system - Energy expenses may be lowered by applying energy recovery sub-systems. - Solid waste disposal is not required. - Water recovery of 75–90%	- High sensitivity to organic and inorganic components in the feed water - Membranes are not able to tolerate feed temperatures over 45 °C. - Multiple back-washing cycles are needed.
RO	To be effective in PW treatment, it is necessary to extensively pre-treat the PW feed. Notably, multiple pilot studies failed to use it due to the poor pre-treating process.	- Fouling prevention occurs by caustic and scale inhibitors. - Agents for cleaning process: NaOH, HCl, H₂O₂, Na₂SO₄, Na₄EDTA, and H₃PO₄.	- Pre-treatment: extensively required for fouling inhibiting. - Post-treatment: may need pH stabilization or remineralization in order to restore SAR values.	3–7	- High pH is tolerable - Automatically operating system - Energy expenses may be lowered by applying energy recovery sub-systems. - Great performance for treating PW that is properly pre-treated	- High sensitivity to organic and inorganic components in the feed water - Membranes are not able to tolerate feed temperatures over 45 °C.

feed softening and is purposed to lower the fouling chances during the membrane processes by adding calcium hydroxide to the sample; large suspended solids can be removed by sand filtration which counts as the second step; the third step targets O&G to be removed and is based on ceramic membranes; using RO technique with PA-TFC membrane is the last step (Atoufi and Lampert, 2020). To boost the capabilities of hybrid systems and reduce the cost and energy, it is crucial to consider a right combination of pre-, main stage, and post-treatments based on the treatment purpose (Ahmad et al., 2020). Hence, thorough understanding is required to achieve the optimized hybrid system for any case.

CONCLUSION AND FUTURE PROSPECT

Due to the environment and health concerns caused by PW discharge and/or leakage, the oil and gas industry is attempting to find better approaches to manage PW. Considering required standards, recycled PW reuse can be partly a great solution to reduce demand for freshwater. PW is being treated and reused in oil and gas field operations such as drilling and EOR. As demand for water increases globally, more governments have started to show interest in PW reuse outside of the oil and gas industry applications (Zolghadr, 2016; McLaughlin et al., 2020). Examples are irrigation, livestock consumption, wildlife watering, and, as an extreme example, drinking water. Regarding health concerns, these ideas of PW reuse require very cautious and responsible consideration of the potential advantages and downsides in a case-by-case manner. What took place in Pennsylvania, where water supplies were polluted by PW which was not properly treated (Webb and Zodrow, 2020), emphasizes the need for careful consideration how to handle PW treatment and reuse. On other hand, this experience also suggests that increasing restrictions on disposal can encourage reuse (Behl et al., 2018; Webb and Zodrow, 2020). A successful example is reusing the treated PW from fields with low concentration of TDS (~750 mg/L), located in Kern County, California (Gray, 2020). This treated PW is used for irrigating by local districts after it is blended with other fresh water supplies. Additionally, PW reuse in drilling operations in the Permian Basin by the Apache Corporation is another successful example of PW reuse in the

United States (US Energy Information Administration, 2016). Notably, Apache Corporation has constructed six recycling systems in the west of Texas since 2016 in order to treat around 90% of PW for drilling operations (Behl et al., 2018; Gray, 2020).

It is predicted that the average ratio of water to oil in the process of oil extraction will be 12 to one by 2025 (Global Water Intelligence, 2011; McCabe, 2020). This ratio is approximately four times larger compared to the average ratios reported in the last decade (Veil, 2011; Dickhout et al., 2017; Al-Ghouti et al., 2019). Even in 2004, an average ratio of 9.5–1 has been reported in the United States (Veil et al., 2004; Veil and Clark, 2011). Moreover, the amount of generated PW increases as the oil well ages. The PW volume could even reach 98% of extracted product in fields that are about to being depleted (McCabe, 2020). As a result, PW characterization, treatment, and reuse are expected to attract more attention in the market, and more guidelines are needed for this significant potential. As the largest contributor in the PW generation worldwide, the United States has a great role to investigate different potential reuse purposes and accordingly different techniques for PW treatment.

AUTHOR CONTRIBUTIONS

EZ contributed to the formal analysis, investigation, visualization, and writing—original draft. MDF contributed to the conceptualization, validation, supervision, project administration, and writing—review and editing. GA contributed to the investigation and writing—original draft. PL contributed to the resources, validation, supervision, funding acquisition, and writing—review and editing. ME contributed to the resources, validation, supervision, funding acquisition, project administration, and writing—review and editing. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2021.629767/full#supplementary-material>

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The Applicability of Big Data in Climate Change Research: The Importance of System of Systems Thinking

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The aim of this paper is to provide an overview of the interrelationship between data science and climate studies, as well as describes how sustainability climate issues can be managed using the Big Data tools. Climate-related Big Data articles are analyzed and categorized, which revealed the increasing number of applications of data-driven solutions in specific areas, however, broad integrative analyses are gaining less of a focus. Our major objective is to highlight the potential in the System of Systems (SoS) theorem, as the synergies between diverse disciplines and research ideas must be explored to gain a comprehensive overview of the issue. Data and systems science enables a large amount of heterogeneous data to be integrated and simulation models developed, while considering socio-environmental interrelations in parallel. The improved knowledge integration offered by the System of Systems thinking or climate computing has been demonstrated by analysing the possible inter-linkages of the latest Big Data application papers. The analysis highlights how data and models focusing on the specific areas of sustainability can be bridged to study the complex problems of climate change.

Keywords: big data, climate change, modeling, systems of systems, data science, climate computing

1. INTRODUCTION

Climate change is a pressing issue of today, for which data-based models and decision support techniques offer a more comprehensive understanding of its complexity. The aim of this paper is to reveal data-based techniques and their applicability in terms of climate researches. More precisely, how can Big Data, through data science answer sustainability climate issues and be applicable in scientific researches and decision sciences in an integrated manner.

The overview is guided through three closely related notions, namely, (1) data science as a novel interdisciplinary field connected to (2) machine learning that is a tool for improving automatic prediction or decision processes, and (3) Big Data which foster processing and connecting large amount of heterogeneous data. The focus point of this research is the interconnectedness of the complex climate-related systems, for which exploration Big Data provides an efficient toolbox.

Research questions formulated three aspects, which answering kept in focus through the whole paper:

- How and when Big Data appears in climate-related studies?
- What researches have been made in regard with Big Data applications in climate studies, and how they are structured?
- How to integrate the knowledge accumulated in diverse specific researches?

The year 2015 brought about further excitement in the field of research directions concerning climate change, as the United Nations declared 17 sustainable development goals, of which SDG13 is “Take urgent action to combat climate and its impacts” (UN, 2016) and the Paris Agreement has been signed, that concerning the mitigation of greenhouse gas emissions, adaptation and finance in 2015 with the specific aim of keeping global average temperature rises well below 2°C above pre-industrial levels and then continuing efforts to keep global temperature rises below 1.5°C above pre-industrial levels, recognizing that this will significantly reduce the risks and impacts of climate change (Rogelj et al., 2016). This kind of organizing principle supports the complex analysis of the classical disciplinary sciences with a holistic, interdisciplinary approach. New types of approaches require much more complex analyses and models and, therefore, several orders of magnitude more data, which brought Big Data to life as a stand-alone scientific discipline.

Big Data-based tools are already widespread in this new complex science, for example, to monitor seasonal changes in climate change (Manogaran et al., 2018), understand climate change as a theory-guided data science paradigm (Faghmous et al., 2014), learn how to manage the risks of climate change (Ford et al., 2016), explore soft data sources, e.g., Twitter (Jang et al., 2015), or demonstrate the potential of Systems of Systems (SoS), for instance, the exploration of the structure and relationships across institutions and disciplines of a global Big Earth Data cyber-infrastructure: the Global Earth Observation System of Systems (GEOSS) (Craglia et al., 2017).

Today, it is obvious that sustainability science is intertwined with data science, however, with the support of the business model of the circular economy (Jabbour et al., 2019), the complexity of the problem repository has further increased, so there is an urgent need to include data and analysis methods in the framework, whereas research results from different fields can be used in other fields. Furthermore, trends in climate and sustainability science are driving models toward higher resolution, greater complexity, and larger ensembles, which calls for multidisciplinary approaches in climate computational sciences (Balaji, 2015). This research provides a higher-level overview of the interconnectedness of disciplines, systems, data, and tools related to climate change, exploring further focal points concerning the need a deeper level of integration, because a disconnection between important industry initiatives and scientific research is still experienced (Nobre and Tavares, 2017). We propose to solve these integration tasks and disconnections by the System of Systems thinking.

This overview seeks to address these shortcomings. Information sources (data, news, scientific databases) can be linked, drawing attention to the future importance of open linked data. The present research draws attention to System of Systems (SoS) thinking, as the drivers and effects of climate change, as well as resilience and adaptation, can only be achieved through the timely recognition and exploitation of synergies and trade-offs between the new research directions.

The research methodology outlines firstly, the identification of sustainability science problems in section 2, which revealed the connected issues and tasks as well as the requirements needed to succeed. It ensured that sustainable operation of nature and society demands the approach of systems of system along with the integration of Big Data applications into climate-related scientific, societal, and political researches. This is in line with the growing risk of uncertainty zones highlighted in the planetary boundary framework (Steffen et al., 2015). Then, the existing applications of the related data analysis in the field was explored. For a deeper and narrowed insight, literature review was based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) method, which contributes to the exploration and evaluation of related articles. The search has a clear and narrowed focus on the multidisciplinary nature of the issue, therefore the generic evaluation is not in purpose. Fifty-seven review articles were individually analyzed to identify focus areas and research gaps in the Big Data applications in climate change researches. Systematic meta-analysis was used to identify how data are clustering into diverse focus areas and to extract valuable structural information. The co-occurrences of keywords were examined with regard to 442 articles describing the relationship between climate change and Big Data.

In the following sections, the aforementioned research questions are being unfolded and answered through revealing the increasing importance of the System of Systems theorem. Synergies between new research directions and disciplines must be explored to determine the drivers and effects of climate issues as well as provide an efficient strategic adaptation and mitigation plan that also consider socio-environmental factors. Our proposed SoS framework is a response to this integrated knowledge management, as a first step toward climate computing.

In section 2, the sustainability science theorem questions are answered considering the essential need of data science applications. In section 3, heterogeneous data management as well as Big Data tools and techniques are emphasized.

The systematic review of climate change analyses can be found in section 4, which includes the connections between Big Data and climate in section 4.1 as well as a critical summary of different methods in section 4.2. The social aspects are highlighted in section 4.3. Based on the overview, from the new climate-related research findings, a specific SoS framework is presented in section 4.4 and the intertwining of the SoS and SDGs are discussed in section 5, where the suggestions for future research directions and applications are summarized.

2. PROBLEMS OF SUSTAINABILITY SCIENCE

The complexity of climate issues requires adaptive strategies for public policy (Di Gregorio et al., 2019), actions to incite social behavior (Xie B. et al., 2019), and the development of regulatory and market-simulating responses to economic life (Wright and Nyberg, 2017). To meet this complex societal need, research has focused on understanding the causes of climate change (Hegerl et al., 2019), the development of predictive models (Du et al., 2019), and mitigation solutions (Gomez-Zavaglia et al., 2020), as well as the exploration of opportunities to shape social attitudes (Iturriza et al., 2020).

An interdisciplinary approach is essential in terms of the identification of almost every climate-related problem and development of their solutions. This interdisciplinary perspective has formed sustainability science theorem to gain a comprehensive understanding of the interrelationship between environment and society (Kates et al., 2001). This theory focuses on transdisciplinary questions, which can only be answered by applying of data science tools.

- *How can the dynamic relationship between nature and society be described and analyzed?*

Systems Dynamics Modeling tends to be a commonly used tool when describing and analysing the dynamic interrelation of environment, economy, and society (Honti and Abonyi, 2019). This concept is clearly characterized by the World3 model, which describes the relationship between population, industrial growth, food production, and ecosystem constraints over time for the Club of Rome in the book entitled “The Limits to Growth” (Meadows et al., 1972). The exploration of the relationship between the state variables of the model requires targeted interdisciplinary research. The tools of data science can render this research more efficient with the automated generation and validation of relationship hypotheses (Sebestyén et al., 2019), as data-based models beyond the exploration of probabilistic correlations can provide information on causation (Dörgö et al., 2018). One of the most significant tasks for the more in depth analysis of climate effects is the integration and joint management of heterogeneous data and information. The proof of this potential approach is a case study that interlinks socio-economic variables to explore the effect of the climate on global food production systems (Fischer et al., 2005).

- *How can delays, inertia, and uncertainty in models be handled?*
To quantify the impact of uncertainties inherent in climate variables, the evaluation of Representative Concentration Pathways RCP 4.5 and RCP 8.5 CMIP models developed to forecast climate change (Taylor et al., 2012; Eyring et al., 2016), by using Monte Carlo simulations can be suitable (Mallick et al., 2018). The most important task ahead is the integrated development of targeted solutions for designing, evaluating and integrating simulation studies to quantify uncertainty and risk in the light of environmental and social data (Climate Change, 2014). For this reason DKRZ carried out extensive simulations with the Earth system model MPI-ESM with

respect to the CMIP5 project and the IPCC AR5, presenting a selection of visualizations for different key climate variables and for the different scenarios (Klimarechenzentrum, 2021).

- *How can the features concerning the vulnerability of socio-environmental systems be explored?*

The conceptual framework of vulnerability is grounded by the Intergovernmental Panel on Climate Change (IPCC). The complex impact chains of vulnerability demand the identification and integration of non-climatic factors into climate models, in addition the development of models describing adaptability as well as the estimation of expected damage (Füssel and Klein, 2006). It is believed that the toolbox of network science will play an increasing role in evaluating vulnerability as the significance of state variables and their relationships can be directly qualified regarding their role in dynamic models (Leitold et al., 2020).

- *How can the increasing risk be measured? What scientifically based “boundaries” and “limits” can be defined?*

The purpose of the planetary boundaries concept is to define operating conditions and to account for adverse or catastrophic abrupt environmental changes in the crossing of one or more planetary boundaries (Rockström et al., 2009). Quantifying the risks of climate-induced changes using climate models shows that the risks will increase over the next 200 years, even if the composition of the atmosphere remains constant (Scholze et al., 2006). The socio-cultural domain plays a crucial role in terms of risk perception (Van der Linden, 2015), therefore, the integration of variables describing socio-cultural factors into the models can be particularly important. Analyses are essential to explore how human-induced perturbations affect the delicate balance of the ecosystem in addition to determining where the limits and boundaries are, the crossing of which would pose an unacceptable level of risk (Steffen et al., 2015). The integrated application of simulation tools and machine learning toolbox can efficiently explore these boundaries (Lenton, 2011).

- *What support/motivation systems can be developed—rules, norms, scientific information—to increase the capacity and sustainability of society? What signs and guidelines are needed to put society on a sustainable path? How can today’s isolated research, analyses, and decision support systems be integrated more efficiently?*

The integration and targeted systematization of scientific knowledge is needed to address the long-term causes of climate change and reduce its effects (Pauliuk, 2020). Research concerning sustainability and socio-ecological systems has been partly interlinked to foster sustainability transformation in a transdisciplinary manner. For bridging the gap between science and society, the involvement of citizens in framing research and processes may be a solution as “through their relationship to a place, bounded often as a social-ecological construct, stakeholders, and people at large play an essential role in sustainability transformation research.” Furthermore, the involvement of external parties can support research into socio-ecological systems and sustainability science (Horcea-Milcu et al., 2020). Methods of the co-production of knowledge, e.g., triangulation, the Multiple Evidence Based

approach and scenario building, by learning about cross-border engagement, help to ensure that transdisciplinarity is not only a precursor of integration (Klenk and Meehan, 2015).

To follow the aforementioned path toward sustainable dynamics of nature and society, the data science toolbox and models must be integrated into climate change-related scientific and societal research as well as political agenda. In the following, the Big Data tools and management are interpreted with a specific focus on their role in climate change and we build a System of Systems (climate computing) framework from the various applications.

3. DATA ANALYSIS TASKS OF CLIMATE CHANGE RESEARCHES

The term Big Data has spread due to new technologies and innovations that have emerged over the past decade (Chen and Chiang, 2012) given the demand for the analysis of large amounts of and rapidly generated diverse data, therefore, collection and processing takes place at a high speed, which is difficult to implement with calcareous analytical tools (Constantiou and Kallinikos, 2015). The explosive leap in the amount of data has also infiltrated health, finance, and education (Benjelloun et al., 2015). With regard to the global economy, Big Data is key to understanding and increasing performance (Maria et al., 2015). Big Data is also gaining ground in the field of sustainability, so it can be used to improve social and environmental sustainability in supply chains (Dubey et al., 2019), augment the informational landscape of smart sustainable cities (Bibri, 2018), and improve the allocation and utilization of natural resources (Song et al., 2017) as well as supply chain sustainability (Hazen et al., 2016).

Big and open data from “smart” government to transformational government can facilitate collaboration. It is possible to introduce real-time solutions into agriculture, health, transport, and other challenges (Bertot et al., 2014). The Big Data approach can be the most effective tool to improve mutual governmental and civic understanding, thus embodying the principles of digital governance as the most viable public management model (Clarke and Margetts, 2014). There is a need to collect large amounts of data that can be used to model and test different scenarios to sustainably transform energy production and consumption, improve food and water security, as well as eradicate poverty. Initiatives such as the Intergovernmental Panel on Climate Change and the Global Ocean Observing System can fill gaps in scientific, technical and socio-economic data (Gijzen, 2013). The analysis of sustainable business performance forecasts through the analysis of Big Data in the context of developing countries shows that “Management and leadership style” and “Government policy” are the most significant factors at present (Raut et al., 2019).

The process of data mining is shown in **Figure 1**.

Big Data is a rapidly generated amount of information from a variety of sources and in a different format. Data analysis is the examination and transformation of raw data into interpretable information, while data science is a multidisciplinary field of various analyses, programming tools, and algorithms, forecasting analysis statistics as well as machine learning that aims to

recognize and extract patterns in raw data. Thus, Big Data primarily looks at ways to analyse, systematically extract or otherwise handle data from datasets that are too large or complex to handle with traditional data processing application software that requires significant scaling (multiple nodes) to process efficiently. In other words, Big Data can be defined by the 5V key characteristics, i.e., volume, velocity, variety, veracity, and value (Laney, 2001).

The storage, sustainability, and analysis of massive content is a challenge that the current state of algorithms and systems cannot handle (Trifu and Ivan, 2014) in an integrated manner, therefore the synergies of the different sources are not sufficiently exploited. The purpose of using Big Data is to provide data management and analysis tools for the ever-increasing amount of data (Anuradha et al., 2015). As is shown in **Figure 2**, data analysis can be divided into four general categories (Erl et al., 2016). In the environments of Big Data analytics, data analytics involves the use of highly scalable distributed frameworks and technologies to extract meaningful information from large amounts of raw data that requires the use of different data analysis methods (Rajaraman, 2016).

Big Data is usually associated with two technologies, cloud computing and the Internet of Things (IoT) (Honti and Abonyi, 2019). Cloud computing accelerates unlimited data storage, parallel data processing, and analysis (Inukollu et al., 2014). The key benefits of cloud computing are improved analysis, simplified infrastructure, and cost reduction. IoT offers the ability to connect computing devices, mechanical and digital machines as well as objects and people (Lavin et al., 2015). With the advent of the IoT, huge amounts of data can be collected using smart devices connected via the Internet (Suchetha et al., 2015).

The applicability of Big Data techniques is also significantly enhanced by the novel tools that support data collection and integration. The interoperability of the systems can be improved by data warehouses and the related ETL (extract, transform, load) functionalities that can also be used to gather information from multiple models and data sources. The benefit of these structure are demonstrated in the EC4MACS (European Consortium for Modeling of Air Pollution and Climate Strategies) data warehouse that establishes a suite of modeling tools for a comprehensive integrated assessment of the effectiveness of emission control strategies for air pollutants and greenhouse gases. In this system the integrated data are loaded into the GAINS (Greenhouse gas-Air pollution Interactions and Synergies) Data Warehouse. This assessment brought together expert knowledge in the fields of energy, transport, agriculture, forestry, land use, atmospheric dispersion, health and vegetation impacts, and it developed a coherent outlook into the future options to reduce atmospheric pollution in Europe (Nguyen et al., 2012).

The integration of different information can also be supported by ontology-based linked data. Ontology Web Language (OWL) models enables the semantic characterization of the different events that can describe the climate change story from multiple perspectives, including scientific, social, political, and technological ones (Pileggi et al., 2020).

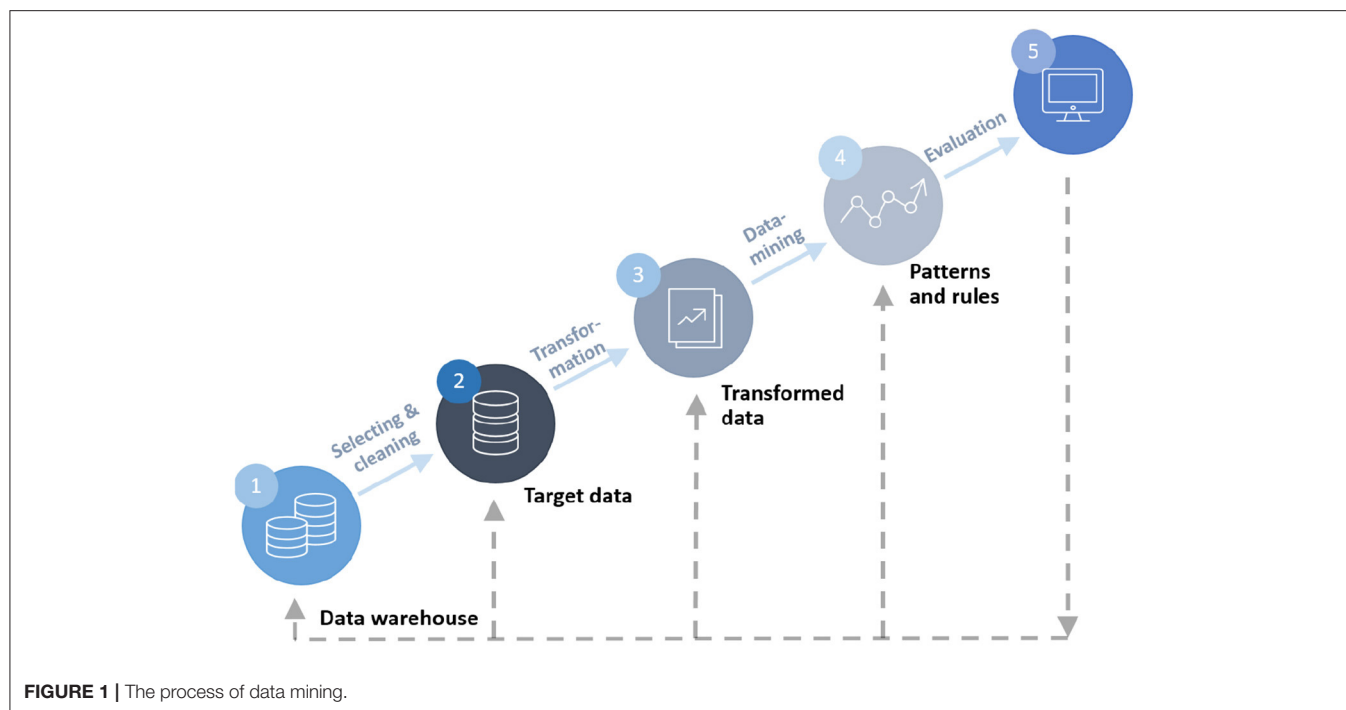


FIGURE 1 | The process of data mining.

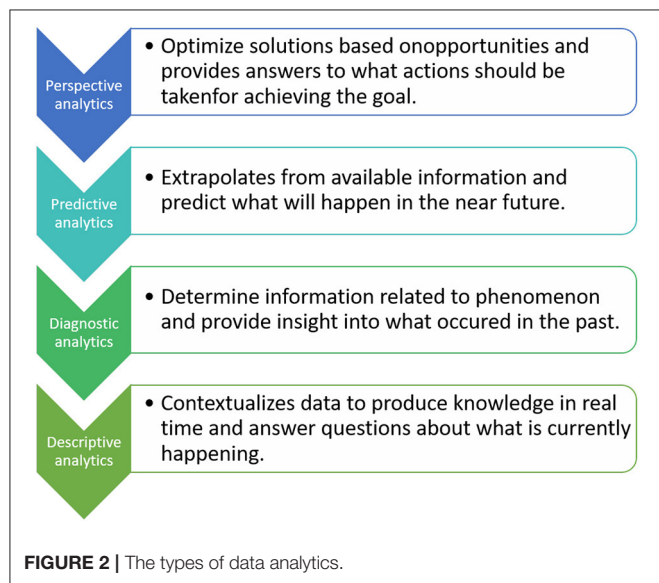


FIGURE 2 | The types of data analytics.

Artificial intelligence (AI) and machine learning (ML) are also the key enabler technologies of big data analysis. This paper focuses on the applicability of ML-based models. AI is mainly used to support decision-making, but it also can skillfully fill observational gaps when combined with numerical climate model data. An example of this application can be found in the extension of historical temperature measurements used in global climate datasets like HadCRUT4 (Kadow et al., 2020).

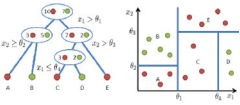
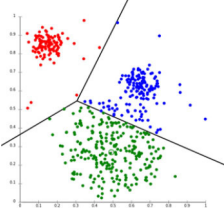
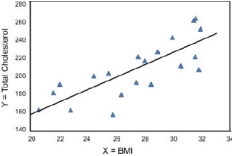
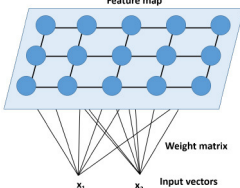
Analysis of Big Data combines traditional methods of statistical analysis with computational approaches. Based on the

complexity between the variables and the type of results required, data analysis can be a simple data set query or a combination of sophisticated analysis techniques (Al-Shiakhli, 2019). The analysis of Big Data is a synthesis of quantitative and qualitative analyses. Climate computing combines multidisciplinary researches in regard to climatic, data and system sciences to efficiently capture and analyse climate-related Big Data as well as to support socio-environmental efforts. Underlying this aspect, a complex model of the earth system is continuously developed by DKRZ using supercomputers relying on Big Data, numerical computations, and simulation models to enable scientists to integrate chemical and biological processes, as well as investigate the interaction of the climate and the socio-economic system (Klimarechenzentrum, 2021).

Exploratory Data Analysis (EDA) techniques are approaches for analysing large data sets. These techniques make the main features clearer by hiding other aspects. Most EDA techniques are graphical in nature, with some non-graphical additions. Some basic EDA tools are histograms, quantile quantile plots (Q-Q-plots), scatter plots, box plots, stratification, log transformation, and other summary statistics (Komorowski et al., 2016). Qualitative models can be classified into qualitative causal models and abstraction hierarchies. The causal models can be classified into Digraphs, Fault Trees, and Qualitative Physics. Abstraction hierarchies consist of two important components: structural and functional (Venkatasubramanian et al., 2003).

Data mining is a set of methods that extracts certain information from large and complex databases. Data discovery uses automated, software-based techniques to eliminate randomness and uncover hidden patterns and trends (Fayyad and Simoudis, 1997). The classification of data mining techniques is summarized in **Table 1** (Zaki and Ho, 2000), including a

TABLE 1 | Data mining techniques and areas of application.

Method	Definition	Data analysis techniques	Areas of application	Climatic examples
Classification 	Discriminating data into different labeled subsets according to class attributes. Retrieving important and relevant information about data and metadata.	Neural network Support vector machine (SVM) Decision tree k-nearest neighbors algorithm Bayesian network Genetic algorithm	Predefined distribution (e.g., identification of differences) Fault detection Anomaly detection problems	Evaluation of hydrological responses Poff et al., 1996, Climate modeling Knutti et al., 2003 Peterson et al., 2002, Mapping mangrove areas Heumann, 2011, land cover Friedl and Brodley, 1997, Vulnerability of the river basin Sharif and Burn, 2006, Forecast uncertainty Gutierrez et al., 2011, Optimizing water distribution system Wu et al., 2010
Clustering 	Grouping the database according to their similarities. Discovering similarities and dissimilarities between the data.	Partition-based algorithms (e.g., K-Means, Fuzzy C-Means) Hierarchical clustering (e.g., dendrograms) Density-based methods Grid-based methods Model-based methods	Data segmentation (division into homogeneous sets) Identification of typical prototypes (e.g., simultaneous identification of time-homogeneous periods and their averages/trends)	Assess soil erosion risk Aslan et al., 2019, atmospheric data Cuzzocrea et al., 2019, wind patterns Wang M. et al., 2020, groundwater level fluctuation Zare and Koch, 2018, Determine drought homogeneous regions Goyal and Sharma, 2016
Regression analysis 	Identifying and analysing the relationship between variables. Predicting and forecasting the process or dependent variables.	Multivariate linear regression Neural networks Regression tree	Creating a model that predicts time (e.g., creating a model for predicting temperatures)	Assess flood risk in urban catchments Jato-Espino et al., 2018, effects on the hydrology cycle Keliang, 2019 and soil organic carbon distribution Olaya-Abril et al., 2017, Determine the shift in climatic trends (temperature) Maheshwari et al., 2020
Frequent itemset/pattern mining 	Determining the association between different datasets. Tracking patterns and creating groups of data that have dependently linked variables.	Frequent itemset search algorithms: Apriori algorithm, FP-grow algorithm sequence search algorithms: refixSpan, Spade, SPAM	Identification of common co-occurring anomalies Exploring the relationships between events and their order	The discovery of spatio-temporal fluctuating patterns with regard to the outbreak of an epidemic Teng et al., 2019 Mapping wind profile patterns Yusuf et al., 2017, atmospheric environment Li et al., 2019, and deforestation Toujani et al., 2020. Predicting climate variability Rashid et al., 2017
Visualization 	Displaying multivariate data. Reducing the number of variables. Exploring the internal context of data.	Principal Component Analysis (PCA) Multidimensional scaling (MDS) t-SNE, Self Organizing Map (SOM)	Exploratory data analysis Identification of factors Preparation of composite indicators Monitoring of complex systems	Analysis of atmospheric circulation patterns and temperature anomalies Gao et al., 2019 and changes in land cover Li et al., 2018, Mapping climate Uddin et al., 2019/ drought Balaganesh et al., 2020, vulnerability and flood hazard mapping in urban environments Rahmati et al., 2019

straightforward description of the method, common analytical techniques, the definition of relevant application areas and examples related to climate studies.

Classification is fundamental in terms of data mining techniques (Zaki and Ho, 2000). Classification models define the similarity structure of the variables and are partitioned into groups (classes) (Aggarwal, 2015). In Big Data-based climate studies, classification models and techniques are greatly utilized. Two streams with different hydroclimatologies were studied in the United States using an artificial neural network (ANN). The analysis identified a large effect on a variety of factors such as average runoff, flow variability, flood frequency and baseline flow stability (Poff et al., 1996). To overcome the great uncertainties inherent in climate models, an alternative neural network-based climate model has been developed that increases the efficiency of large climate model sets by at least one order of magnitude. Based on this, it can be concluded that heating exceeds the surface heating range estimated by the IPCC for almost half of the members of the ensemble (Knutti et al., 2003). This neural network is an effective tool for dealing with such difficult and challenging problems, moreover, has been widely used to explore the mechanisms of climate change and predict trends in climate change that take full advantage of the unknown information hidden in climate data, however, it cannot decipher it.

General Circulation Models (GCMs)—the most advanced tools for estimating future climate change scenarios—operate on a coarse scale, which can be downscaled by support vector machine (SVM) approaches, training meteorological subdivisions (MSDs) and developing a downscaling model (DM) that has been shown to be better than conventional downscaling using multilayered regenerative artificial neural networks (Tripathi et al., 2006). The utilization of solar energy is evolving dynamically in connection with SDG 7, but power plant performance may fluctuate due to the diversity of meteorological conditions, which can be compensated by satellite imagery and SVM learning scheme to predict the motion vector of clouds (Jang et al., 2016). Object-based image analysis (OBIA) and support vector machine (SVM) combined with a decision-tree classification are suitable for mapping mangrove areas that was impossible by traditional remote sensing methods other than rough spatial resolution (Heumann, 2011). Decision tree algorithms consistently outperform maximum likelihood and linear discriminant function classifiers in terms of land cover mapping problems classification accuracy (Friedl and Brodley, 1997). Using a weather-generating model, which allows the nearest neighbor to be re-sampled by disturbing historical data, it is possible to create a set of climatic scenarios based on probable climatic scenarios to produce meteorological data that can be used to assess the vulnerability of the river basin to extreme events (Sharif and Burn, 2006). The ability of the Bayesian Network (BN) to predict long-term changes in the shoreline associated with rises in sea level and quantitatively estimate forecast uncertainty renders it suitable for research into the effects of climate change (Gutierrez et al., 2011). It has been used successfully to assess the effects of climate change disturbances on the structure of coral reefs (Franco et al., 2016) and in terms of belief updating concerning the reality of climate

change in response to presenting information concerning the scientific consensus on anthropogenic global warming (AGW) (Cook and Lewandowsky, 2016). Using genetic algorithm and occurrence data from museum specimens, ecological niche models were developed for 1,870 species occurring in Mexico and projected onto two climatic surfaces modeled for 2055 (Peterson et al., 2002). A multi-objective genetic algorithm for optimizing water distribution systems (WDS) was used as a discovery tool to examine trade-offs between traditional economic goals and minimize greenhouse gas emissions (Wu et al., 2010). The European territory was subdivided into similar regions of predicted climate change based on simulations of total daily precipitation as well as recent (1986–2005) and long-term future (2081–2100) temperatures using K-mean cluster analysis (Carvalho et al., 2016). An automated procedure based on a cluster initialization algorithm is proposed and applied to changes in the 27 climatic extremes. The proposed method requires, on average, 40% fewer scenarios to meet the 90% threshold than k-means clustering (Cannon, 2015).

Clustering-based analyses are widely accepted data mining techniques, however, improvements in terms of time and cost savings are constantly required due to the management of an increasing amount of data (Shirkhorshidi et al., 2014). Regarding its usage in climatic analyses, a clustering-based spatio-temporal analysis framework of atmospheric data was developed to support both governmental and industrial decision-making processes (Cuzzocrea et al., 2019). To assess erosivity risk, clustering and classification analyses were applied on the national level in Turkey, moreover, an artificial neural network-based prediction was also made. The results identified an increasing risk of soil erosion in the southern and western regions of Turkey, which demands erosion control practices (Aslan et al., 2019). Research has been conducted to regionalize Europe according to similar surface temperatures based on data between 1986 and 2005. The differences between long-term predictive data (CMIP5) and historical data were analyzed with k-means clustering analyses to determine grid points (Carvalho et al., 2016). A fuzzy c-means approach regionalization was determined in western India for the analysis of meteorological drought homogeneous regions to provide effective support for water resources planning and management during droughts (Goyal and Sharma, 2016). Clustering techniques can support simulation and predict models by grouping large-scale data. “Wind energy production is expected to be affected by shifts in wind patterns that will accompany climate change.” In California, wind patterns have been clustered using model simulations from the variable-resolution Community Earth System Model (VR-CESM) and analyzed according to the change in the frequency of clusters and changes in winds within clusters. The changes in capacity factor have significant influence with regard to energy generation (Wang M. et al., 2020).

Regression analysis sought to reveal functional relationships between variables that can further support predictive and forecasting models. Urbanization tends to have a significant impact on climate change, as underlined by an Australian study which determined that changes in land use and vegetation as a result of shifts in urbanization that affect the local climate

and water cycle as well as its impacts are considered to be local specific (Maheshwari et al., 2020). Multiple regression-based analysis has been used to determine flood risk in urban catchments by combining multiple linear regression, multiple nonlinear regression and multiple binary logistics regression. This framework sought to support action plans concerning drainage management and maximize the impacts of flood susceptibility strategic implementations (Jato-Espino et al., 2018). Regarding water management, the influence of climate change on the hydrological cycle in the Yangtze River Basin has been analyzed using a regression analysis model and geographic information system (Keliang, 2019). Soil plays a significant role in carbon sequestration, therefore, moderate undesired climatic effects. A model has been designed regarding the top 25 cm of topsoil of the Sierra Morena (Red Natura 2000) area to determine the relationship between independent variables and soil organic carbon (SOC), moreover, by the use of multiple linear regression analysis examined the effects of these variables on SOC content. The results indicated that “SOC in a future scenario of climate change depends on average temperature of coldest quarter (41.9%), average temperature of warmest quarter (34.5%), annual precipitation (22.2%), and annual average temperature (1.3%).” The comparison between the current (2016) and future situations reflects a reduction of 35.4% SOC content and a trend in northward migration (Olaya-Abril et al., 2017).

Frequent itemset/pattern mining is a commonly used technique to extract knowledge from databases. The handling of an increasing amount of heterogeneous data is becoming ever more difficult, therefore, “an efficient algorithm is required to mine the hidden patterns of the frequent itemsets within a shorter run time and with less memory consumption while the volume of data increases over the time period” (Chee et al., 2019). Association rule mining (ARM) models have been built for atmospheric environment monitoring based on the Apriori algorithm and D-S theory/ER algorithm. These techniques provide both technical and theoretical support to prevent as well as manage air pollution (Li et al., 2019). Association rule mining has also been used in terms of monitoring weather behavioral data to develop a prediction model for climate variability (Rashid et al., 2017). Furthermore, climate variability has an impact on agriculture, which demands a greater understanding with regard to the impact of the climate on crop production and food security. Therefore, the impact of seasonal rainfall on rice crop yield was determined based on ARM techniques (Gandhi and Armstrong, 2016). For the understanding of wind conditions, multidimensional sequential pattern mining is used that can define which pattern is suitable for wind energy (by taking into consideration the factors of space, time, and height). According to a study on the Netherlands, 68.97% of the country covered by a suitable wind pattern (at 128 m) and already has wind turbines installed (Yusof et al., 2017). A spatio-temporal pattern-based sequence classification framework was built to estimate the extent of deforestation. This approach was applied on a Tunisian case study that took into consideration 15 years of satellite images and historical wildfire GIS data (Toujani et al., 2020).

Visualization methods sought to explore the interconnections between data by simplifying multivariate data. Self-organizing

map neural network (SOMN) method has been used to analyse anomalous atmospheric circulation patterns in China with regard to surface temperature anomalies between 1979 and 2017 (Gao et al., 2019). This method is greatly used for mapping changes, e.g., regarding urban flood hazards (Rahmati et al., 2019). A study on the city of Amol in Iran was conducted and according to the aforementioned model of urban flood hazard mapping, 23% of the land area of the city is expected to high or very high levels of flood risk, which demands efficient flood risk management. SOMN and grid cells method were applied to determine changes in spatio-temporal land cover in Inner Mongolia between 2004 and 2014 (Li et al., 2018). The Principal Component Analysis (PCA) technique has been used to assess the vulnerability of the coastal region of Bangladesh while taking into consideration the IPCC framework. The study used 31 indicators (24 socio-economic, 7 natural). PCA was applied and determined seven eigenvectors [Demographic Vulnerability (PC1), Economic Vulnerability (PC2), Agricultural Vulnerability (PC3), Water Vulnerability (PC4), Health Vulnerability (PC5), Climate Vulnerability (PC6), and Infrastructural Vulnerability (PC7)] that take into consideration climate change scenarios from 2013 to 2050 (Uddin et al., 2019). PCA has also been used to build the composite drought vulnerability index (Balaganesh et al., 2020).

4. SYSTEMATIC REVIEW OF CLIMATE CHANGE-RELATED ANALYSES

4.1. Overview of Big Data-Based Climate Change Analysis

The significance of Big Data in climate-related studies is greatly recognized and its techniques are widely used to observe and monitor changes on a global scale. It facilitates understanding and forecasting to support adaptive decision-making as well as optimize models and structures (Hassani et al., 2019).

Review articles can provide a better organized structure of previous studies, so the major focus areas are determined with regard to previous review articles concerning the connection between climate change and Big Data. The major objective is to reveal how diverse disciplines appears in the related researches, therefore narrowing when and how Big Data applications and the relation with data science are appeared in climate studies.

A comprehensive overview was conducted based on the Scopus database. Fifty-seven articles were retrieved from the following search: [TITLE-ABS-KEY(“climate change”) AND TITLE-ABS-KEY(“Big Data”)] AND [TITLE-ABS-KEY(“overview”) OR TITLE-ABS-KEY(“review”)].

Articles were reviewed and selected individually for the final sample. **Table 2** shows the number of articles selected and excluded.

The 47 articles of the final sample are shown in **Tables 3–5**, where a straightforward description and focus area of the research are indicated as well as categorized accordingly. It is notable that mostly specific climate issues are observed (e.g., decarbonization of energy or land ecosystem) and their potential with regard to Big Data determined. The two most affected

TABLE 2 | Selection of articles related to the review of climate data.

Scopus database search	No. of articles	No. of duplicates	No. of unavailable	No. of excluded	No. of reviewed
"climate change" AND "Big Data" AND "review/overview"	77	3	8	19	47

categories are agriculture and studies of sustainable cities and communities. This is a good illustration of how intertwined research on climate action is with sustainable development goals.

The quality and safety of agricultural products can be assured through solutions provided by the Internet of Things (IoT) and cloud computing (Marcu et al., 2019). Remote sensing and Artificial Intelligence technologies enables to integrate Big Data into predictive and prescriptive management tools, to improve e.g., the resilience of agricultural systems (Jung et al., 2020). Big Data virtualization in the field of agriculture enables physical objects to be virtualized, e.g., sensors and devices used for defining soil moisture, water flows, or salinity, where these objects can provide diverse meaningful information in each phase of a data chain to support decision-making and information handling (Mathivanan and Jayagopal, 2019). Furthermore, Big Data techniques are utilized in terms of plant breeding (Taranto et al., 2018), crop ideotypes for food security (Christensen et al., 2018), or in precision agriculture framework (Demestichas et al., 2020). Climate Smart Agriculture framework aims to enhance the capacity of the agricultural systems to support food security, supporting adaptation, and mitigation into sustainable agriculture development through latest technologies as IoT, AI, geo-informatics, and Big Data analytics (Gulzar et al., 2020). The interdisciplinary and systematic approach of soil use and management to achieve related sustainability goals has also been explored (Hou et al., 2020).

Alignment with regard to the focus area of sustainable cities and communities with the 11th sustainable development goal (Sustainable cities and communities) has been explored through reviews. Big Data management can enhance the opportunity for organizations to respond to the risk of climate change in time (Seles et al., 2018) as well as offers possibilities to consider sustainable production and lower emission rates. Furthermore, machine learning can be effectively utilized for low-carbon urban planning (Milojevic-Dupont et al., 2020). Outside the field of industry, co-operation, legislation, and environmental agreements are essential to realize a sustainable manufacturing environment (Hämäläinen and Inkinen, 2019). The concept of smart cities seeks to overcome and prevent climate change and issues concerning urbanization (Sharifi, 2019), moreover, smart transportation policies can utilize the advantages of Big Data (De Gennaro et al., 2016). In this smart environment, civil engineers are seen as future risk and uncertainty managers to improve community resilience through smart infrastructure programs (Berglund et al., 2020).

Climate resilience studies assess how to prepare for, recover from and adopt to climate-related risks (Center for Climate and Energy Solutions, 2019). Big Data seeks to support these activities by providing a large volume, variety, and quality data

to reveal patterns and enables data democratization (Faghmous et al., 2014). Therefore, Big Data approach can serve as a source of key information for decision-makers in terms of creating and adapting appropriate strategies, determining current, and upcoming issues, as well as identifying stages of recovery for taking actions in time (Sarker et al., 2020). News media can serve as a near-real-time geolocated information, which can support the understanding of social movements and early-warning systems. "Combining news media with social and biophysical data is important to verify results and limit biases in analysis" (Buckingham et al., 2020). One of the issues concerning urban environments is energy efficiency and carbon emissions, for which net zero energy movements seek to bring about a solution as well as the application of a resilience ecological framework for net zero energy research (Hu and Pavao-Zuckerman, 2019). Furthermore, Big Data techniques with regard to machine learning enable the attitude of people toward and recognition of environmental changes to be determined (Park et al., 2020). Big Data and machine learning approaches are vital in comprehensively merging heterogeneous genomic and ecological datasets (Cortés et al., 2020).

However, review articles have explored the potential for utilizing Big Data techniques in diverse areas, moreover, comprehensive overviews about climate change are becoming less of a focus. Even though data-intensive research applications may seem to be unbalanced among disciplines (Hassani et al., 2019), the dynamism and complexity of climate issues must not be neglected. This complexity brings about an interdisciplinary approach and the intertwining of diverse disciplines, to which the System of Systems concept (climate computing) is the urgent answer.

4.2. Meta-Analysis With Regard to the Methods of Climate-Related Analyses

Co-word analysis examines the relationships between keywords to reveal the structure and development of methodologies or applications. The relationships between keywords in research papers "contains valuable information about knowledge structure of the field, its relevant concepts, and their connections" Lozano et al. (2019). It is our aim to determine diverse focus areas, methodologies and techniques regarding Big Data-driven climate change analyses and harmonize these to allow better utilization of the achieved field-specific results.

The Scopus database was used to identify the corresponding papers using the following search: [TITLE-ABS-KEY("climate change") AND TITLE-ABS-KEY("Big Data")]. As a result 442 articles were retrieved and the co-occurrence of their keywords analyzed using VOSviewer. The time period in which the

TABLE 3 | Overview of articles analysing Big Data usage with climate change issues categorized into the domains of Agriculture, Cleaner production, and Climate resilience.

Agriculture			
Focus area	Description	Usage	References
Soil	The article provides a comprehensive overview about soil in connection with sustainability issues—several SDGs.	The overview highlights that interdisciplinary studies which incorporate such advances may lead to the innovative sustainable use of soil and management strategies that seek to optimize soil health and achieving the SDGs.	Hou et al., 2020
Land ecosystem	The article analyses the developmental characteristics and trends of research into global land ecosystem services using the Bibliometrix software package.	The overview highlights the diverse facets of land ecosystem services and the practical application of land ecosystem services.	Xie et al., 2020
Virtualization, soil, water, crops, plants	The article provides a comprehensive review of Big Data virtualization in the agricultural domain.	The overview highlights the potential in information a the virtual object as it has large volume of data which helps data analysis or to create application services like decision-making, problem notification, and information handling.	Mathivanan and Jayagopal, 2019
Crop production, food security	The article examines modeling strategies for the development of crop ideotypes and scientific visualization technologies that have led to discoveries in “Big Data” analysis.	The overview highlights that integrative modeling and advanced scientific visualization may help overcome challenges in agricultural and nutritional data as large-scale and multidimensional data become available in these fields.	Christensen et al., 2018
Soil	The article explores trends in the development of pedotransfer around the world and considers trends between data and methods to build pedotransfer relationships.	The overview highlights that the physics-based interpretation of pedotransfer functions (PTFs) is expected to be in demand.	Pachepsky et al., 2015
Plants, biotechnology	The article describes technologies concerning plant breeding and provides examples of their application to breed climate- resilient cultivars.	The overview highlights that technological improvements in phenotypic and genotypic analysis, as well as the biotechnological and digital revolution, will reduce the breeding cycle in a cost-effective manner.	Taranto et al., 2018
IoT, cloud technology, Smart farming	The article explores the potential in IoT technology with regard to the agricultural sector—plants are sensitive to changes, in climate change context and monitoring, IoT can bring about dramatic progress.	The overview can be used as a basic tool for choosing an IoT platform solution for future telemonitoring systems.	Marcu et al., 2019
Smart farming, crops	The article presents a review of some areas involved in the definition of an alert system for diseases and pests in terms of Smart Farming, based on machine learning and graph similarity.	The article proposes an architecture for coffee disease and pest detection.	Lasso and Corrales, 2017
Food safety	The article presents a review of the likely consequences of climate change for foodborne pathogens and associated human illnesses in higher-income countries.	The overview highlights that climate change may have important effects of foodborne illnesses.	Lake and Barker, 2018
Agricultural systems, AI, remote sensing	This article focuses on the use of recent technological advances in remote sensing and AI to improve the resilience of agricultural systems.	The review presents a unique opportunity for the development of prescriptive tools needed to address the next decade's agricultural and human nutrition challenges.	Jung et al., 2020
Smart farming	The article conducts a literature review of prominent ICT solutions, focusing on their role in supporting different phases of the lifecycle of precision agriculture-related data.	The article also introduce a developed data lifecycle model as part of a novel categorization approach for the analyzed solutions.	Demestichas et al., 2020
Food safety	The article discuss some of the forefront issues in food value chains with a focus on using technology.	The article highlights that the cultural awareness and social innovation to prevent food waste and therefore improve food security and sustainability will also prove to further complexities.	Chapman et al., 2020
Smart agriculture	This article presents an analytical review of smart agriculture (SA) and climate smart agriculture (CSA) along with a thorough CSA architectural taxonomy.	The article surveys CSA and devise its architectural taxonomy in terms of technological components of SA as well as climate change mitigation to ensure food security, environment sustainability and lesser CO2 emissions.	Gulzar et al., 2020

(Continued)

TABLE 3 | Continued

Cleaner production			
Focus area	Description	Usage	References
Cleaner production	The article provides an overview of the scope and trends in venture capital-funded innovation in Cleantech.	The overview explores trends in venture capital-funded innovation in Cleantech, the broad scope of the basic science and technology, and the impacts of Cleantech that affect global climate change.	Huang, 2015
Energy	The article provides a comprehensive review that assesses the current as well as the potential impact of digital technologies within cyber-physical systems (CPS) on the decarbonization of energy systems.	The overview highlights advances in CPS and Artificial Intelligence (AI) with regard to real-world adaptation in energy systems.	Inderwildi et al., 2020
Climate resilience			
Focus area	Description	Usage	References
Satellites, remote sensing	The article explores the potential of Big Data with regard to implementing a proper strategy against the effects of climate change as well as enhancing the resilience of people in the light of the adverse effects of climate change.	The overview enables policymakers and related stakeholders to implement appropriate adaptation strategies for enhancing the resilience of the people from the affected areas.	Sarker et al., 2020
Machine learning	The article explores the attitude of people toward climate change issues based on news analysis.	The article highlights the potential in using this method for monitoring functions, recognition and that detection of opinion.	Park et al., 2020
Satellites, simulation, weather, water, land ecosystem, air	The article explores the advances of climate change studies based on Earth observation Big Data and provides examples of case studies that utilize Earth observation Big Data in climate change research.	The overview suggests that the management of data resources should be strengthened and the construction of the global change Earth observation data-sharing platform for the realization of the effective sharing of data resources accelerated.	Guo et al., 2015
Energy, climate resilience	The article provides an initial step in terms of understanding the research activities of the past five decades in these two areas (NZE and resilience) as well as their connection to their ecological roots.	The overview highlights the major difference between the net zero movement and resilience theory in terms of the urban environment and their respective relations to their ecological origins.	Hu and Pavao-Zuckerman, 2019
Water	The article explores some important impacts on the development of hydrology and water resources in Australia.	The overview highlights that the value and distribution of water resources will change.	Fitzharris, 2016
Forestry	The article discuss predictive genomic approaches that promise increasing adaptive selection accuracy and shortening generation intervals.	The article discuss how trees' phylogeographic history may affect the adaptive relevant genetic variation available for adaptation to environmental change. Encouraging "Big Data" approaches (machine learning—ML) capable of comprehensively merging heterogeneous genomic and ecological datasets.	Cortés et al., 2020

papers were written was between the years 2012 and 2020. In **Figure 3**, seven clusters are indicated by a diverse range of colors that overarch topics related to climate change and application methods of Big Data.

Each cluster refers to a focus area including its attributes of interrelationships as well as methodologies and techniques applied in the field.

The "Red" cluster denotes the connections between Big Data technologies and methods applied for optimization procedures, measures the impact of climate change and resilience as well as makes predictions. Technologies are considered, e.g., artificial intelligence, learning algorithms such as machine learning and deep learning, data analytics, neural networks, and cluster computing. Neural networks are used to analyse climate change, weather prediction, and visualization (Buszta and Mazurkiewicz,

2015), while machine learning techniques are used for intelligent recognition (Demertzis and Iliadis, 2016) and to define the impact of climate change and resilience (Rolnick et al., 2019). In addition, they are used to predict epidemics and diseases in both social (Rees et al., 2019) and environmental contexts e.g., in the case of crops (Fenu and Mallocci, 2019), coffee disease and pest (Lasso and Corrales, 2017), or pedotransfer functions (Benke et al., 2020). Clustering techniques on cloud computing infrastructure have been applied, e.g., to map changes in glaciers (Ayma et al., 2019). A novel machine learning approach has been developed by the U.S. Department of Energy's National Renewable Energy Laboratory using adversarial training in climate forecasting, in which the model provides a "physics-informed variation to the super resolution generative adversarial network (SRGAN) model, which extends proven performance

TABLE 4 | Overview of articles analysing Big Data usage in terms of climate change issues categorized into the domains of Cyberinfrastructure (IoT), Impact assessment and Methods.

Cyberinfrastructure (IoT)			
Focus area	Description	Usage	References
Climate models	The article provides an overview of some Big Data problems of climate science's and the technical solutions being developed to advance data publication, climate analytics as a service as well as interoperability within the Earth System Grid Federation (ESGF), which is currently the primary cyberinfrastructure supporting global climate research activities.	The overview highlights how improved machine-to-machine interoperability can lead to increased analytical capabilities across distributed storage systems.	Schnase et al., 2016
Plants, biotechnology	The article describes technologies applied to plant breeding and provides examples of their application to breed climate-resilient crop cultivars.	The overview highlights that technological improvements in phenotypic and genotypic analyses, as well as the biotechnological and digital revolution, will reduce the breeding cycle in a cost-effective manner.	Taranto et al., 2018
IoT, cloud technology, Smart farming	The article explores the potential of IoT technology in the agricultural sector—plants are sensitive to changes in terms of climate change and monitoring, IoT can bring about dramatic progress.	The overview can be used as a basic tool for choosing an IoT platform solution for future telemonitoring systems.	Marcu et al., 2019
Smart farming, Crops	The article presents a review of some areas involved in the definition of an alert system for diseases and pests in terms of Smart Farming, based on machine learning and graph similarity.	The article proposes an architecture for coffee disease and pest detection.	Lasso and Corrales, 2017
Water, IoT	The article provides a review of the application of the Internet of Things in the field of marine environment monitoring.	The overview highlights that Big Data analytics can be used not only as feedback for agencies and control center of marine environment but also for autonomous vessels and remotely developed devices in order to take real-time actions.	Xu et al., 2019
Agricultural systems, AI, remote sensing	This article focuses on the use of recent technological advances in remote sensing and AI to improve the resilience of agricultural systems.	The review presents a unique opportunity for the development of prescriptive tools needed to address the next decade's agricultural and human nutrition challenges.	Jung et al., 2020
Remote sensing, urban development, ML	The article shows that the emergence of Big Data and machine learning methods enables climate solution research to overcome generic recommendations and provide policy solutions at urban, street, building and household scale, adapted to specific contexts, but scalable to global mitigation potentials.	The article suggests a meta-algorithmic architecture and framework for using machine learning to optimize urban planning for accelerating, improving and transforming urban infrastructure provision.	Milojevic-Dupont et al., 2020
Impact assessment			
Focus area	Description	Usage	References
Climate models	The article provides a critical overview and synthesis of issues related to climate models, data sets, and impact assessment methods pertaining to islands which can benefit decision-makers and other end users of climate data in island communities.	The overview explores challenges of islandness in terms of top-down, model-led climate impact assessment and bottom-up, vulnerability-led approaches.	Foley, 2018
Risk management, water, energy, food safety	The article examines the challenge facing risk assessment posed by the transmission of climate risk.	The overview aims to support future national risk assessments, ensuring that they adequately account for the transmission mechanisms of climate risk.	Challinor et al., 2018
Water	The article explores some important impacts on the development of hydrology and water resources in Australia.	The overview highlights that the value and distribution of water resources will change.	Fitzharris, 2016
Food safety	The article presents a review of the likely impacts of climate change for foodborne pathogens and associated human illnesses in higher-income countries.	The overview highlights that climate change may have important effects on foodborne illnesses.	Lake and Barker, 2018

(Continued)

TABLE 4 | Continued

Focus area	Description	Usage	References
Climate change	The article identifies the potential of this new data source for the increasingly important role that computational social science can play alongside established biophysical data in monitoring largescale environmental change.	The article highlights that combining news media data, such as GDELT, with other social and biophysical data sources is an important method for verifying results and limiting biases in data collection and analysis.	Buckingham et al., 2020
Methods			
Focus area	Description	Usage	References
Machine learning, crowdsourcing, data fusion, cluster analysis	The article provides an overview of techniques and approaches with regard to climate studies.	The overview provides brief knowledge of a few strategies in terms of supporting Big Data administration and investigation in the domain of geoscience for climate studies.	Radhika et al., 2016
Water	The article presents the advances in machine learning and deep learning through novel classification methods.	The overview outlines present state-of-the-art machine-learning and deep-learning methods used to model and identify application areas.	Ardabili et al., 2019
Water, weather, air quality, Hazard management	The article provides a review of crowdsourcing-related papers in seven domains: weather, precipitation, air pollution, geography, ecology, surface water and natural hazard management.	The overview outlines knowledge development in terms of crowdsourcing within the specific domain of geophysics as well as similarities and differences.	Zheng et al., 2018
Plants	The article reviews phenology models as an important component of earth system modeling.	The overview highlights that the mechanistic development of phenological observation is essential.	Tang et al., 2016
Climate models	The article explores space-time analytics dealing with spatial processes, examples of space-time concepts and tools to analyse data.	The overview suggests movement-based space-time analytics by addressing processes across multiple levels with constraints of boundary conditions and initial conditions for the processes at the focal level.	Yuan and Bothwell, 2013
Remote sensing, urban development, ML	The article show that the emergence of Big Data and machine learning methods enables climate solution research to overcome generic recommendations and provide policy solutions at urban, street, building and household scale, adapted to specific contexts, but scalable to global mitigation potentials.	The article suggests a meta-algorithmic architecture and framework for using machine learning to optimize urban planning for accelerating, improving and transforming urban infrastructure provision.	Milojevic-Dupont et al., 2020
Land ecosystem	The article provides an overview on Integrated Climate Sensitive Restoration Framework that recognizes the local participation in mapping degraded lands, identification of species for supporting species modeling to better understand climate uncertainty.	The article highlight that the framework potentially helps in sustainable land restoration by transformative changes for achieving UN decade on Ecosystems Restoration (2021–2030), SDGs 15 and addressing the post 2020 Global Biodiversity Framework.	Dhyani et al., 2020

on super resolution of natural images to scientific datasets” (Stengel et al., 2019). This breakthrough is capable of saving computational time and data storage, moreover, can provide more accessible high-resolution climate data that can be utilized in a wide range of climate scenarios. These techniques seek to assess risk management in terms of human and environmental health by providing vital information concerning the present conditions and making predictions about the future.

Keywords included in the “orange” cluster, mainly describe agriculture-related climate issues and adaptations. IoT technologies, information systems and sensor networks tend to be applied in a field. Big Data increase the heterogeneity “across farms, farmers, climates, crops, soils, natural resources, models, management strategies and outcomes, post production

value chain system, and other economic variables of interest” that can boost knowledge with regard to the concept of climate-smart agriculture (Rao, 2018). IoT technologies have been proven to be beneficial in improving efficiency in the complex field of agriculture. Sensors are used to collect vital information about soil, fertilizer, moisture, sunshine, temperature, and geographic information of farmland for monitoring as well as to link to other databases for identifying attributes (Yan-e, 2011). The combination of automation and IoT technologies broad perspectives in smart agriculture, as remote controlled robots to perform tasks, smart and intelligent decision making based on real time data as well as warehouse management (Gondchawar and Kawitkar, 2016).

TABLE 5 | Overview of articles analysing Big Data usage in terms of climate change issues categorized into the domains of Sustainable cities and communities, Water, and Biodiversity.

Sustainable cities and communities			
Focus area	Description	Usage	References
IoT, visualization, water, air, energy, crowdsourcing	The article explores the role of civil engineers with regard to conventional and smart infrastructure programmes—managers of risk and uncertainty—as well as considers climate change mitigation	The overview incites inventive thinking to develop research agendas and creatively integrate new technologies across infrastructures.	Berglund et al., 2020
Smart city	The article provides a critical analysis of 34 selected smart city assessment tools to highlight their strengths and weaknesses as well as examine their potential contribution to the evolution of the smart city movement.	The study can be used by interested target groups such as smart city developers, planners, and policy makers to choose tools that best fit their needs.	Sharifi, 2019
Emissions tracking	The article illustrates that Big Data is utilized in various industries, and explores a large variety of pollutants.	The overview addresses the need for using and combining data resources, particularly at the industrial level, in order to develop more efficient tools for environmental monitoring and decision-making.	Hämäläinen and Inkinen, 2019
Energy	The article builds complex uncertainty models of power demand and the cost of renewable energy generation, as well as proposes an improved IRSP model based on complex uncertainty simulation.	The overview highlights the necessity to look at the development of electricity from the perspective of energy, moreover, additional primary energy limitations will be introduced into the model in the future.	Zheng et al., 2019
Remote sensing, weather, climate model, air quality, machine learning,	The article reviews the current state of urban data science in the context of climate change investigates the contribution of urban metabolism studies, remote sensing, Big Data approaches, urban economics, urban climate and weather studies.	The overview highlights that data-based approaches have the potential to upscale urban climate solutions and bring about change on a global scale.	Creutzig et al., 2019
Air quality, energy	The article develops a framework for reducing dust emissions and energy consumption on construction sites.	The article highlights that the proposed framework can be used on construction sites to conduct real-time monitoring, evaluation and the minimization of dust emissions and energy consumption.	Hong et al., 2019
Air quality, energy	The article explores the application of Big Data in terms of road transport policies in Europe, namely—minimize the environmental impact, handle climate change mitigation and sustainability challenges, as well as maximize system efficiency.	TEMA designed for supporting EU transport policies via Big Data.	De Gennaro et al., 2016
Risk management	The article analyses the challenges and opportunities that the climate crisis presents for organizations and how organizations respond to this scenario, while examining the implications of Big Data management.	The overview highlights that Big Data is a key component to understand the opportunities and challenges of the climate crisis and organization responses.	Seles et al., 2018
Energy, climate resilience	The article provides an initial step in understanding the research activities over the past five decades in these two areas (NZE and resilience) and their connections to their ecological roots.	The overview highlights the major difference between the net zero movement and resilience theory in the urban environment and their respective relations to their ecological origins.	Hu and Pavao-Zuckerman, 2019
Remote sensing, Urban development, ML	The article show that the emergence of Big Data and machine learning methods enables climate solution research to overcome generic recommendations and provide policy solutions at urban, street, building and household scale, adapted to specific contexts, but scalable to global mitigation potentials.	The article suggests a meta-algorithmic architecture and framework for using machine learning to optimize urban planning for accelerating, improving and transforming urban infrastructure provision.	Milojevic-Dupont et al., 2020
Water			
Focus area	Description	Usage	References
Water	The article provides a systematic review of the literature on the ecological models and eutrophication.	The overview aims to improve the level of application with regard to ecological models in the field of water eutrophication and to better serve environmental water science research.	Hu et al., 2019

(Continued)

TABLE 5 | Continued

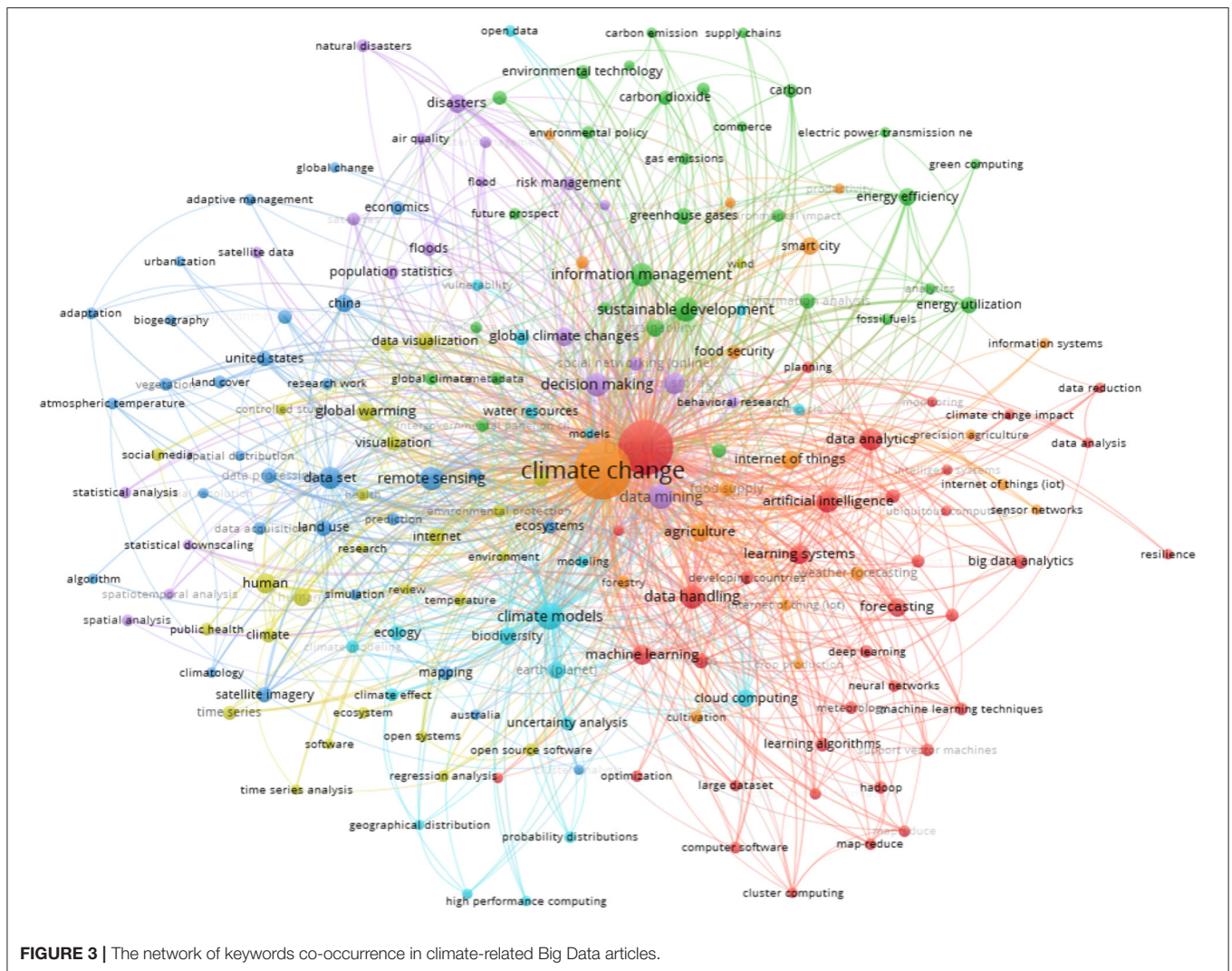
Focus area	Description	Usage	References
Water	The article explores and compares global wetland-related datasets and suggest a synthetic method for wetland mapping.	The overview suggests that this synthetic method of wetland mapping should be applied.	Hu et al., 2017
Water	The article explores the development of watershed management, potential uses of new technologies, current issues as well as the future direction of watershed management and research.	The overview highlights the importance of employing integrated watershed management strategies and outlines methods for improving management strategies.	Wang et al., 2016
Water	The article explores some important impacts on the development of hydrology and water resources in Australia.	The overview highlights that the value and distribution of water resources will change.	Fitzharris, 2016
Water, IoT	The article provides a review of the application with regard to the Internet of Things in the field of marine environment monitoring.	The overview highlights that Big Data analytics can be used not only as a source of feedback for marine environmental management agencies and control centers but also for autonomous vessels and remotely developed devices to take real-time actions.	Xu et al., 2019
Water	The article reviews the evolution of Managed Aquifer Recharge (MAR) concept, and then captures its current research in terms of MAR tech-nologies, process of the MAR implementation, applications of MAR, as well as common problems and challenges that are associated with MAR.	The article recommends that further studies on MAR should focus on systematic clogging mechanism and prevention, the theory of seepage calculation, theory of infiltration for MAR, purification mechanism, and application of Big Data and artificial intelligence in MAR	Zhang et al., 2020
Water	The article uses information visualization technology of CiteSpace to present a systematic review of published literature on the application of eco-models to eutrophication from 1968 to 2018.	The article highlights that eco-models range from dimension-models to time-dependent dynamic models and that the recent trend of close coupling between modeling and the acquisition of new types of experimental data (i.e., remote sensing, high-frequency field sensors) provides a higher prediction ability of ecological models.	Hu et al., 2019
Biodiversity			
Focus area	Description	Usage	References
Biodiversity	The article reviews the current state of lichen conservation in Canada and the United States.	The review highlights the effective usage of Big Data in informing and monitoring species.	Allen et al., 2019

The “purple” cluster represents natural disasters caused by climate change, e.g., floods or deteriorating air quality, and the related risk management. Decision-making processes are supported by data mining techniques and statistical as well as spatial analysis. The frequency of natural disasters in the Philippines increased by 147% from 1980 to 2012 and continues to rise (Garcia and Hernandez, 2017). Big Data through data mining plays a significant role in creating real-time feedback loops on natural disasters to support disaster management in prevention, protection, mitigation processes as well as response and recovery, moreover, in increasing the resilience of citizens (Yang et al., 2017).

“Light blue” clusters climate models that define interactions of the drivers of climate change. Topics like ecology, biodiversity, vulnerability, and the issue of water resources are included. Big Data-based techniques are widely used and the importance of open data must be recognized. Cloud computing and uncertainty analysis tend to support the modeling of life cycles and climatic effects. The open data science approach ensures a transparent and collaborative environment for multi-model climate change

data analytics (Fiore et al., 2018). Information about the geographic distribution of greenhouse gas emissions can be useful in terms of high-resolution modeling (Charkovska et al., 2019).

The “green” cluster defines topics with regard to sustainable development, dealing with gas emissions, greenhouse gases, energy efficiency, and environmental policies. Information analytics and environmental technologies as well as green computing seek to minimize hazardous waste while maximizing energy efficiency and recyclability to foster the concept of a circular economy. Data mining, generic algorithms, and neural networks are gradually applied in sustainable consumption research, that enables more accurate and better visualized results (Wang et al., 2019). Managing efficient energy use is a commonly discussed issue that takes into consideration the climate change impact analysis with regard to the energy use of campus buildings (Fathi and Srinivasan, 2019), life-cycle assessment of energy-consuming products (Ross and Cheah, 2019) as well as the adaptation of green computing to reduce the carbon footprint of ICT (Airehrour et al., 2019).



The “blue” cluster seems to reveal methodologies considered in climatology, urbanization, and adaptive management. Remote sensing and satellite imagery make it possible to collect a large amount of data that supports mapping and is used to make further predictions. Satellite remote sensing quantifies processes and spatio-temporal states of the atmosphere, land, and oceans (Yang et al., 2013), moreover enables, for example, climate change and the impact of human activities on cropland productivity to be detected (Yan et al., 2020) and changes in water resources to be mapped (Senay et al., 2017). The monitoring of carbon by satellite observation provides information about greenhouse gases and emissions that can be utilized in estimation processes regarding the investigation of CO_2 (Zhao et al., 2019).

The “yellow” cluster consists of the global climate change-related data analyses, visualization methods, regression analysis, and time series analysis. Open systems and open sources are gaining ever more attention in this field. A web-based visualization of complex climate data can assure scientists, resource managers, policymakers, and the public to explore

climate-balance projections even at the local level (Alder and Hostetler, 2015). The assessment of spatiotemporal data to gain knowledge from it is a complex challenge, however, a well-developed visual analytical system can support performance improvement methods and techniques (Li et al., 2013). A high performance query analytical framework that proposes grid transformation can provide a complex climate data observation and model simulation (L et al., 2017). For climate environmental analyses, a 3D visualization simulation of cloud data is gaining attention in the fields of computer graphics and meteorology (Xie Y. et al., 2019).

The application of contemporary technologies like Big Data analytics and IoT-based models is sought to gain a knowledge base in any field by collecting and analysing large complex heterogeneous data sets. This enables evidence-based policy making to be encouraged and serves as a decision support tool for risk assessment and resilience adaptation, while forecasting future socio-economic as well as aiding environmental conditions caused by climate-related change. The

Big Data researches are important in itself and contribute to the understanding of climate change, but managing their results in an integrated way increases the level of problem extraction and provides new solutions for decision makers.

4.3. The Role of Social Sciences in Climate Change Studies

Most articles on climate change belong to the field of environmental science, closely followed by Earth and planetary sciences, then agricultural and biological sciences. Interestingly, the number of articles published in the social sciences precedes the fields of engineering and energy.

The growing amount of information and knowledge renders multidisciplinary analyses covering the whole field of science and the development of such analytical tools indispensable as the knowledge accumulated cannot be directly utilized without systematization and targeted processing.

Climate change issues tend to connect different disciplines as well as research ideas, models, and solutions related to these issues. In the following, significant connection between climate and social sciences is discussed. The Scopus database was used to extract relevant information for meta-analysis.

The search for a connection with social sciences yielded 1,203 documents: [TITLE-ABS-KEY("climate change") AND TITLE-ABS-KEY("social sciences")]. The networks concerning the co-occurrence of keywords referring to the interrelationship between climate change and social sciences is shown in **Figure 4**.

Based on the intersections presented in **Figure 4**, seven communities are detected. The red community includes emissions, energy and economic hubs. The yellow community includes habitat-related nodes. The light blue community covers regulators and issues concerning water management, while the purple community summarizes concepts related to "change," e.g., vulnerability, adaptation, etc. The green community includes interdisciplinary subject areas, while the dark blue one represents political keywords and the orange community describes sustainable mergers.

A complex relationship exists between human and natural processes involving social, political, geographic, and cultural contexts that demands a multidisciplinary concept (Fiske et al., 2018). Environmental changes call for socio-economic transformation to mitigate the effects caused by humans and increase resilience. Changes are observed in a diverse range of areas such as agriculture and food security, air quality, waters, energy consumption, land ecosystem as well as global warming. These issues must be managed through strategic planning and management with a high degree of focus on long-term sustainable operation. Socio-ecological-economic models must integrate social and biophysical information in order to develop sufficient mitigation and adaptation strategies (Sullivan and Huntingford, 2009). The impact of climate change on water resources is critical as it is related to floods, droughts, tidal waves, and humidity. Big Data-based processes are used to determine, for example, soil conditions and humidity (Anton et al., 2019) to estimate energy consumption (Seyedzadeh et al., 2018) or greenhouse gas emissions (Hamrani et al., 2020) that

enable optimal processes and interventions to be predicted. Decision support algorithms, models, and databases are used to provide evidence-base for policymaking and legislation (Aragona and De Rosa, 2019) as well as disaster management (Akter and Wamba, 2019). These can be considered at organizational (Kouloukoui et al., 2019), local (Giest, 2017), sub-national (Hsu et al., 2019), national (Iacobuta et al., 2018), or even global levels (Flato et al., 2014).

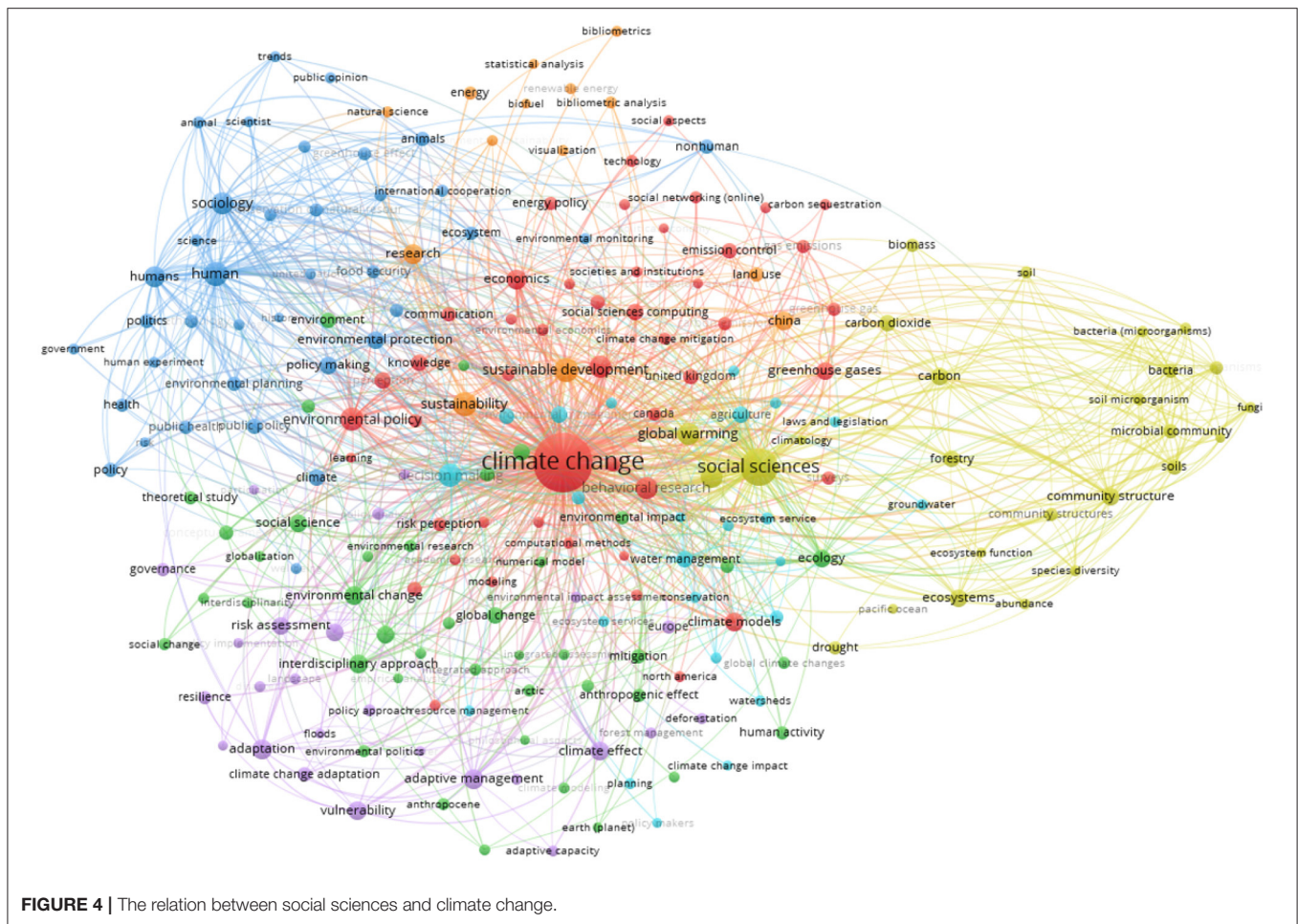
Socio-environmental sciences are sought to explore the systematic cause-effect relationship following the environmental impact of human induced climate change. By providing heterogeneous data and supportive models, positive changes can be achieved through interdisciplinary data-driven perceptions that contribute to a better understanding of the complex issue, monitor changes, support decision-making, and bring about in-time interventions.

4.4. The Importance of the System of Systems Approach

Climate change is one of the most significant global challenges that need to be managed. To resolve any of the climate change-related challenges, "it is essential to elicit and integrate knowledge across a range of systems, informing the design of solutions that take into account the complex and uncertain nature of the individual systems and their interrelationships" (Little et al., 2019). The system of system (SoS) framework enables to analyse the interdependencies between various systems (e.g., human, information, environmental, and physical systems), therefore provides a clear understanding of the complex nature of the issue (Fan and Mostafavi, 2019). The trends in data science and information technology (Tannahill and Jamshidi, 2014) supports the integration of various disciplines and research outcomes to represent a socio-environmental system holistically inform policy and decision-making processes (Iwanaga et al., 2020), which can be referred as climate computing.

To highlight the importance of the application of the system of systems approach, the latest Big Data-based works in the field of climate change were reviewed, based on which we identified a SoS framework (**Figure 5**). In the network of applications, the nodes show the different researches, and the edges represent the relationships of the research results. The BigData applications have been grouped according to sustainable development goals, thus showing the possible scientific contributions with the other fields.

By processing satellite data, the system developed in Semlali and El Amrani (2021) can monitor changes in air quality, which can also be used to monitor agricultural areas (Majidi et al., 2021). Cloud tracking (He et al., 2020) further helps to assess the evolution of air pollution, the reliability of which can be further enhanced with statistical downscaling solutions (Wang Q. et al., 2020). The time-series data (Joshi et al., 2019) extracted from satellite images support long-term forecasts, but the description of cloud motion (Xie Y. et al., 2019) can also be used to refine shorter-term analyzes. The use of satellite imagery as a data source in urban planning also helps identify climate-friendly solutions (Milojevic-Dupont et al., 2020).



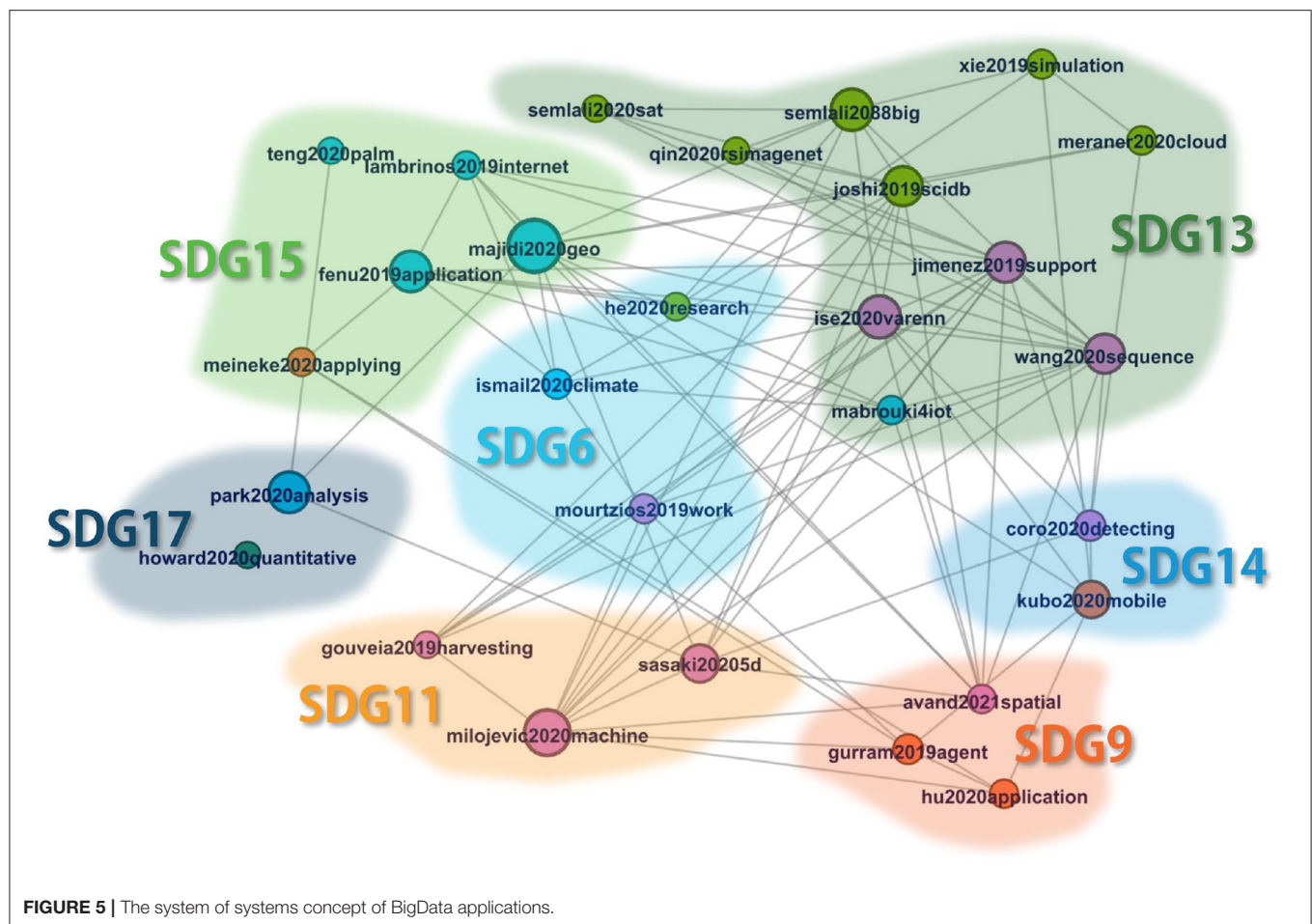
Web-based water management (Mourtzios et al., 2021) can be supported with trends identified from time-series data (Ise et al., 2020), but remotely sensed water flow data also complements the agricultural water management model (Ismail et al., 2020). And if we increase the resolution of the data (Jimenez et al., 2019), we can also understand the causal relationships related to consumption. In terms of infrastructure load, patterns of population movement (Gurram et al., 2019) offer exciting opportunities, but can also be integrated with the condition of buildings (Gouveia and Palma, 2019), which also supports the satisfaction of urban planning tasks (Milojevic-Dupont et al., 2020) at a higher level.

Agricultural satellite imagery applications (Majidi et al., 2021) can be transferred to air quality satellite monitoring (Semlali and El Amrani, 2021), or time-series data (Ise et al., 2020) can be used to plan better agricultural interventions. By implication, satellite-based support plays an important role in modeling agricultural water management (Ismail et al., 2020), but disaster news (Park et al., 2020) also helps provide a deeper understanding of social involvement. In assessing disaster resilience in different areas, (Sasaki et al., 2020) satellite imagery provides feedback on risks that can even be revealed over time (Joshi et al., 2019). Satellite-based results can be supported by on-site special (Lambrinos,

2019) and meteorological (Mabrouki et al., 2021) sensor data, and flood protection of valuable agricultural areas can also be planned with flood models (Avand et al., 2021).

Identifying patterns in time-series data (Ise et al., 2020) helps with research in many other areas, whether it is agricultural water management (Ismail et al., 2020) or marine habitat protection (Coro et al., 2020). It allows (Kubo et al., 2020) forecasting and a better understanding of coastal traffic and increases the reliability of disaster resilience estimation (Sasaki et al., 2020). By extracting time series data (Joshi et al., 2019) from satellite imagery, we can indirectly validate the models by comparing the time series or identify the factors of potato disease (Fenu and Mallocci, 2019). In urban developments (Milojevic-Dupont et al., 2020) and in building condition surveys (Gouveia and Palma, 2019) the forecast shows the development of infrastructure expansion and maintenance, to which the probability of flood protection problems (Avand et al., 2021) can also be linked.

Statistical downscaling (Wang Q. et al., 2020) helps to find the external variables of Mourtziou et al. (2021) consumption patterns identified based on remote sensing and is comparable with the results of satellite image-based analyzes (Semplali and El Amrani, 2021). And comparable to other approaches (Jimenez et al., 2019), which strengthens confidence in the models (Qin



and Chi, 2020). Better resolution data supports marine habitat protection planning (Coro et al., 2020), risk assessment input (Fenu and Malloci, 2019), but can also be used (Gouveia and Palma, 2019) to analyze building consumption data. The efficiency of downscaling techniques can be increased with the Internet of Things (Lambrinos, 2019) toolbar. The increase of the number of observations allows a more accurate description of local climatic conditions to estimate floods (Avand et al., 2021) and heat island effects, as well as other sustainable urban planning (Milojevic-Dupont et al., 2020) aspects.

Coastal tourism monitoring (Kubo et al., 2020) can be integrated with traffic data (Hu et al., 2020) to optimize traffic management and thereby reduce pollutant emissions. The effect of transport on plant damage can be included (Meineke et al., 2020) as a factor to be analyzed, or we can use it (Gurram et al., 2019) to identify patterns in population movement.

Population movements (Gurram et al., 2019) affect water consumption (Mourtzios et al., 2021), can damage plants (Meineke et al., 2020), show the popularity of coastal areas (Kubo et al., 2020), but are also suitable for improving transport planning (Hu et al., 2020). Because the movement of residents is closely related to the infrastructure (Milojevic-Dupont et al., 2020), it is a very valuable input in urban planning.

The data of the Internet of Things sensors (Mabrouki et al., 2021) allow the conclusions drawn from the satellite images to be verified (Majidi et al., 2021), as a measuring station (Jimenez et al., 2019) increases the number of observations, thus better downscaling solutions (Wang Q. et al., 2020) can be made. It can be used for causal exploration of plant morphological damage (Fenu and Malloci, 2019) and supports agricultural irrigation water demand planning (Ismail et al., 2020), but can also be imported into flood models (Avand et al., 2021).

In the Big Data application, that supports the energy demand management of buildings (Gouveia and Palma, 2019), we can use water consumption data (Mourtzios et al., 2021) as an extension, development alternatives can be ranked based on time series data (Ise et al., 2020), or based on time series extracted from satellite images (Joshi et al., 2019), which can be supported by a deeper understanding of energy demand downscaled data (Wang Q. et al., 2020), because the resolution of the input data can be improved (Jimenez et al., 2019).

Based on the presented system of systems framework, it can be seen how the new results of Big Data applications related to climate change contribute to other areas. Remote sensing of water consumption (Mourtzios et al., 2021), analysis

of cloud water content (He et al., 2020), and the agricultural water management model (Ismail et al., 2020) contribute to the goal of clean water and sanitation (SDG6). Planning based on the analysis of traffic data (Hu et al., 2020), studying population movements (Gurram et al., 2019) and flooding models (Avand et al., 2021) support the goal of industry, innovation and infrastructure (SDG9). Climate-friendly urban planning (Milojevic-Dupont et al., 2020), monitoring the energy demand of buildings (Gouveia and Palma, 2019), and defining disaster resilience (Sasaki et al., 2020) play an important role in achieving sustainable cities and communities (SDG11). The Climate Action goal (SDG13) tackles most data gaps, so research such as linking satellite images to Semlali and El Amrani (2021) with air quality, preprocessing them (Meraner et al., 2020; Qin and Chi, 2020; Semlali et al., 2020), the analysis of time series data (Ise et al., 2020) and its exploration (Joshi et al., 2019), downscaling (Wang Q. et al., 2020) techniques, enrichment of precipitation and temperature data (Jimenez et al., 2019), tracking the movement of clouds (Xie Y. et al., 2019), or just using IoT sensors (Mabrouki et al., 2021) are all key in creating a strategy to support the achievement of the climate goal. For the sustainability of life below water (SDG14), marine life prediction models (Coro et al., 2020) and human coastal activity (Kubo et al., 2020) can be integrated. Of course, the goal of life on land (SDG15) also requires new research, where a satellite-based study of agriculture and forestry (Majidi et al., 2021), deployment of IoT sensors (Lambrinos, 2019), analysis of climatic factors of potato damage (Fenu and Mallocci, 2019), studying the morphology of plants (Meineke et al., 2020), or social media based illustration of palm oil consumption (Teng et al., 2020) are promising. Partnerships for the goals (SDG17) is critical in several ways, on the one hand we recommend the grouping of climate services (Howard et al., 2020), which fits the SoS concept we propose, and on the other hand we need to integrate the knowledge and give feedback to society. An exciting tool for measuring the effectiveness of climate and sustainability related measures is the analysis of news comments (Park et al., 2020).

It is essential to highlight that Big Data research on climate change can be used in other areas and as shown by the SDG grouping in **Figure 5**. Thus, based on the recommended SoS viewpoint, the specific results of sustainability-related research and development projects can be integrated, enhancing knowledge accumulation and utilization.

5. DISCUSSION

This paper described the essential need for research and development objectives to realize and manage the complex issues of climate change through Big Data tools. Data-driven applications were reviewed through the co-occurrence analysis of keywords, which showed the widespread application of Big

Data technologies and tools, however, comprehensively utilized and integrative analyses are less prevalent.

This research aimed to highlight the perspective of systems of systems (SoS) as the drivers and effects of climate as well as that their resilience and adaptation cannot be determined without the exploration of the synergies between new research trends and disciplines. Based on the recommended SoS viewpoint, the specific results of sustainability-related research and development projects can be integrated, enhancing knowledge accumulation and utilization. The tools of data and systems sciences can play a crucial role in recognition of climate challenges and mitigation opportunities thanks to the integration of heterogeneous data and models, and the exploration of the relationship between environmental and social factors. This integrated thinking lays the groundwork for promising future trends in climate computing.

It can be claimed that the exclusive analysis of climatic factors cannot bring about sufficient strategic adaptation by itself, rather the socio-environmental factors must be integrated the climate change models.

Mitigating the impacts of climate change and successful adaptation requires effective climate change strategic planning by countries worldwide whose decision-making requires complex models and sources of information. The Big Data toolkit enables the systematization, processing, and evaluation of heterogeneous data and information sources, which is unfeasible with traditional disciplinary analysis tools. The harmonization of the ever-expanding scientific knowledge and diversified data sources related to climate change may be one of the most urgent tasks for researchers in the future. This research presented Big Data analytics tools and their contribution toward exploring the characteristics of climate change as well as climate action-related counterparts such as sustainability and social sciences that are essential for the successful development and implementation of strategies.

AUTHOR CONTRIBUTIONS

VS: conceptualization, validation, investigation, writing-original draft, visualization. JA: conceptualization, validation, resources, writing-review and editing, supervision, and funding acquisition. TC: writing-original draft, investigation, visualization, and validation. All authors contributed to the article and approved the submitted version.

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From Acceptance Snapshots to the Social Acceptability Process: Structuring Knowledge on Attitudes Towards Water Reuse

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Water reuse is considered a technologically viable option to meet the increasing demands of the domestic, industrial and agricultural sectors. Alongside challenges such as environmental health, infrastructure and regulations, water reuse is often hindered by lack of acceptance and dismissive attitudes. This paper seeks to structure knowledge about acceptance of water use. It provides a systematic look at the overall reuse challenges and social attitudes towards water reuse considering the three integrative elements of water reuse, namely the water source, the technology, and the end use. It first maps the challenges and common insights that constitute the enigma of water reuse acceptance. Later, it conceptualizes acceptance as a social process consisting of the interdependent components of public perception, politicization, individual acceptance, and use adaptation. Using this conceptual framework, solutions to increasing water acceptance stemming from different bodies of acceptance studies are reviewed. The paper reiterates the need for a nuanced view on water reuse acceptance that incorporates spatio-temporal considerations as well as knowledge from different disciplines.

Keywords: water reuse, water reuse acceptance, potable water reuse, water supply, water recycling, water reuse strategy

INTRODUCTION

Water reuse is on the rise worldwide as a viable option for countering scarcities of local water resources. The benefits of water reuse are manifold; e.g., the provision of additional water supply, environmental improvements through decreased discharge of polluted water, opportunities to expand agriculture or recharge groundwater, and additional benefits through the valorization of wastewater for the production of fertilizers or energy (Barnes 2014; Angelakis and Snyder 2015; Duong and Saphores 2015; McClaran et al., 2020). These benefits often exceed the capital costs and negative impacts in many areas, such as Beijing, where the total benefits of wastewater reuse are 1.7 times the costs (Fan et al., 2013). Alongside economic costs, the environmental and social costs (e.g., related to environmental health and public safety) are largely manageable, while the benefits often outweigh these costs (Garcia and Pargament 2015). Water reuse can be a viable option for achieving improved water-use efficiency, reducing water scarcity and mitigating environmental shocks such as droughts (Garcia and Pargament 2015; Brown et al., 2018; Lee and Jepson 2020). As such, it can be seen as an instrument for achieving key sustainability targets such as the Sustainable Development Goals (SDGs) Target 6.3 on sustainable water quality, wastewater treatment and safe reuse, and Target 6.4 on water-use efficiency and water scarcity.

Urban water reuse networks have been growing for the last 50 years and are increasingly deployed in areas experiencing water scarcity (Wilcox et al., 2016). Wastewater treatment, for example, is evolving rapidly, particularly in countries affected by water scarcity and arid conditions as well as the accumulating impacts of climate change (Mu'azu et al., 2020; Scruggs and Thomson 2017; Bichai et al., 2018; Lee and Jepson 2020). As a result, it has been increasing by an annual average of 25% in countries such as the United States, China, Japan, Spain, Israel and Australia (Angelakis and Snyder 2015). Different types of water (e.g., greywater, seawater, wastewater, brackish water, and produced water) can be reused by using different technologies for various subsequent uses such as landscaping, edible and non-edible agriculture, groundwater recharge, industrial purposes and domestic use. Multi-purpose and multi-source water reuse systems exist nowadays in some reuse-pioneering countries such as Singapore, United States, Australia, Japan, some EU countries, Namibia, and increasingly, in countries of the Middle East.

The growth and diversity of reuse supply infrastructure are determined by the ability to overcome the multi-dimensional challenges of large-scale water reuse (van Rensburg 2016; Scruggs and Thomson 2017; Bichai et al., 2018; Lee and Jepson 2020). Despite these challenges, the academic literature reports a positive reception of the idea of water reuse among policymakers, business leaders and public authorities as a solution to a range of external and internal water-use problems (Chen et al., 2015a; Smith et al., 2015). While there is largely positive reception to water reuse as a water supply option, empirical evidence often indicates less favorable attitudes towards the direct use of water for food, drinking, showering or swimming (Fielding et al., 2018). Furthermore, attitudes towards water reuse have been unstable, and this presents a fundamental problem for large-scale water reuse applications. In fact, studies on reactions and attitudes towards water reuse are scattered across individual cases, selected reuse options, and specific technologies. Water reuse acceptance is studied through assessing momentary attitudes (being for or against water reuse at a certain moment), which is a “thin narrative” of the complex acceptance problem (Ching 2016). Furthermore, simple remedies for water reuse (e.g., awareness and campaigns), binary frames (e.g., a yes/no acceptance problem), and narrow disciplinary perspectives have often failed to capture the complex and multidimensional problem of acceptance or to promote water reuse (Russell and Lux 2009; Beveridge et al., 2017). Generalizations from water reuse studies are also difficult due to the wide variety of methods deployed and the lack of contextualization of results.

There is a need to provide insights into improving water reuse acceptance based on examples from a wide range of academic disciplines and a broad understanding of acceptance. This study aims to explore the advancement of the understanding of water reuse attitudes by structuring state-of-the-art knowledge on water reuse acceptance. The paper also proposes a novel, holistic framework for analyzing attitudes towards water reuse as a complex process of social acceptability. This study maps the recent literature referring to

(social) acceptance and (public or individual) attitudes depending on water reuse source/technology/end use. First, it introduces water reuse acceptance as one of many interrelated water reuse challenges. Secondly, it structures knowledge of common elements as well as the heterogeneity of perceptions of or attitudes to water reuse across different case studies. While doing this, it also conceptualizes the relationships between the three constituent elements of water reuse (water source, technology, and reuse purpose) and acceptance of the reuse. Thirdly, the paper proposes a framework for understanding acceptance as a long-term social acceptability process consisting of interactional debates on the sub-components of acceptability, namely public perception, politicization, individual acceptance, and use adaptation. Using the conceptual framework, it summarizes perspectives coming from different bodies of research on water reuse acceptance and relates them to the social acceptability process. Finally, remedies to increase the social acceptability of water reuse are presented, and overarching insights are summarized.

IN COMPANY WITH ACCEPTANCE: COMPLEMENTARY CHALLENGES OF LARGE-SCALE WATER REUSE

Water reuse acceptance does not represent the only challenge facing the expansion of water reuse. It is one among several major challenges, which are often interrelated. These challenges bridge technology, science/knowledge, finance, and policymaking, and understanding them is an important initial step in contextualizing the water reuse acceptance issue. If some of these challenges are not solved or are perceived to constitute a problem in a certain case, water reuse acceptance can diminish greatly. As will be explained later in this paper, these challenges present key contextual factors that can affect the outcomes of the public or/and private debates in the social process that engulfs the acceptability of water reuse. This makes it difficult to frame the acceptance or acceptability problem without first examining the prevalence of the challenges on a local level. Based on a review of key literature, this paper identifies six key challenges that can hinder the development of large-scale water reuse or negatively influence the public perception of its safety and usefulness (Table 1). While these are common critical challenges of water reuse, their importance can change from one case study to another.

Long-term impacts of water reuse on human health and the environment particularly soil are arguably at the forefront of research and public debates. However, evidence from state-of-the-art applications shows that, with appropriate monitoring and multi-barrier technology, public health risks are quite low (Ong 2016; Binz et al., 2018). Successful case studies such as Singapore, or Windhoek in Namibia, confirm the importance of comprehensive regulatory frameworks and clear technological guidelines (Cotruvo 2016; Lee and Tan 2016). Often, the reuse of water for potable purposes is achieved indirectly through, for example, discharge into sources such as rivers and streams in

TABLE 1 | Challenges of large-scale water reuse.

Challenge	Description of identified barriers	Literature
Long-term impacts: health	Public health threats due to pollutants	Jasim et al. (2016), Ouda (2016), Miller (2015), Duong and Saphores (2015)
	Availability of appropriate treatment technologies and multiple treatment barriers to IDR and DPR	Cotruvo (2016)
	Safety, reliability and the minimization of health impacts of production failures through standards, monitoring and international guidelines	Ong (2016), Binz et al. (2016), Miller (2015)
	Microbial risk to the environment and public health by the presence of antibiotics in reclaimed water	Hong et al. (2013)
Long-term impacts: soil	Increase in soil salinity because of reused wastewater and agricultural drainage water	Al-Hamaiedeh and Bino (2010), Carr et al. (2011), Barnes (2014)
	Improvement in soil nutrient and microorganism levels with longer reclaimed water irrigation	Chen et al. (2015b)
	Effects of wastewater reuse on soil productivity, soil fertility, human health and environmental health	Becerra-Castro et al. (2015)
	Treatment technology in relation to soil functions and planned crops	Abegunrin et al. (2016)
Regulations and standards	Cross-sectoral regulations and guidelines	Fawell et al. (2016), Bahri (2009), European Commission (2015), Scruggs and Thomson (2017)
	Safety culture and regulations incorporating low and high probability risks as well as low and high consequences of safety failures	Binz et al. (2018), Meehan et al. (2013)
	Clear water reuse criteria for environmental and human health	Paranychianakis et al. (2015)
	Specific legal frameworks at national and local levels	Sanchez-Flores et al. (2016), Kayhanian and Tchobanoglous (2016)
Market-related issues	The need for systematic assessments including hazard identification, control measures, monitoring, incident protocols and regulation surveillance	World Health Organisation (2017)
	Adequate rates for reused water	Jensen and Yu (2016), Aleisa and Al-Zubari (2017)
	Lack of economic feasibility or Willingness to Pay (WTP); Need for subsidies to achieve cost recovery; Demand management of reused water	Molinos-Senante et al. (2013), Sgroi et al. (2018), Ravishankar et al. (2018), Duong and Saphores (2015)
	Lack of adequate competition policies	Horne (2016)
Infrastructure and distribution	The need for incentives for water reuse projects and public leadership	Garcia and Pargament (2015), Miller (2006)
	Inadequate infrastructure and distribution networks	van Rensburg (2016), Aleisa and Al-Zubari (2017), Brown et al. (2018)
Participatory and comprehensive reuse strategies	Path dependence on centralized, large-scale infrastructure	Bichai et al. (2018)
	Lack of stakeholder engagement, public participation and communication	Lautze et al. (2014), Sanchez-Flores et al. (2016), Aldaco-Manner et al. (2019)
	Lack of embeddedness within larger water security plans and water supply strategies; The need for expanding strategies to incorporate wider circular economy concepts	van Rensburg (2016), Sgroi et al. (2018)
	The need for systems thinking, linkages with other sectors, and a holistic water security approach; Overcoming regulatory fragmentation	Voulvoulis (2015), Bichai et al. (2018)
	The need for political will, legal frameworks, public policies and public utilities as role models	Tortajada and Nam Ong (2016), Aleisa and Al-Zubari (2017), Lee and Jepson (2020)
	Adequate governance; Coordination across different administrative levels	Keremane (2017), Aldaco-Manner et al. (2019), Meehan et al. (2013)

order to augment these sources (sometimes called blended water). Water users can consume reused water indirectly by using these rivers, streams or canals. This Indirect Potable Reuse (IPR) is less associated with social acceptance problems than the Direct Potable Reuse (DPR) (reusing treated water without any blending of it with other water sources). IPR is thus more feasible despite the availability of adequate technologies, policies and regulations to make DPR widely available; e.g.,

(Horne 2016) for the case of Australia, or (López-Ruiz et al., 2020) for Southern Spain.

Health concerns are found in cases where water reuse infrastructure is still emerging and DPR is not practiced; e.g., Australia (Horne 2016) or the Gulf Cooperation Council (GCC) region (Aleisa and Al-Zubari 2017). For example, studies from Saudi Arabia and Qatar show that the presence of pathogens, chemicals, heavy metals or antibiotics is the primary limiting

factor for the reuse of treated wastewater for recharging of aquifers and irrigation (Hong et al., 2013; Jasim et al., 2016; Ouda 2016). There is, nonetheless, agreement on the beneficial potential of water reuse for the GCC region and the technical feasibility of expanding this important option (Aleisa and Al-Zubari 2017; Brown et al., 2018).

The long-term impacts of reused water on different types of soil are relevant considerations for many countries. For example, in Qatar, a salinity of about 1,000 mg/L at treatment plants means that water use for agriculture is problematic (Ministry of Development Planning and Statistics 2016), while excessive nutrients in treated wastewater represent a threat to soil health (Jasim et al., 2016). In reality, more research is required into the long-term impacts of different types of reused water and associated technologies on local soil and crops. Such impacts are largely specific to the case study and the related technology. Studies show that water reuse can have contradictory effects such as an increase in both soil salinity and soil fertility (Al-Hamaiedeh and Bino 2010; Barnes 2014; Chen et al. 2015b). In order to manage such effects, the treatment technology needs to match soil characteristics and the envisioned cropping pattern (Carr et al., 2011; Abegunrin et al., 2016). At the same time, long-term effects of reused water on the water-holding capacity of different local soil types need to be monitored and analyzed (Mohtar 2015).

The complex interactions in water reuse between the required inputs and the impacts or benefits for humans, nature and the economy require comprehensive, multi-level regulations. This need for regulation is more evident in regions with large reuse systems such as in the European Union (EU) or the United States. For example, in the EU, some member states are lagging behind in terms of potable water reuse regulations, while the EU has been reluctant to develop a unified regulatory framework (Fawell et al., 2016). Indeed, the lack of uniform water reuse criteria has been the most important obstacle to the exploitation of the high potential for water reuse in the EU (Paranychanakis et al., 2015). Regulation is the issue most demanded by the sector's practitioners in the EU (European Commission 2015). While the United States might have more reuse regulations than some EU countries, some national and local regulations require revisions and adaptation to more direct uses as well as emerging issues such as climate variability (Sanchez-Flores et al., 2016). Particularly in the case of DPR, it is important to develop a safety culture in the reuse industry as well as comprehensive regulations for different risks and probabilities (Binz et al., 2018).

Other important and interrelated challenges are those related to markets, infrastructure and strategies/participation (summarised in Table 1). Monetary considerations such as the correct pricing policies, the provision of subsidies and incentives for water reuse projects, and the encouragement of competition represent critical components of any reuse strategies. However, a financial approach to water reuse might fail if not embedded within a broader policy context taking into consideration the overall use of resources. Water reuse strategies should be based on holistic planning to incorporate key issues such as stakeholder participation, public awareness, and integration among policies. The public sector has a key leadership role in such policies since it can act as a regulator, initiator and major financier of water reuse

projects. Water reuse needs also to be seen as a major supply option, especially in dry regions, within a broader water security plan. The emergence of water reuse as a viable and renewable supply option is largely dependent on a greater political appreciation of water issues, especially of water quality (Biswas 2016), and the abilities of public sector agents such as the utilities to lead by example (Tortajada and Nam Ong 2016). For example, a strong political will and strict and comprehensive reuse regulations, together with persistent public engagement, can help make DPR a reality, as in famous cases such as Singapore (Lee and Tan 2016) or Windhoek, Namibia (van Rensburg 2016; Lahnsteiner et al., 2018).

A SYNTHESIS OF THE REUSE ACCEPTANCE ENIGMA

With water reuse attracting increased attention as a viable solution to supply shortages, the acceptance challenge has been investigated in many surveys and in-depth case studies. In reviewing this literature, an important question can be formulated: What are the key lessons learnt and the implications from empirical research on water reuse across different case study locations and contexts? In response, in this section, this paper synthesizes some common observations regarding both similarities and discrepancies. This synthesis shows that while there are general acceptance problems across the globe, the solutions need to be based on scientific evidence collected locally and tailored to local circumstances. Comparative studies of water reuse acceptance have been conducted across different locations in the United States (Hartley 2006; Garcia-Cuerva et al., 2016; Scruggs et al., 2020), Turkey (Buyukkamaci and Alkan 2013) and across different countries (Crampton et al., 2016; Hurlimann and Dolnicar 2016). Single-case studies on local acceptance surveys are also available for comparison of similarities and differences. The key insights into similarities and discrepancies in response to water reuse are summarized in the following section.

Similarity Observations

Similarity observations relate to highly aggregated observations from empirical studies on similar public and individual attitudes to water reuse. They have been stable over the majority of surveys, and they represent important starting points for understanding the enigma of water reuse acceptance.

High Support for Water Reuse

In the majority of cases, there is a positive perception of, and a level of high support for, the overall idea of water use as an alternative water source. This might be due to growing scarcities and stresses on water resources. For example, regions experiencing recent droughts are more likely to support reclaimed water (Garcia-Cuerva et al., 2016). Key institutional water stakeholders (e.g., managers, researchers or bigger users, etc.) regard water reuse highly (Chen et al. 2015a). There is an increasing receptivity towards recycling water, and this is especially true for non-potable purposes and in developed

regions of the world, e.g., in Europe (Smith et al., 2015). In particular, wastewater reuse is supported by many stakeholders as an important option for water supply augmentation (Duong and Saphores 2015). However, being for or against water reuse might be a misleading “thin narrative”, which needs to be replaced by a more nuanced understanding of individual stances on recycled water (Ching 2016). Furthermore, public responses can be unstable and susceptible to change in the wake of contamination accidents, public debates, rumors, media reports, etc. (Russell and Hampton 2006).

Decrease in Support for Reuse With Close-to-Person Uses

Acceptance of water reuse decreases in cases of close-to-person uses such as bathing, cooking or drinking. This similarity pattern emerges across acceptance case studies (Fielding et al., 2018). Despite the complexity of individuals’ attitudes to contacts with reused water, the less favorable view of close-to-person contacts is stable across locations and users (Chen et al. 2015a). For example, farmers surveyed in Jordan reported a negative opinion on touching reused water or using it for fruit and vegetable cultivation despite the perceived benefits for soil productivity (Carr et al., 2011). Similarly, in Iran, the acceptance of non-personal use of treated wastewater was much higher than for close-to-person uses; e.g., 87% acceptance for public consumption such as landscaping or firefighting vs. 8% for cooking or drinking (Baghapour et al., 2017). Similar attitudes are found in Latin America, e.g., Chile (Segura et al., 2018). This is due to many reason such as the famous “yuck” effect of coming in contact with previously “dirty” water (Duong and Saphores 2015; Wester et al., 2016). This attitude of negativity or disgust can be psychologically, culturally and/or socially constructed; e.g., influenced by the media (Ching 2010). It can also be linked to a complex set of individual and contextual factors, or what Bennett et al. (2010) called “fright factors”, which include issues such as risk exposure and distribution, the level of damage and the level of trust in others and in public and civil organizations. However, ever since the beginnings of research on public attitudes during the 1960s and 1970s, the decrease in acceptance with close-to-person use has been a stable observation that is less affected by psychological or water price factors (Baumann 1983).

Low Awareness of Water Quality and Associated Risks

Awareness and knowledge on water quality and associated risks of water reuse seem to be low across regions. This finding is combined with a high demand from consumers for more information. This is because knowledge of water recycling processes has been confirmed to be quite low across cases (Fielding et al., 2018). Hurlimann and Dolnicar (2016) found knowledge about recycled and desalinated water to be low across all nine surveyed locations and suggested a higher communication and engagement level with communities. Similarly, Buyukkamaci and Alkan (2013) reported a high demand by 375 respondents in Turkey to be told more about the health and food safety impacts of water reuse. In Jordan, farmers’ perceptions did not correspond to the water quality

delivered to their farms (Carr et al., 2011). In reality, awareness of health risks is generally low across many surveys of farmers in other locations (Kerai et al., 2010). While low awareness is a salient factor in many surveys, it does not apply to some location such as in Singapore where engagement with the community, the media and the public seems to be relatively high (Lee and Tan 2016). There, the attitudes towards water reuse are more influenced by economic interests, ecological limitations and national security rather than by norms or information (Ching 2016).

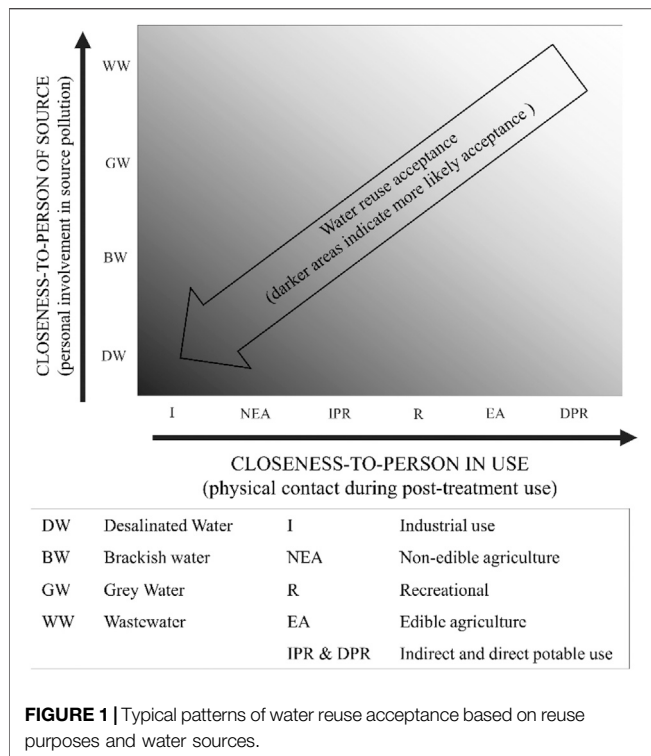
Divergence Experiences

Acceptance of water reuse projects is primarily a context-specific issue. Therefore, divergent experiences are expected, while improving acceptance should be addressed with the participation of local stakeholders. In the following section, insights from comparative research are presented with regard to the key reasons behind the locale-specificity and divergence of water reuse acceptance.

Variance With Sources and use Purposes

Water reuse acceptance varies with different water sources and reuse purposes. While acceptance decreases with close-to-person uses, the exact preference order is dependent on the location and the characteristics of the water presented to consumers. Hurlimann and Dolnicar (2016) found that rainwater is more often preferred to desalinated water for the least personal uses (garden watering and toilet flushing), but the differences in the preferred source depend on the purpose use and vary in certain locations. In fact, water originating from desalination (i.e., reused seawater) is widely accepted in many parts of the world for all types of purposes including DPR. Desalination is the main source of domestic water in GCC countries, while it is also used to a limited extent in agriculture (Aleisa and Al-Zubari 2017). In Australia, the factors behind the likelihood of using desalinated water can be similar to those behind the likelihood of using recycled water (wastewater) (Dolnicar et al., 2011). However, this conclusion is site-specific, since accepting one resource type does not automatically indicate a higher willingness to accept other types. In the GCC region, for example, wastewater reuse in agriculture is largely not accepted or practiced despite the overwhelming endorsement of desalination (Brown et al., 2018).

The consumer perception of the quality and health risks of reused water for a specific purpose is affected by other factors beyond the water source (location of collection), use purpose, or treatment technology. For example, Menegaki et al. (2009) found that labeling treated wastewater as “recycled water” would increase its use acceptance. Inaccurate labels such as “toilet to tap” (T-to-T) are used by opponents of water reuse to scare potential consumers. At the same time, events including public figures drinking beer brewed with reused water can help overcome the “yuck factor” (Katz and Tennyson 2018). **Figure 1** explains how acceptance of water reuse usually changes with the source and the use purpose of such water. Here, brackish water is understood as being water from lagoons or collected from rain, while greywater is the collected and treated wastewater gathered at household level. The collection source



differs, but all water sources need a high level of treatment (e.g., tertiary treatment) to be used for most purposes, especially the close-to-person types.

A Plethora of Awareness Determinants

Factors that can increase awareness, acceptance, and trust of water reuse schemes are site-specific; e.g., education, religion, role models, media coverage, etc. The contribution of these specific factors to increasing awareness and ultimately acceptance of water reuse has been reviewed by Fielding et al. (2018), Hurlimann and Dolnicar (2016). Empirical studies show the importance of local conditions, while results are mixed for all determinant categories. One reason for the mixed results might be the heterogeneity of methods and the vagueness of the measured conceptual objects; i.e., whether studies seek to determine the incidents/perceptions/risks related to acceptance, or simply the attitudes of individuals, communities, stakeholders, or the public.

Among the many determinants of awareness and acceptance and despite the inconsistent results across all studies, education, economic and climatic factors seem to stand out in large acceptance surveys, while some other factors can be less substantive factors. These three factor groups (economic wellbeing, education and climate) were the most influential in a survey with 2,800 people in the United States by Garcia-Cuerva et al. (2016), while sex, age, location and the last monthly water bill showed no significant effect. Robinson et al. (2005) show that both men and women in the United States view wastewater reuse for personal uses unfavorably, while high income and education can lead to having greater knowledge on water reuse. Menegaki

et al. (2007) showed in the case of Creta, Greece, that factors influencing willingness to reuse water are related to scarcity condition, information and awareness, income, and water price, but that age and sex still played a role. Gender did not, however, play a significant role in surveys in Turkey (Buyukkamaci and Alkan 2013). Aitken et al. (2014) found that demographic factors such as the membership of the Muslim community can lead to less support for water reuse in South-East England, while religion was seen not seen as a limiting factor in the study in Jordan (Carr et al., 2011).

Another often-cited determinant of awareness is the media. Buyukkamaci and Alkan (2013) identified the media as the most important method for informing the community. However, Goodwin et al. (2017) found that media coverage of a proposal to introduce IPR in London (United Kingdom) had no impact on online public reaction. In the case of Australia and New Zealand, Crampton et al. (2016) founded the media to be the least likely information source on drinking water quality for the respondents and stressed the role of expert knowledge, technical plans, and the involvement of communities. Similarly, Dolnicar et al. (2011) did not find any influence of TV watching on attitudes towards water reuse and stressed broader issues such as perception and prior knowledge.

Site-specific Risk Factors and Perception

A key discussion in water acceptance experiences is related to risk perception of recycled water. Risk perception and risk factors differ from location to location. This is also true for trust in public authorities to mitigate and adapt to these risks. Such authorities are largely behind large-scale, technical and centralized water reuse projects (Meehan et al., 2013) and their (lack of) competence is a key factor in assuring the public of safe water reuse. Doria (2010) examined risk and acceptance variables of water reuse and emphasized individual factors related to risk perception as well as contextual factors such as the supply system in general, trust in the suppliers, quality of information provided by media, and public trust. Crampton et al. (2016) found risk perception to be significantly correlated with employment status and sex, with location being insignificant in the case of New Zealand and Australia. Dolnicar et al. (2011) reviewed acceptance surveys and identified trust in the authorities as a key category for determining acceptance. Similarly, Fielding et al. (2018) identified risk perception and trust among many “psychological” factors of acceptance surveys, alongside other issues in this broadly defined category such as social norms, fairness, disgust, environmental issues, knowledge, etc.

Influence of Local Resource Types on Acceptance

The acceptance of water reuse cannot be separated from local factors such as the specific potential depending on available water resources, the exposure to certain forms of water reuse and the reuse practicability in terms of associated costs of treatment and distribution. In some regions, some water sources might be more accepted due to scarcity conditions, and awareness of water scarcity can even increase the likelihood of accepting water reuse (Dolnicar et al., 2011; Fielding et al., 2018; Scruggs et al., 2020). In regions of water abundance, some water sources such as

desalination might not be accepted. Other regions have a different acceptance pattern due to differences in resource availability. In the GCC region as an example, desalinated water is largely accepted as it supplies more than 90% of domestic and industrial needs in the region, but it is too costly for irrigation (Brown et al., 2018). In this region, brackish water is available in limited amounts in some cases and can be used for recharging ground water and then reused for drinking or agriculture. Produced water is significant in the oil and gas industry and can be utilized locally or provided in limited amounts for least-personal uses. Greywater represents a high potential due to the very large water use footprints per capita in the region, and it could be reused in the future on a household level for recreation or agriculture. Wastewater reclamation is expanding and is used for all kinds of purposes such as landscaping or aquifer recharge (Aleisa and Al-Zubari 2017). Finally, acceptance of certain water types can be high in urban areas. For example, in Australian cities, the use of stormwater for aquifer recharge and supply augmentation is largely accepted and more positively perceived than recycled wastewater (Mankad et al., 2015).

A STRUCTURING PROPOSITION: SOCIAL ACCEPTABILITY INSTEAD OF ACCEPTANCE

The acceptance of water reuse is understood in this section as a complex and dynamic social process. Following on from viewing water reuse acceptance as one of several interrelated reuse challenges (*In Company with Acceptance: Complementary Challenges of Large-Scale Water Reuse*) and a critical and site-specific intervention (*A Synthesis of the Reuse Acceptance Enigma*), this section structures reuse acceptance phases and studies. It seeks to move the debate beyond studying and comparing incidents of acceptance and towards understanding water reuse as a complex social acceptability process. Such a process is structured in order to break down the different concepts and ideas regarding the multi-disciplinary and multi-sphere issue of the social acceptability of water reuse.

The Social Acceptability Process of Water Reuse

In this section, the relevance of understanding attitudes towards water reuse within a broader process of social acceptability is explained. Furthermore, the contents of this process are presented.

Justification and Relevance

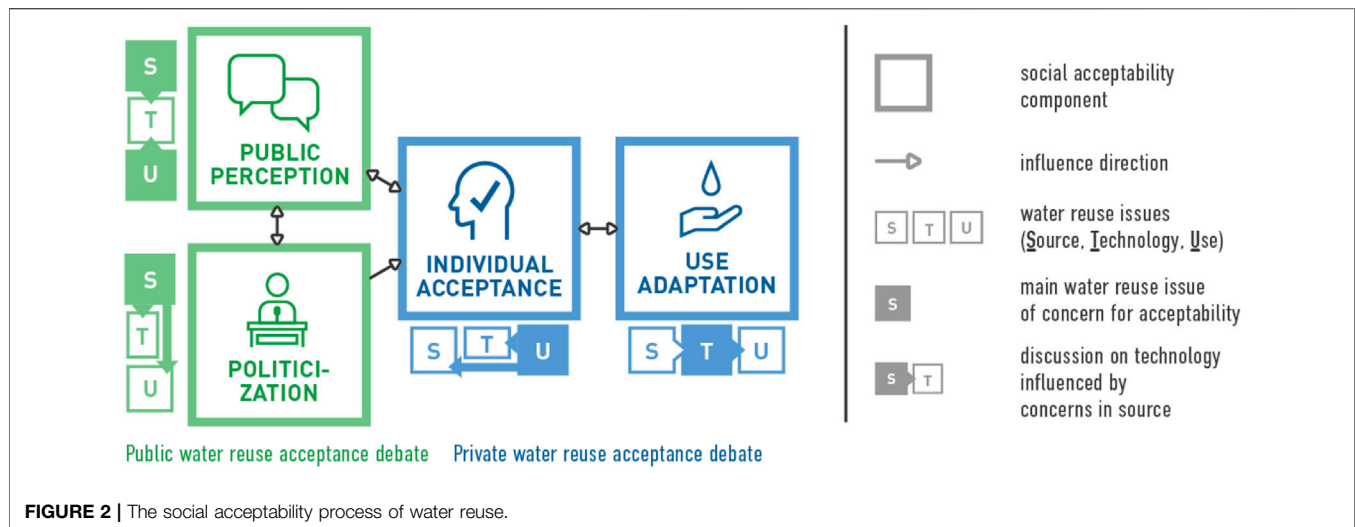
Water acceptance studies deliver divergent conclusions with few overarching commonalities, but many local specific factors. The resulting insights from comparisons of acceptance studies cover a large breadth of issues. Often, review studies (e.g., Dolnicar et al., 2011; Duong and Saphores 2015; Hurlimann and Dolnicar 2016; Wester et al., 2016; Fielding et al., 2018) will stress factors including socio-demographic characteristics of respondents, reuse technologies and locations, contextual challenges facing

water reuse (**Table 1**), (risk) perceptions and trust, socio-psychological factors (e.g., disgust), and socio-political environments (e.g., politics, role models, etc.). The key conceptual difficulty here is that these categories of acceptance factors/determinants relate to different aspects of the water acceptance phenomenon. Many studies do not define key concepts such as the acceptance by whom? (e.g., acceptance by “users/consumers”, “stakeholders”, the “reuse sector”, or the “public”) and of what? (i.e., which source, use or technology, and in which sites). Furthermore, the divergence of results is indicative of the temporality of water reuse acceptance: i.e., water reuse acceptance varies with the change in innumerable site-specific factors (e.g., adaptation to use, trust, policies, awareness, modernization, etc.). At the same time, the standalone approach of water reuse surveys is often inadequate for capturing the complexity of acceptance, as it gauges mere incidents or snapshots of supports, acceptance, or other momentary attitudes (e.g., favorable or skeptical).

As will be explained in the following sections, water reuse acceptance should be framed as an acceptability problem that is not merely related to individual or group attitudes, nor is it entirely a matter of public opinion. The demand for a more nuanced and contextual understanding of acceptability based on social norms and processes was also reiterated by other studies (e.g., Ching 2016; Crampton et al., 2016). Such a broad understanding of acceptance can lead to broader recommendations and can be used for structuring knowledge from acceptance case studies (*Discussion*). In a similar sense, Harris-Lovett et al. (2015) viewed potable water reuse from the broader perspective of whether a reuse technology is seen as legitimate or desirable in a certain social context. Beveridge et al. (2017) consider the whole water reuse issue as inherently social, with important spatial and political dimensions. Using a review of the relevant literature, this paper argues that the acceptability of water reuse projects requires a holistic understanding as a multi-layered social process of interactions between humans, technology and nature that takes place in norm-based and interlinked debates in both the public and private spheres.

Framing the Social Acceptability Process

The framework presented here to understand social acceptability (the willingness to accept) distinguishes four components (public perception, politicization, individual acceptance, and use adaptation), in which the debate about water reuse takes place and thus the view on water reuse is shaped (**Figure 2**). Policymakers can address each social acceptability component through various instruments, to be presented later in *Mapping solutions across the social acceptability components*. For a high acceptability of water reuse, the majority of debates on all components need to end with positive outcomes, since these components are largely interdependent. In each of the respective social acceptability components, one or more of the three issues or aspects of water reuse (water source, treatment technology and reuse purpose) figures prominently. As argued previously in *A Synthesis of the Reuse Acceptance Enigma*, water reuse experiences and acceptance vary significantly based on these three issues (source, technology and use). These issues are embedded in



debates on other reuse challenges (*In Company with Acceptance: Complementary Challenges of Large-Scale Water Reuse*), which, in turn, can also affect the social acceptability of water reuse.

The social acceptability process of water reuse is divided into two main debates, the public debate and the private one. These debates are interrelated, while acceptance studies usually provide snapshots of sub-topics in one of these debates. These studies can be classified as placing more emphasis on either the private debate (*Evidence highlighting the private debate dimension*) or the public debate (*Evidence highlighting the public debate dimension*), but they often start from one acceptability component and ultimately recommend remedies beyond this particular component. This is due to the fact that improving water acceptance is rarely possible through a simple set of interventions (See *Discussion*).

The private acceptance debate of water reuse is influenced by considerations regarding the use purpose and treatment technology of the recycled water. This is repeatedly highlighted in bodies of literature using methods such as surveys, perceptions, or narratives and psychological tests (*Evidence highlighting the private debate dimension*). Individual acceptance depends on risk perceptions associated with certain reuse purposes such as drinking, cooking or irrigation. As mentioned previously, this is a highly individual process influenced by previous experiences, attitudes towards the environment, personal values or discussions with others (Bennett et al., 2010; Doria 2010; Ching 2016). Individual acceptance is susceptible to influences from political debates and public perception, but is also independent from these debates to a certain extent. For example, economic factors such as the cost of recycled water, incentives or subsidies for different reuse purposes can help to increase individual acceptance; e.g., (Molinos-Senante et al., 2013; Duong and Saphores 2015; Ravishankar et al., 2018; Sgroi et al., 2018). Similarly, use adaptation entails a private debate concerned with the technology used to recycle water. Consumers try to acquire information about the quality of delivered water and the associated risks (Binz et al., 2018). They form their opinions on the use safety and the required

measures before the final use, e.g., filtration mechanisms or heating of water, depending on the source. Debates about the reliability of water treatment or other previously mentioned challenges of the safety of water reuse influence the determined use of recycled water by consumers. Ultimately, the ability to adapt to the use of recycled water is highly relevant for the individual acceptance, and often independent from other factors such as ideology and values.

Public perception is the most difficult, but arguably the most important, component of social acceptability. Indeed, under this component, all kinds of political, technical or scientific, religious and cultural debates take place in public and with the participation of various actors such as policymakers, scientists and experts as well as public and religious figures. The importance of this public debate is highlighted by several bodies of research on reuse acceptance, such as economic, institutional and social studies (See *Evidence highlighting the public debate dimension*). Ideas and norms compete in these debates to form an important public perception of water reuse. Here, the media is an important factor in this regard, while the debates are often less technical and rather broad; e.g., benefits vs. advantages of reuse, or reuse as desirable or alarming (Russell and Hampton 2006; Ching 2010; Lee and Tan 2016). The outcomes of public debates can be determined by a number of locally specific issues such as scarcity conditions, socio-political systems, role models and religious/cultural aspects (Po et al., 2003; Robinson et al., 2005; Hartley 2006; Doria 2010; Garcia-Cuerva et al., 2016; Hurlimann and Dolnicar 2016). During public debates on water reuse, politicization represents a deliberative process to highlight reuse as relevant for a society's development, peace and order, relationship with nature, etc. At the same time, water reuse can become politicized in the wake of contamination incidents, project failures or constraints in the reuse system (Russell and Hampton 2006). However, in line with securitization theories (e.g., Allouche et al., 2011; Fischhendler 2015), politicization often takes place as a way to promote water reuse and prioritize action towards achieving it. Issues related to the water source such as

water quality, source control, trust in supply authorities and effects of communities at the source are at the forefront of politicization debates (e.g., Fischhendler 2015; Beveridge et al., 2017). In cases of politicization incidents, these can significantly affect public perception.

Evidence Highlighting the Private Debate Dimension

Several bodies of acceptance literature have highlighted one or another component of what this paper calls the social acceptability process of water reuse. In this section, the academic studies are presented with regard to their key approaches and potential contributions to illuminating the acceptance problem from the starting point of the private debate in the social acceptability process.

Traditional Acceptance Studies

Traditional perception studies (e.g., Robinson et al., 2005; Hartley 2006; Russell and Hampton 2006; Carr et al., 2011; Buyukkamaci and Alkan 2013; Aitken et al., 2014; European Commission 2015; Crampton et al., 2016; Garcia-Cuerva et al., 2016; Hurlimann and Dolnicar 2016; Baghapour et al., 2017) look at public responses to water reuse projects using perception and opinion surveys. This body of literature represents the most common approach in water acceptance research. The surveys *de facto* target the individual acceptance component of the social acceptability process. As highlighted previously in the review of similarities and divergences of experiences of acceptance of water reuse, (*A Synthesis of the Reuse Acceptance Enigma*), issues related to the particular use (i.e., Which source and technology is used for which use purpose?) dominate debates in acceptance surveys. Since individual acceptance lies at the center of the social acceptability process of water reuse, it is influenced by all of the other components. In this sense, acceptance surveys often link incidences of the acceptance or rejection of a certain water reuse issue to factors that might arise from other acceptability components.

In reality, traditional acceptance studies use individual acceptance or perceptions as a starting point of analysis. They then gauge a plethora of determinants related to the characteristics of individuals, debates in the public sphere, and contextual issues that represent the challenges related to water reuse (earlier described in **Table 1**). Some overviews of the determinants of acceptance and contextual challenges found in acceptance surveys are provided in Fielding et al. (2018), Dolnicar et al. (2011). Although some studies (e.g., Duong and Saphores 2015; Ravishankar et al., 2018; Smith et al., 2018) investigate “public” or “social” perceptions or acceptance, the focus is less on analyzing discourses or debates happening in the public sphere; rather, these mostly survey-based studies center on users’ acceptance and its determinants. Finally, since the focus of the traditional studies is on the conceptual constructs of perception, attitudes and acceptance incidents, the suggested remedies are mostly informational; i.e., stressing awareness, debates, education campaigns, etc.

Narrative and Psychological Studies

Psychological and narrative studies (e.g., Hampton 2004; Menegaki et al., 2009; Ching 2010; Ching 2016; Leong 2016; Wester et al., 2016; Greenaway and Fielding 2020; Nemeroff et al., 2020) look at factors affecting individual attitudes towards water reuse. These factors include internalization processes of certain opinions, individual experiences, discourses, semantics, framing of water reuse information, etc. The methods deployed include surveys, observations or narratives and conceptualizations based on grounded theory. In the social acceptability process, the starting point for psychological studies is often the use adaptation component; e.g., disgust experiences (the yuck factor), emotions, or lived experiences. Often, psychological determinants such as disgust are presented as the most relevant determinants or predictors of acceptance (e.g., Duong and Saphores 2015; Wester et al., 2016). However, disgust is also seen as resulting from other factors. For example, education, prior knowledge, psychologically prepared information, labeling and public debates can mediate disgust experience (Ching 2010; Wester et al., 2016; Goodwin et al., 2017). In this sense, the recommendations of psychological or narrative-based studies often go beyond the component of the use adaptation.

Other studies in this body of literature relate emotions, attitudes and willingness to use to environmental values and personal profiles. For example, one approach in psychological studies is to link disgust with peoples’ sensitivity/aversion towards contagion/cleanliness (Fielding et al., 2018). While these approaches are highly revealing about processes in the private acceptance debate of water reuse, some insights, e.g., on the “yuck discourse”, is of limited value for explaining how to change peoples’ responses towards water reuse (Russell and Lux 2009). Therefore, it is important to translate psychological insights into actionable implications. For example, psychological insights can help to improve the design of environmental nudging (Byerly et al., 2018), the targeting of awareness campaigns, the framing/labeling of water reuse strategies (McClaran et al., 2020), or interventions to facilitate the adaptation of households (Nemeroff et al., 2020).

Evidence Highlighting the Public Debate Dimension

In this section, bodies of academic literature are presented with the public debates as a point of departure. They include studies focusing on the influence of institutional, regulatory and social issues. Although not all studies highlighting the public debate on reuse acceptability can be classified, the two categories presented represent the commonest approaches in the literature on acceptance.

Economic and Institutional Studies

Economic and institutional studies (e.g., Lloyd Owen 2016; Molinos-Senante et al., 2013; Menegaki et al., 2007; Fan et al., 2013; Garcia and Pargament 2015) reiterate the role of economics and contractual (rights-based) approaches in improving acceptance of water reuse. The basic premise here is

understanding such acceptance as a regulation problem. This means that better rules and incentives can improve the outcomes of challenges directly affecting reuse acceptance, particularly those challenges of safety, distribution, participation, economics and governance. In this sense, the literature on these related challenges presented in **Table 1** can also be seen as a part of this body of academic literature; e.g. framing water reuse as a function of better communication among agencies (Aldaco-Manner et al., 2019), or holistic circular economy strategies (Sgroi et al., 2018). Furthermore, improving regulation and the institutional set-up of water reuse can yield favorable outcomes in debates happening in all components of the acceptability process. However, the focus is rather on the public acceptance debate. Regulating resource allocations and (private) incentives for water reuse is a task centered in the overall governance or public policy context.

Social Legitimacy and Justice Studies

Closely related to the economic and institutional studies are social legitimacy and justice studies (e.g., Russell and Lux 2009; Harris-Lovett et al., 2015; Binz et al., 2016; Beveridge et al., 2017), which stress the complementary aspects of legitimate processes and decision-making, participation, consultations and transparency. The focus here is rather related to the public debate on reuse acceptance and whether the public perceives the system and technologies of water reuse as adequate, necessary, legitimate, transparent, or trustworthy. Often, studies in this body of literature use mixed methods and provide critical insights into classic approaches to understanding acceptance. For example, Russell and Lux (2009) criticized the premises that disgust and lack of information or awareness are deterministic for people's responses. Instead, they propose a cultural (rather than psychological) approach in which people's responses to reuse are seen as amendable through understanding local contexts and complexities and designing a participatory/deliberative planning structure for water reuse schemes. Another example is the framing of water reuse in California as a broad endeavor (beyond acceptance) to achieve the legitimacy of this new technology/innovation through institutional work and legitimacy strategies (Harris-Lovett et al., 2015; Binz et al., 2016).

DISCUSSION

In this section, the reviewed literature and the structured process of water reuse acceptability will be used to advance current discussions on the promotion of water reuse. First, some solutions or remedies for advancing the social acceptability of water reuse are summarized. Later, some key insights and contextualized lessons are presented.

Mapping Solutions Across the Social Acceptability Components

The previously presented analysis framework for the social acceptability of water reuse shows the complexity of the

acceptance question as a process, and the need for nuanced solutions across different components of this process. It can be used for mapping solutions to water reuse acceptance problems based on recommendations regarding immediate action put forward by different bodies of literature. In fact, there are numerous solutions proposed in the academic literature to help increase the acceptance of water reuse. However, suggestions from broad, descriptive studies on water reuse (e.g., Po et al., 2003; Doria 2010; Yi et al., 2011; Duong and Saphores 2015; Wilcox et al., 2016; Fielding et al., 2018; Smith et al., 2018) are difficult to interpret in terms of which components of reuse acceptability should be addressed by which instruments, and when. There is also a conceptual difficulty in the use of same terms in different scientific and contextual frames. Furthermore, it can be difficult to mainstream or gauge the meaning of insights coming from different bodies of literature. For example, surveys indicating discomfort with the idea of DPR do not necessarily result in a negative public perception or a lack of individual acceptance. Even if we interpret discomfort as a problem for reuse acceptance, such a survey-based result might not predict acceptance in a certain location, as this is a long-term social process with many intermediate outcomes and debates across several components. Instead of focusing on single (and often unstable or intermediate) roots of the acceptance problem or seeking cross-case panaceas for increasing acceptance, remedies should be sold as a package of measures based on interdisciplinary knowledge and tailored to the complex and long-term process of the social acceptability of water reuse. **Table 2** provides a high-level overview of such solutions to increasing acceptability based on remedies arising from different bodies of studies. Although these studies usually focus on specific components or debates within social acceptability (*Evidence highlighting the private debate dimension* and *Evidence highlighting the public debate dimension*), **Table 2** summarizes most relevant remedies across the interrelated components. Traditional acceptance studies often stress the importance of marketing or information, public campaigns, debates, and community involvement. Psychological and narrative studies complement these insights through tools related to role models, testimonials, labels, brands and group-based reuse promotions. Economic studies highlight the role of incentives, prices, regulations and private-sector participation as measures to achieve optimal allocation, instill trust and incentivize individual adaptation and acceptance. Finally, social justice and institutional studies introduce remedies related to good governance, legitimacy and representation.

Contextualization and Overarching Insights

The academic literature has shown that there are many different perspectives from which to look at water reuse acceptance. The most common ones are related to polling studies, psychology, economics, institutionalism and social justice, while important perspectives such as those of politics/politicization and security studies are still lacking. Similarly to other authors (e.g., Russell and Lux 2009; Beveridge et al., 2017), this paper stresses that water reuse is a multi-dimensional process that is inherently

TABLE 2 | Solutions for increasing social acceptability from different acceptance studies.

Components of the social acceptability process of water reuse		Traditional acceptance studies	Narrative and psychological studies	Economic and institutional acceptance studies	Social legitimacy and justice studies
The public acceptance debate	Public perception	Awareness campaigns	Engagement through role models	Information on economics of recycling, risk-cost/benefit trade-offs, valuations and externalities	Impartiality and credibility of information
		Information on control, multi-barrier systems, safety and quality criteria Public consultations	Promotion of trust in authorities and decision-making processes		Transparency and promotion of quality of suppliers (e.g., quality standards, certification, training)
	Politicization	Media engagement to increase public interest	Making recycling issues "influenceable", i.e., participatory planning and policymaking Independent panels of prominent experts	Complaint and monitoring arrangements Participatory economic regulation (e.g., boards, public-private partnerships)	External accountability in monitoring and evaluation Technical publications on quality and success stories Involvement of local authorities and advisory boards
		Effective crisis and emergency management, and communication Consensus-building and political activism			
The private acceptance debate	Individual acceptance	Stakeholder and consumer engagement Community involvement	Branding and terminology (e.g., recycled instead of treated water)	Adequate pricing policies Information on environmental costs and benefits	Frequent contacts with utility managers Increasing consumer representation in reuse schemes
	Use adaptation	Educational programs	Working with focus groups on different reuse purposes	Use of financial incentives such as subsidies to water bills or to production costs of small recycling schemes	Tasting, demonstration and self-experimentation
		Guidelines and information for household level and farm level	Testimonies of users		Different products of recycled water (e.g., bottled water)

political, cultural and social, as well as being influenced by the classic factors of technology, climate and resource availability, economy, and infrastructure, or individual values and psychology. This process needs to be better structured with regard to its temporal and spatial components, and also better embedded in its contextual environments. In this context, two overarching insights can be highlighted from the previous analysis. Firstly, the reuse acceptance issue cannot be studied or addressed in isolation from other challenges hindering large-scale reuse. Case studies on water reuse reveal that acceptance increases if the other challenges highlighted earlier in this paper are addressed adequately (e.g., health and safety, markets, regulation and guidelines). Thus, water reuse (acceptance) cannot be separated from the local water governance arrangements, the socio-political culture, and economic development at large. In this sense, promoting large-scale water reuse requires broader policies that tackle the interrelated challenges, including a set of measures to promote acceptability. A stronger public role can help tackle many of these challenges (e.g., infrastructure, safety regulations, monitoring arrangements, investments, campaigns, debates, or politicization/securitization). Public policies can also embed

the promotion of water reuse in overarching developmental policies as well as global targets to increase wastewater treatment (Indicator 6.3.1. of the SDGs agenda), improve water use efficiency (e.g., Indicator 6.4, SDGs) and decrease the stress facing freshwater resources (Indicator 6.4.1, SDGs).

Secondly, this paper argues for a holistic and nuanced understanding of water reuse acceptance as an acceptability process that should be understood within a specific context. Here, it is important to analyze local reuse acceptability processes and examine debates, challenges and remedies. Such holistic and nuanced analyses of water reuse acceptability should look at different spatial (i.e., households, sources, treatment systems, public arenas) and temporal aspects (e.g., initial debates, politicization, reception of people, adaptation and feedbacks) and incorporate insights from different academic disciplines. Indeed, descriptions of deployed solutions in successful case studies on water reuse (e.g., Horne 2016; Lee and Tan 2016; van Rensburg 2016; Lahnsteiner et al., 2018) emphasize the importance of multi-layered approaches to increasing social acceptability. The long-standing emphasis on "acceptance" as merely a stance or attitudes attached to individuals and groups has been misleading. Such a premise

merely produces snapshots of a complex social reality, while giving the false hope that reluctant people can be “convinced otherwise” through simply remedies, or that “rejection” can be “switched” into “acceptance” through some short-term interventions. As this paper has previously argued, it is highly relevant to contextualize the local context by examining the overall challenges and the site-specific acceptability debates. Alongside improving the enabling environment through enhancing reuse technologies, infrastructure, policies and regulations, it is also important to invest in a set of reuse acceptability measures, including various socio-economic, political and psychological instruments. At the same time, the acceptance issue should be perceived as a social acceptability process of complex components that are best addressed and studied on a local level through gradual, iterative and long-term efforts. Such a perception can have important implications for how to design context-specific measures that are decided locally. Future case study research on reuse acceptability can highlight these locally embedded processes and describe interesting efforts to push site-specific measures; e.g., by involving influential actors (e.g., from civil society or media), empowering certain user groups (e.g., women), providing targeted incentives, solving certain adaptation problems, or politicizing the importance of water reuse.

CONCLUSION

Responses by individuals and the general public to water reuse endeavors might be the most restrictive challenge facing the wide adoption of water reuse. There are other (more manageable) concerns such as the effects of water reuse on human and environmental health, the need for infrastructure and regulations as well as the long-term impacts on soil quality. These challenges are recurrent issues in acceptance surveys, and they can also affect the overall acceptance of water reuse. Although numerous studies have shown a positive appreciation of the idea of water reuse, people are skeptical of the use of recycled water for close-to-person purposes such as drinking, cooking, or irrigating edible agriculture. Evidence shows that appropriate technologies for each water source, combined with multi-layered and strict regulation and monitoring, can significantly increase the safety of water reuse, which, in turn, can improve trust and help improve its public perception. However, there is a need for further studies at the local level that examine the occurrence of, and interrelations between, specific water reuse challenges as well as the suitable technology standard-sets. The acceptance gap (between the positive perception of the idea of water reuse and dismissive attitudes towards certain reuse purposes) represents a complex phenomenon requiring nuanced analysis.

Negative response to water reuse can be related to the lack of knowledge of aspects of water quality and state-of-the-art advancements in water reuse, but more importantly, to perceptions about the ability of the utilities and public authorities to deliver on water reuse. Here, there is mounting

criticism related to the absence of common and updated standards on water reuse for different uses in many regions, as well as a lack of comprehensive and participatory reuse strategies embedded in larger water-sector policies. Overall, water reuse acceptance cannot be separated from the broader challenges facing the upscaling of water reuse. It can also be incentivized and promoted through measures such as adequate pricing schemes, strong public leadership, and financing or subsidies for consumers. In light of the expansion of water reuse projects to new countries and cities, we need to analyze the issue of water reuse in the totality of its interrelated challenges while investing more in social research in order to understand individual attitudes and collective responses at the local level.

Water reuse represents a significant and untapped potential. For example, it can alleviate the pressures on scarce water resources in dry areas. In arid regions, water recycling is on the rise due to deteriorating conditions of water stress. The reuse of treated wastewater is increasing, while reuse can be expanded to other water sources such as produced water or greywater, exploiting the high consumption level of these water types. Much can be gained by studying successful experiences or applying methods to increase awareness and knowledge of the local water reuse options.

This paper has structured knowledge on water reuse acceptance and shown the diversity of approaches and results, so generalizations are quite difficult. Reuse acceptance levels will differ according to the source, the reuse purpose and the deployed technology. Therefore, acceptance needs to be investigated with local populations using interdisciplinary knowledge, while it can be best promoted gradually and iteratively. At the same time, it is important to move beyond incidents of negative responses to consider attitudes of acceptance or the willingness to accept (acceptability) as a social process comprising public and private debates taking place at different spatial and temporal points. On the one hand, debates regarding the public perception of water reuse can be influenced through positive messages and discourses, thus increasing trust in the authorities and the supply system. Such debates require a wide engagement of technocrats and role models. Furthermore, water reuse can (and sometimes has to) be politicized in the context of water security and development needs. On the other hand, individual acceptance is a subjective component that can be influenced by public debates or framings or personal values, but also by the ability to adapt to the use of recycled water. It can be targeted by measures to decrease the perception of risks associated with certain uses. At the same time, pathways to adapt to the use of this new renewable water resource can be demonstrated.

Several instruments for increasing reuse acceptance are found in the academic literature. Detailed water reuse strategies need to incorporate insights from traditional awareness studies as well as socio-economic, psychological, discursive and narrative analyses. Such insights can be integrated into multi-level and participatory planning approaches during water reuse projects. Non-acceptance of certain water reuse projects can be temporary, and often decrease with awareness, trust and adaptation. Until water reuse becomes a normal practice in water supply, we need to promote its acceptance as a generally sustainable, safe and

renewable water source. Furthermore, for water reuse to become a serious supply option, it has to be mainstreamed into water governance frameworks. Similarly to water conservation or water efficiency issues, water reuse can be an integral part of national water sector strategies, management plans, regulatory frameworks and public debates on resource governance. The treatment of water reuse in many countries as a side issue or an emerging topic is masking its potential as well as the repertoire

of knowledge and technologies available for public authorities to enforce and promote its implementation.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Ramsar Wetlands of International Importance—Improving Conservation Outcomes

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The Ramsar Convention (or the Convention on Wetlands), signed in 1971, was one of the first international conservation agreements, promoting global wise use of wetlands. It has three primary objectives: national designation and management of wetlands of international importance; general wise use of wetlands; and international cooperation. We examined lessons learnt for improving wetland conservation after Ramsar's nearly five decades of operation. The number of wetlands in the Ramsar Site Network has grown over time (2,391 Ramsar Sites, 2.5 million km², as at 2020-06-09) but unevenly around the world, with decreasing rate of growth in recent decades. Ramsar Sites are concentrated in countries with a high Gross Domestic Product and human pressure (e.g., western Europe) but, in contrast, Ramsar Sites with the largest wetland extent are in central-west Africa and South America. We identified three key challenges for improving effectiveness of the Ramsar Site Network: increasing number of sites and wetland area, improved representation (functional, geographical and biological); and effective management and reporting. Increasing the number of sites and area in the Ramsar network could benefit from targets, implemented at national scales. Knowledge of representativeness is inadequate, requiring analyses of functional ecotypes, geographical and biological representativeness. Finally, most countries have inadequate management planning and reporting on the ecological character of their Ramsar Sites, requiring more focused attention on a vision and objectives, with regular reporting of key indicators to guide management. There are increasing opportunities to rigorously track ecological character, utilizing new tools and available indicators (e.g., remote sensing). It is critical that the world protect its wetlands, with an effective Ramsar Convention or the Convention on Wetlands at the core.

Keywords: Ramsar Convention, International agreement, ecological character, river conservation, risk assessment

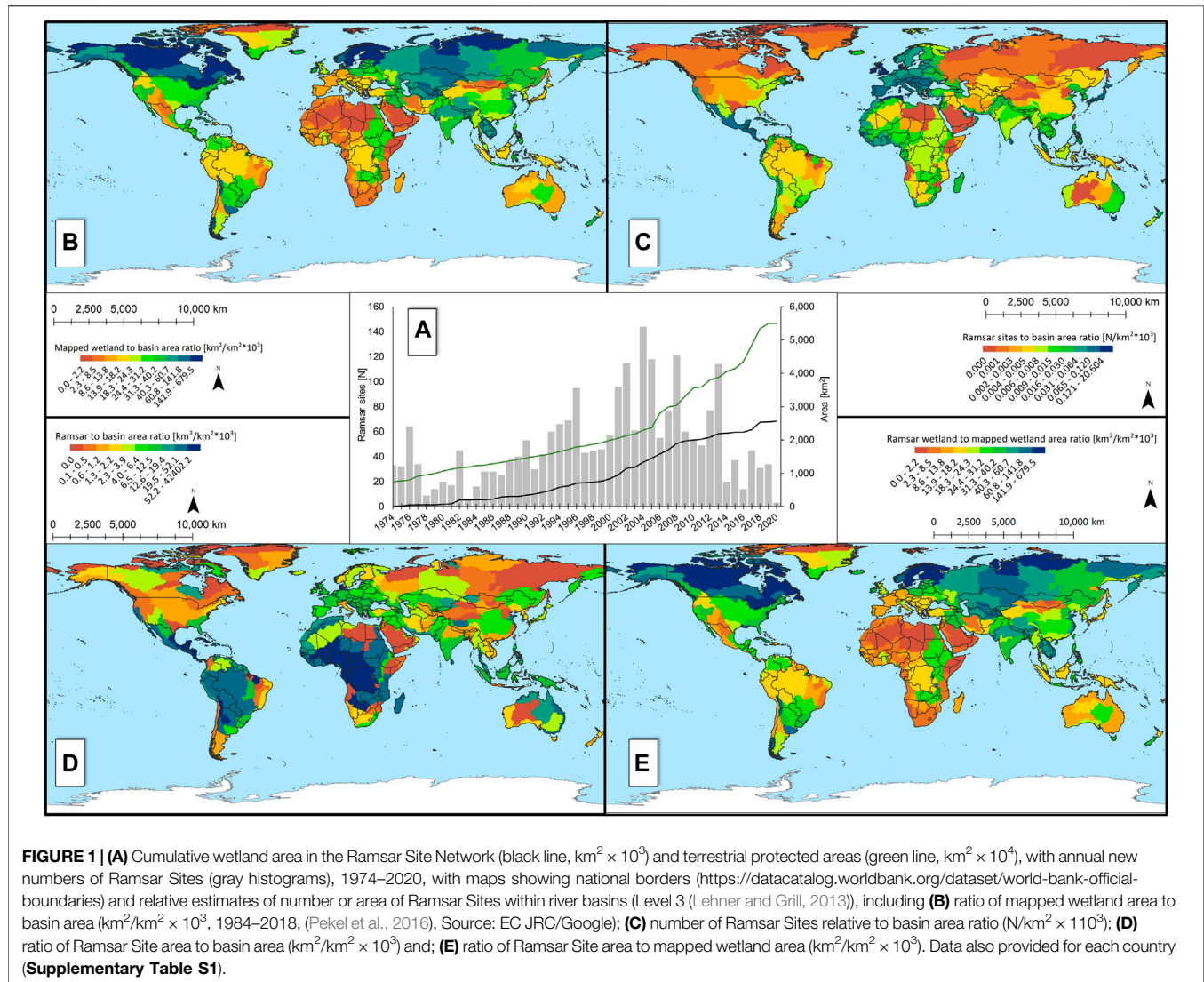


FIGURE 1 | (A) Cumulative wetland area in the Ramsar Site Network (black line, $\text{km}^2 \times 10^3$) and terrestrial protected areas (green line, $\text{km}^2 \times 10^4$), with annual new numbers of Ramsar Sites (gray histograms), 1974–2020, with maps showing national borders (<https://datacatalog.worldbank.org/dataset/world-bank-official-boundaries>) and relative estimates of number or area of Ramsar Sites within river basins (Level 3 (Lehner and Grill, 2013)), including **(B)** ratio of mapped wetland area to basin area ($\text{km}^2/\text{km}^2 \times 10^3$, 1984–2018, (Pekel et al., 2016), Source: EC JRC/Google); **(C)** number of Ramsar Sites relative to basin area ratio ($\text{N}/\text{km}^2 \times 10^3$); **(D)** ratio of Ramsar Site area to basin area ($\text{km}^2/\text{km}^2 \times 10^3$) and; **(E)** ratio of Ramsar Site area to mapped wetland area ($\text{km}^2/\text{km}^2 \times 10^3$). Data also provided for each country (Supplementary Table S1).

INTRODUCTION

Water crises and the collapse of biodiversity and ecosystems are among the top global risks to human well-being (World Economic Forum, 2019). Globally, wetlands have declined by 35% from 1970 to 2015, where data are available (Darrah et al., 2019). Such loss and degradation of wetlands and their ecosystem services is increasingly expressed in global initiatives (e.g., Convention on Biological Diversity Aichi targets, Sustainable Development Goals 6 and 15 and associated targets). The Ramsar Convention (the Convention on Wetlands), signed in 1971 and legally effected in 1975, was one of the first modern multilateral environmental agreements advancing protection of wetlands through international collaboration and effective management.

The Convention includes palustrine, riverine, estuarine, lacustrine wetlands and near shore systems, including reefs. Over nearly five decades, 171 (as at 2020-06-09) contracting parties (i.e., countries) have prioritized the conservation and wise

use of wetlands, and by listing wetlands assessed as internationally important and committing to maintaining their ecological character (Gardner and Davidson, 2011; Davidson et al., 2019). However, ecological character, the combination of ecosystem components, processes and benefits (Davidson et al., 2019), is increasingly deteriorating in Ramsar Sites (Davidson et al., 2020). These can be tracked by measuring key ecological indicators, including threats, which cause degradation (Davis and Brock 2008).

The *Ramsar Strategic Plan 2016–2024* has three strategic goals, to: 1) address the drivers of wetland loss and degradation; 2) effectively conserve and manage the Ramsar Site Network; and 3) wisely use all wetlands (Ramsar Convention Secretariat, 2016). Targets include increasing the area, numbers, and ecological connectivity of Ramsar Sites, focusing on under-represented regions or types, and effective management. We provide a perspective on achieving these goals and targets, focusing on two key objectives: 1) identification of biases in the current global distribution of the Ramsar Site Network and 2) a conceptual

adaptive management framework, linking maintenance of ecosystem dynamics with drivers of change.

RAMSAR SITE NETWORK

The number of Ramsar Sites (2,391) and their area (2.54 million km² as at June 2020) have increased, but the rate of increase in area has slowed, contrasting a steady growth in protected area (**Figure 1A**), reflected in various regional analyses (Mauerhofer et al., 2015; Fritz et al., 2017). Given that effective management of most inland wetlands is dependent on flow and flooding regimes of rivers (Saunders et al., 2002; Kingsford et al., 2016), we examined the distribution of Ramsar Sites and their areas, at the river basin scale (Lehner and Grill, 2013), relative to maximum extent of inland surface water between 1984 and 2018 (<https://www.ngdc.noaa.gov/mgg/shorelines/gshhs.html>; Pekel et al., 2016, Source: EC JRC/Google). Such mapping generally excluded vegetative flooded areas, while Ramsar Site areas included terrestrial ecosystems.

The world's mapped wetlands are predominantly in the Northern Hemisphere (**Figure 1B**; **Supplementary Table S1**), different to the distribution of numbers of Ramsar Sites (**Figure 1C**) with relatively higher numbers of Ramsar Sites in Europe, parts of South America, Central America, Northern, Central and Western Africa, Southern Asia and eastern Australia (**Supplementary Table S1**), reflecting Northern Hemisphere bias (Rebello et al., 2009). Growth in numbers of Ramsar Sites has continued linearly in Europe since the beginning of the Convention on Wetlands, contrasting most other regions of the world where significant growth only began after about 2000 (**Supplementary Figure S1**). In contrast, Ramsar Site area, relative to basin area, is higher in the Southern Hemisphere (**Figure 1D**; **Supplementary Table S1**) and at a higher density relative to mapped wetland area (**Figure 1E**; **Supplementary Table S1**). Much of this growth in Ramsar Site area has occurred in Africa and Latin America (**Supplementary Figure S1**). Numbers and area of Ramsar Sites has continued to grow in Europe, Africa, Asia and Latin America but plateaued in North America and Oceania (**Supplementary Figure S1**).

We also explored possible associations among countries between the number and area of Ramsar Sites, area of mapped inland surface water (Pekel et al., 2016), area of protected areas (UNEP-WCMC and IUCN, 2020), Gross Domestic Product (GDP) (GIS processing World Bank DECRG, 2010) and Human Footprint Index (HFP, sum and mean, (Venter et al., 2018); **Supplementary Figure S2**). The number of Ramsar Sites was also positively related to HFP and surface water area (Pekel et al., 2016), slightly correlated to protected area and not to GDP (**Supplementary Figure S2**). This could reflect the high number of Ramsar Sites in the Northern Hemisphere, with relatively high HFP, contrasting to Ramsar Site area potentially associated with countries with lower GDP and HFP.

Adaptive Management Framework

Effective management and reporting is a significant challenge, given many Ramsar Sites lack management plans, and there is

inadequate reporting of ecological character trajectories (Davidson et al., 2019; Davidson et al., 2020). Social-ecological interconnectedness and feedbacks are essential for hydrologically connected freshwater ecosystems, requiring collaboration of managers, scientists and stakeholders (Lee, 1999; Huitema et al., 2009; Novellie et al., 2016). Conceptually, adaptive management of a Ramsar Site needs a vision of a desired future state, involving stakeholders, clear objectives, triggers, risk assessment supported by co-design and co-management actions, and transdisciplinary science, with evaluation, reporting and updating (Kingsford et al., 2011; Westgate et al., 2013; Davidson et al., 2019; Davidson et al., 2020; **Figure 2A**).

Direct and indirect biotic and abiotic (natural and anthropogenic) drivers affecting ecosystem processes, characteristic biota and ecosystem services need to be incorporated (**Figure 2B**). In social-ecological systems such as Ramsar Sites, there should also be a focus on how demographic, economic, sociopolitical and cultural factors link to natural biotic and abiotic drivers, incorporated into management (**Figure 2B**; Carpenter et al., 2006). Scientific analysis can then track change, diagnosing causes, and informing management, informed by understanding of the interactions of these drivers on changes to ecological character (**Figure 2C**). Ecosystem dynamics need to be conceptually simplified, characterizing requisite simplicity of an ecosystem (Holling, 2001; Stirzaker et al., 2010). Simple conceptual models can be used to identify primary drivers (Keith et al., 2020), which usually include flow (surface and ground water) and/or flooding regimes, nutrients and water quality. Such models guide choice of indicators, with data available locally (e.g., waterbirds, fish), regionally (e.g., rainfall, river flows) or globally (e.g., remote sensed inundation and land cover) (**Figure 2C**). Indicator data can be used to report on the condition of the wetland or ecological character over time, guiding management. For example, loss of a key bird species, a measure of ecological character, in a Ramsar wetland in Chile was legally judged to have been caused by paper mill discharge impacting an aquatic plant, the bird's main food supply (Marín et al., 2018).

DISCUSSION

After nearly five decades, the listing of 2,391 Ramsar sites is an impressive contribution to global biodiversity conservation (**Figure 1A**). Over the same period, our population has more than doubled, increasingly threatening fresh water for wetlands, diverted for drinking, irrigation and energy. Many ecosystem services provided by wetlands are in decline (Gardner and Finlayson, 2018; Darrah et al., 2019; McInnes et al., 2020). A declining rate of growth in Ramsar Site area in some regions (**Figure 1A**; **Supplementary Figure S1**) may reflect this inability to maintain ecological character, highly dependent on flow and flooding regimes (Kingsford et al., 2016), potentially hampering achievement of Aichi Biodiversity Target 11 (Juffe-Bignoli et al., 2016).

More effective implementation of the Convention on Wetlands is urgent (Geijzendorffer et al., 2019), particularly given the relatively poor condition of the world's wetlands, compared to other realms (Gardner and Finlayson, 2018). This can be done by increasing the area and number of Ramsar Sites;

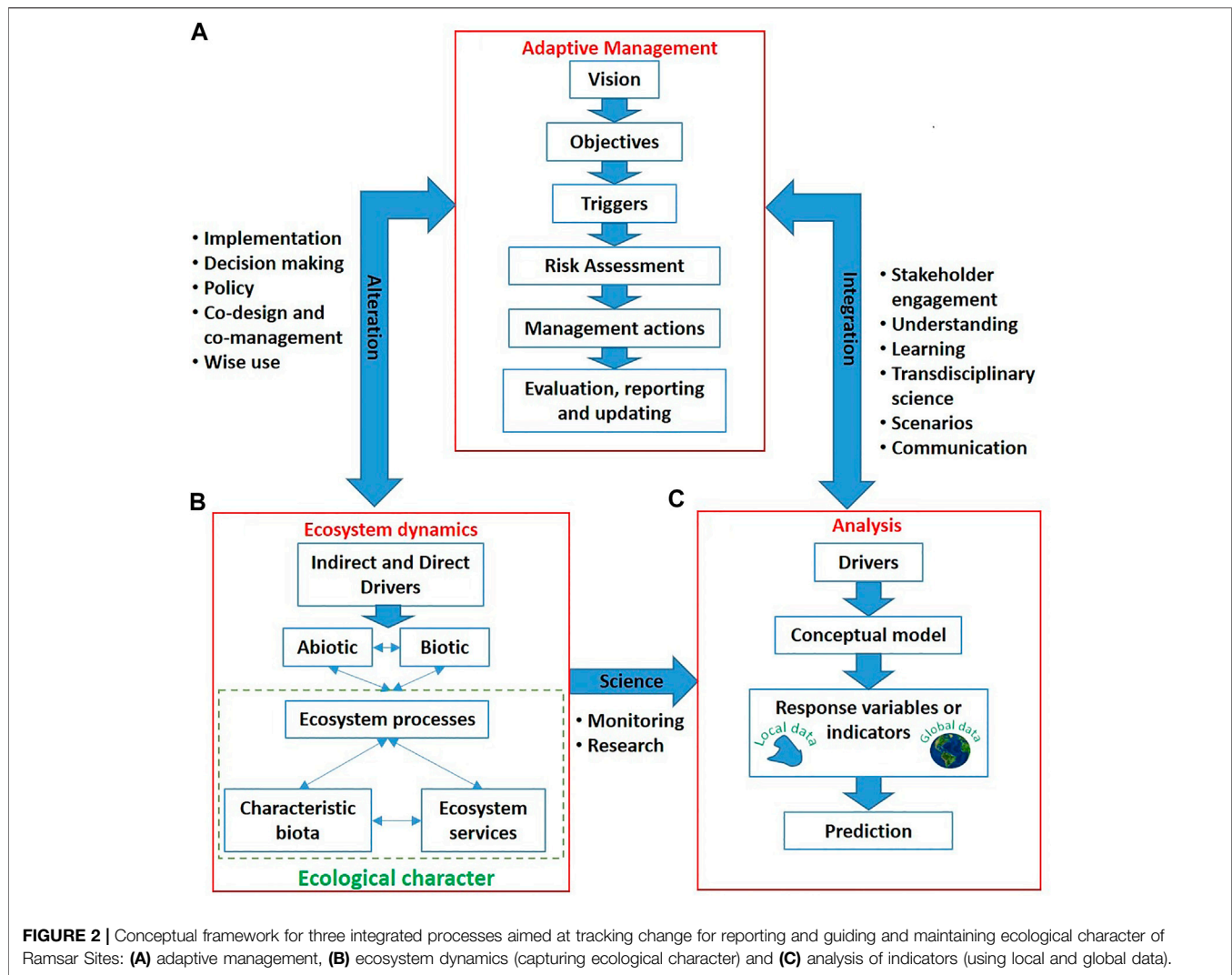


FIGURE 2 | Conceptual framework for three integrated processes aimed at tracking change for reporting and guiding and maintaining ecological character of Ramsar Sites: **(A)** adaptive management, **(B)** ecosystem dynamics (capturing ecological character) and **(C)** analysis of indicators (using local and global data).

improving representativeness (geographically, functionally, biologically); and implementing effective adaptive management. These issues areas are not new (e.g., Davidson et al., 2019; Davidson et al., 2020; Gaget et al., 2020), undermining the goals and targets of the Ramsar Strategic Plan (Ramsar Convention Secretariat, 2016).

Increases in the Ramsar Site Network could be achieved by adopting national representative targets. This could be informed by using typological classifications (e.g., new IUCN typology: Keith et al., 2020, <https://global-ecosystems.org/>) to improve representativeness (Figures 1B–E; recognising mapping shortcomings, such as those in Pekel et al., 2016). Although highly dependent on national will (Davidson et al., 2019), opportunities exist to integrate targets across different commitments (e.g., United Nations Sustainable Development Goals and Aichi targets and Convention on Biological Diversity targets).

To improve management, we recommend implementing adaptive management frameworks, with stakeholders, targeting measurable indicators responsive to drivers that guide management and track ecological character effectively

(Figure 2). This means incorporating social-ecological dynamics (Cilliers et al., 2013), with indicators representing ecological character (Figure 2), tracking ecosystem change (Keith et al., 2013; Bland et al., 2017). With globally available historical data (e.g., palaeoecological, remote sensing), past and future scenario analyses can guide management choices (Kopf et al., 2015).

The Convention on Wetlands provides nearly five decades of commitment to sustainable development and wise use of wetlands, but considerable challenges remain for the world's wetlands. There are significant opportunities to improve national and global wetland conservation by better incorporating representativeness and implementing adaptive management. This would improve effectiveness and streamline and improve transparent reporting, which guides management for conservation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RK conceived the idea and led the writing of the paper, supported initially by GB, including data analyses, and CMF, and by other authors, listed alphabetically.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2021.643367/full#supplementary-material>.

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Effects of Microplastic Fibers on Soil Aggregation and Enzyme Activities Are Organic Matter Dependent

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Microplastic as an anthropogenic pollutant accumulates in terrestrial ecosystems over time, threatening soil quality and health, for example by decreasing aggregate stability. Organic matter addition is an efficient approach to promote aggregate stability, yet little is known about whether microplastic can reduce the beneficial effect of organic matter on aggregate stability. We investigated the impacts of microplastic fibers in the presence or absence of different organic materials by carrying out a soil incubation experiment. This experiment was set up as a fully factorial design containing all combinations of microplastic fibers (no microplastic fiber addition, two different types of polyester fibers, and polyacrylic) and organic matter (no organic matter addition, *Medicago lupulina* leaves, *Plantago lanceolata* leaves, wheat straw, and hemp stems). We evaluated the percentage of water-stable aggregates (WSA) and activities of four soil enzymes (β -glucosidase, β -D-celluliosidase, N-acetyl-b-glucosaminidase, phosphatase). Organic matter addition increased WSA and enzyme activities, as expected. In particular, *Plantago* or wheat straw addition increased WSA and enzyme activities by 224.77 or 281.65% and 298.51 or 55.45%, respectively. Microplastic fibers had no effect on WSA and enzyme activities in the soil without organic matter addition, but decreased WSA and enzyme activities by 26.20 or 37.57% and 23.85 or 26.11%, respectively, in the presence of *Plantago* or wheat straw. Our study shows that the effects of microplastic fibers on soil aggregation and enzyme activities are organic matter dependent. A possible reason is that *Plantago* and wheat straw addition stimulated soil aggregation to a greater degree, resulting in more newly formed aggregates containing microplastic, the incorporated microplastic fibers led to less stable aggregates, and decrease in enzyme activities. This highlights an important aspect of the context dependency of microplastic effects in soil and on soil health. Our results also suggest risks for soil stability associated with organic matter additions, such as is common in agroecosystems, when microplastics are present.

Keywords: microplastic, organic matter, soil aggregate stability, enzyme activity, soil structure, soil health, plastic pollution

INTRODUCTION

Microplastics as a group of anthropogenic contaminants are pervasive and persistent. Microplastic is widely studied in marine ecosystems (Eriksen et al., 2014; Bergmann et al., 2015; Jambeck et al., 2015), and only in recent years has attention shifted to terrestrial ecosystems (Rillig, 2012; Bläsing and Amelung, 2018; de Souza Machado et al., 2018a; Lozano and Rillig, 2020; Zhou et al., 2020). In fact, microplastic has been proposed as a new global change factor (Rillig and Lehmann, 2020). We adopted the definition that microplastics are plastics with size smaller than 5 mm (Moore, 2008; Barnes et al., 2009), with various shapes (e.g., fiber, fragment, film) and polymer types (e.g., polyester, polyethylene, polyacrylic, polypropylene), which are intentionally produced (e.g., microplastic beads in cosmetics) or fragmented into micro-sized plastics by natural or anthropogenic factors, such as photooxidation (Yakimets et al., 2004; Gewert et al., 2015), microbial degradation (Zettler et al., 2013; Moharir and Kumar, 2019) or plowing (Hann et al., 2016).

In microplastic polluted soil, microplastic fibers are a dominant shape (Singh et al., 2020; Zhou et al., 2020). Microplastic fibers derived from textiles are commonly discovered in wastewater (Pirc et al., 2016; Athey et al., 2020), they can span a length range of 0.3–25.0 mm. These microplastic fibers derived from textiles are therefore present in sludge and biosolids, which are applied on agricultural fields as fertilizer (Henry et al., 2019; Crossman et al., 2020; Zhang et al., 2020), likely leading to the accumulation of microplastic fibers in agricultural soils (Corradini et al., 2019; van den Berg et al., 2020). It is estimated that 1.56×10^{14} microplastic particles could enter the soil and other natural environments through sludge per year in China alone (Li et al., 2018). The majority of those fibers are made of polyester and polyacrylic. Moreover, atmospheric deposition of microplastic fibers is an important source of soil contamination, as hundreds of particles are deposited from the atmosphere per square meter per day (Cai et al., 2017; Dris et al., 2017; Brahney et al., 2020). Further anthropogenic activities, such as tillage, and also movement by soil animals can accelerate the incorporation of microplastic fibers into the soil (Huerta Lwanga et al., 2017; Rillig et al., 2017).

Microplastic fibers have been shown to influence soil quality and health by being detrimental to soil aggregate stability (de Souza Machado et al., 2018b; Lehmann et al., 2019a) and altering microbial activity (Liu et al., 2017; de Souza Machado et al., 2018b; Huang et al., 2019). Stability of soil aggregates, a fundamental soil physical property, is crucial to resist erosion, support water infiltration, water retention, aeration, and fertility (Bryan, 1968; Tisdall and Oades, 1982; Boix-Fayos et al., 2001; Li et al., 2016). Soil is a dynamic and complex system, and in particular the stability of soil aggregates is influenced by many factors including organic matter input, soil texture, clay mineralogy, and microbial populations (Seta and Karathanasis, 1996; Bossuyt et al., 2001; Wagner et al., 2007). Among these, organic matter is one of the most important factors determining aggregation (Abiven et al., 2008). The addition of organic matter promotes stable aggregation for example by stimulating microbial growth and metabolism, leading to increases in

microbially derived metabolites such as polysaccharides and proteins, which, together with plant-derived polysaccharides act as gluing agents that facilitate aggregate stabilization (Tisdall, 1994; Wright and Upadhyaya, 1998; Caesar-Tonthat, 2002). The degradability of organic matter/litter is an inherent property of the material, leading to different breakdown products being released. Therefore, the effects of organic matter on aggregation varied among different types of organic matter (Abiven et al., 2008). We have previously shown that under soil conditions favorable to the formation of aggregates, microplastic fiber addition could reduce the stability of aggregates (Liang et al., 2019), because newly formed aggregates are likely to have incorporated microplastic fibers which can reduce stability of aggregates, likely by introducing fracture points. Given that organic matter addition facilitates the formation of aggregates, we assume that microplastic fibers have a more detrimental effect on WSA when organic matter is added, and that the magnitude of the effect depends on the type of organic matter.

Soil enzyme activity is key to biological processes, driving nutrient cycles in terrestrial ecosystems. Enzyme activity as an important indicator of microbial activity is frequently altered by microplastics (de Souza Machado et al., 2018b; Liang et al., 2019). As microplastic fibers tend to reduce bulk density and increase soil porosity (de Souza Machado et al., 2018b; Zhang et al., 2019a), microplastic fibers are expected to improve aeration, and thus increase enzyme activity. Moreover, microplastics themselves as organic carbon might introduce an artificial carbon source (Rillig, 2018), possibly influencing enzyme activity by being a potential substrate. However, the overall outcome of the interactive effects of microplastic fibers and organic matter on aggregates and enzyme activity remains unknown, and is the subject of our investigation here.

We carried out a soil incubation experiment with all combinations of 3 types of microplastic fibers and 4 types of organic matter. We hypothesize: (1) soil aggregation will be decreased by microplastic fiber, but effects will depend on the type of organic matter; (2) microplastic fibers will increase soil enzymatic activities.

MATERIALS AND METHODS

Microplastic Fibers

Two polyester products (polyester1, PE1: Rope Paraloc 137 Mamutec polyester white, item number, 8,442,172, Hornbach.de, diameter: 0.03 mm, density: 1.45 g cm^{-3} ; polyester 2, PE2: Dolphin Fine $5 \times 100 \text{ g}$ Himalaya Knitting Wool, Baby Wool, 500 g Super Bulky Wool, diameter: 0.008 mm, density: 1.37 g cm^{-3}) and one polyacrylic, PA (100% acrylic “Bravo” yarn¹, diameter: 0.026 mm, density: 1.31 g cm^{-3}) product were used in this study (Supplementary Figure 1). These types of plastics are widely used in textiles (Carney Almroth et al., 2018), and have previously been shown to decrease soil aggregation (de Souza Machado et al., 2018b). We produced microplastic fibers by manually cutting them into fragments of approximately 5 mm in

¹schachenmayr.com

length, the average lengths of PE1, PE2, and PA are 4.56 ± 0.94 , 4.20 ± 1.37 , 4.05 ± 0.14 mm, respectively, the size distributions are given in **Supplementary Figure 2**. The estimated particle numbers of PE1, PE2, and PA are 193, 2.9×10^4 , 2.87×10^3 items g^{-1} dry soil, respectively. These lengths were chosen to match the criteria of microplastic upper limit and the general size range of secondary microplastic fibers produced by washing of clothes made from synthetic fibers (Pirc et al., 2016). We rinsed the fibers with tap water for 5 min to remove soluble chemicals, then dried them at 60°C for 24 h, and subsequently microwaved them for 3 min to reduce any microbial populations adhering to the material. The microwaving does not alter the physical appearance of the treated microplastic products (de Souza Machado et al., 2018b). The microplastic fibers were mixed into the soil at a concentration of 0.3% (w/w), toward the upper limit of concentrations used in a previous study (de Souza Machado et al., 2018b), such concentration was also applied in other study (Zhang et al., 2019b). The concentration 0.3% we used is within the contamination range in a plastic industrial area, the soil in which contained 0.03–6.7% of microplastic (Fuller and Gautam, 2016).

Organic Matter

We chose four different types of organic matter: *Medicago lupulina* leaves, *Plantago lanceolata* leaves, wheat straw (MultiFit, Item no.: 1,008,159, Krefeld, Germany), and hemp stems (REAL NATURE, Item no.: 1,259,176, Krefeld, Germany) (**Supplementary Figure 3**). *Medicago lupulina* and *Plantago lanceolata* leaf material were chosen as typical species from the local grassland, which were collected from plants previously grown in our greenhouse, wheat straw was chosen as the type of organic matter widely applied in agriculture for soil amendments, hemp was used to represent woody plant litter. *Medicago*, *Plantago*, straw and hemp formed a gradient of litter quality with C:N ratios as 12.85 ± 0.11 , 14.76 ± 0.29 , 133.03 ± 2.18 , $153.04 \pm 0.70\%$, respectively, the decomposition of *Medicago* was the fastest, followed by *Plantago*, and straw, with hemp being the slowest to decompose (**Supplementary Figure 4**). Before adding organic matter to our experiment, we ground the material using a blender (Philips Pro Blend 6 RD, Germany) and sieved to keep size between 0.5 and 2 mm. We added organic matter with a concentration of 0.8% (w/w) to our test systems. The concentration of 0.8% was below the saturation level of organic matter addition in our test soil (**Supplementary Figure 5**).

Experimental Design

This experiment was set up as a fully factorial design and contained all combinations of microplastic fibers [no microplastic fiber addition (No mf), PE1, PE2, PA] and organic matters [no organic matter addition (No OM), *Medicago*, *Plantago*, wheat straw, hemp stems], resulting in 20 treatments including the controls. Each treatment had 8 replicates for a total of 160 experimental units.

Soil Incubation

Fresh soil was collected from a local grassland (Berlin, Germany) with a sandy loam texture, an Albic Luvisol (Rillig et al., 2010).

Soil was sieved < 0.5 mm in order to reduce the amount of larger soil aggregates, thus intensifying the effect of organic matter addition on aggregate formation. Reducing aggregates beforehand is commonly used to measure macroaggregate formation in laboratory incubations (De Gryze et al., 2005). We mixed 20 g of the dry soil with 60 mg of microplastic fibers by steel spoon for 3 min, achieving a homogeneous distribution of fibers as no obvious fiber clusters could be observed by eye, then we mixed the prepared soil with 160 mg of organic matter of the different types for 30 s which is sufficient time to achieve an even distribution of organic matter. We applied the 3.5 min of mixing time to the soil without microplastic or organic matter addition aiming for the same level of disturbance. The soil mixture was transferred to a 50 ml falcon tube by using a steel spoon, the soil mixture was placed into the tube carefully in order to maintain the distribution of microplastic fiber in soil we achieved after mixing. Then we slowly wetted soil with distilled water by injecting water into soil by using a syringe, the water passively spread throughout our test system. We kept water content at 60% water holding capacity. Tubes were then closed with a hydrophobic vented cap to allow gas exchange. We centrifuged all tubes with soils at 100 rpm/s for 1 min to minimize any cracks in the soil (Brackin et al., 2013), and then incubated them at 25°C in the dark for 42 days, we assumed organic matter might achieve an intensive effect on aggregate stability after 42 days (Abiven et al., 2008).

Aggregate Stability Measurement

To measure soil aggregate stability, we followed the protocol by Kemper and Rosenau (2018): the air-dried soils were sieved through a 2 mm sieve and 4.0 g of soils were placed into sieves for capillary rewetting in deionized water for 5 min. We used 0.25 mm sieves to test the stability of the soil fraction > 0.25 mm (macroaggregates) against water as a disintegrating force. For the test, sieves carrying the soil samples were placed in a wet-sieving machine (Eijkelkamp, Netherlands) and moved vertically (stroke = 1.3 cm, 34 times min^{-1} for 3 min. The fractions left on the sieves were dried at 60°C for 24 h. After weighing the dry fractions, sand particles and organic debris larger than 0.25 mm were extracted from the fractions as coarse matter. The calculation of percent water-stable aggregates (WSA) was: $\% \text{ WSA} = (\text{water stable aggregates} - \text{coarse matter}) / (4.0 \text{ g} - \text{coarse matter})$.

Enzyme Activity Measurement

To assess the ability of the microbial community to acquire nutrients, we measured activities of β -glucosidase (cellulose degradation), β -D-celluliosidase (cellulose degradation), N-acetyl-b-glucosaminidase (chitin degradation), and phosphatase (organic phosphorus mineralization) (Delgado-Baquerizo et al., 2017). These enzymes are key for microbes to acquire C, N, and P, and are thus at least partially indicative of the function of the microbial community in terms of organic matter processing and decomposition (Waldrop et al., 2000). Soil enzymatic activity was determined using a high throughput microplate assay, according to Jackson et al. (2013). Activities of above enzymes were measured using p-nitrophenyl

(pNP)-linked model substrates: *p*NP- β -D-glucopyranoside (Sigma no. N7006), *p*NP- β -D-cellobioside (Sigma no. N5759), *p*NP-N-acetyl- β -D-glucosaminide (Sigma no. N9376), *p*NP-phosphate disodium salt hexahydrate (Sigma no. 71,768), respectively. Briefly, 3.5 g of frozen stored (-20°C) soil of each sample was placed in a sterile 50 ml centrifuge tube and mixed with 10 ml of 50 mM acetate buffer, and then the mixture was vortexed for 30 s to produce a soil slurry. After vortexing, the soil slurry was transferred to 96-well microplates. Reaction mixtures in each cell contained 0.150 mL soil slurry and 0.150 mL substrate dissolved in 50 mM sodium acetate buffer (pH 5.0). Reaction mixtures were incubated at 25°C for 2–4 h. After incubation, microplates were centrifuged at 3,000 rpm for 5 min, then 0.100 ml of suspension was transferred into a new microplate and mixed with 0.200 mL 0.1 M NaOH to stop the reaction. Absorbances were determined spectrophotometrically at 410 nm using a microplate reader (BioRad, Benchmark Plus, Japan). Enzyme activity was defined as the amount of released μmol of *p*-nitrophenol per gram of dry soil per hour.

Statistical Analysis

All statistics were conducted in R 3.5.3 (R Core Team, 2017). We analyzed the effect size of microplastic fibers on WSA and enzyme activity using the package “dabestr” (Ho et al., 2019) to generate unpaired mean differences and 95% confidence interval (CI) by a bootstrapping approach (5,000 iterations). This type of analysis estimates the magnitude and precision of an effect. To support our findings, we additionally applied two-way ANOVA by using generalized least square models in the “nlme” package (Pinheiro et al., 2020). The plots were created with the graphic package “ggplot2” (Wickham, 2016).

RESULTS

Stability of Soil Aggregates

All types of organic matter increased WSA substantially compared to the control, with wheat straw having the most positive effect on WSA (30.7% [95%-CI: 26–35.1]), followed by *Plantago* (24.4% [95%-CI: 20.6–29.1]), hemp stems (24.2% [95%-CI: 19–28.4]), and *Medicago* (16.4% [95%-CI: 11–22.8]).

The effects of microplastic fibers on WSA strongly depended on the type of added organic matter (Figure 1 and Table 1). Microplastic fibers had neutral effects on WSA in soil without organic matter addition and soil with *Medicago* addition. By contrast, all types of microplastic fibers exerted negative effects on WSA in soil with *Plantago* and wheat straw; PE2 had negative effects in soil with hemp stem. The most negative effect of microplastic fibers on WSA was found in soil with PE1 and *Plantago* (-13.3% , [95%-CI: -18.7 to -6.55]), followed by PE1 and wheat straw (-10.9% , [95%-CI: -16.2 to -6.43]). Generally, microplastic fibers were more detrimental for WSA in soils with added organic matter, while organic matter addition was more beneficial to WSA (Figure 2).

Enzyme Activities

Organic matter addition stimulated enzyme activities, while microplastic fibers decreased enzyme activities in some cases. The effects of microplastic fibers on enzyme activities were neutral in soil without organic matter addition, while with organic matter addition, negative effects of microplastic fibers appeared (Figure 3 and Table 1). We found the highest loss of β -Glucosidase activity in soil with PE1 and *Plantago* ($-1.64 \mu\text{mol pNP g}^{-1} \text{h}^{-1}$ [95%-CI: -0.03 to -3.27]), cellobiohydrolase activity in soil with PE2 and hemp stem ($-0.19 \mu\text{mol pNP g}^{-1} \text{h}^{-1}$ [95%-CI: -0.07 to -0.35]), phosphatase in soil with PE2 and wheat straw ($-1.22 \mu\text{mol pNP g}^{-1} \text{h}^{-1}$ [95%-CI: -0.47 to -1.96]), N-acetyl-glucosaminidase in soil with PA and wheat straw ($-0.65 \mu\text{mol pNP g}^{-1} \text{h}^{-1}$ [95%-CI: -0.07 to -1.24]).

We found a similar relationship between effects of microplastic fibers in the presence of different types of organic matter and effects of organic matter on β -Glucosidase activity as we found in WSA; meaning, the more positive an effect a specific organic material, the more detrimental was the impact of added microplastic fibers (Figure 4).

DISCUSSION

Effects of Microplastic Fibers on the Stability of Soil Aggregates (WSA)

As we hypothesized, microplastic fibers reduced the stability of aggregates in the presence of specific types of organic matter, with the magnitude of the microplastic fiber effects dependent on the type of organic matter. This was likely because the addition and subsequent microbial processing of organic matter accelerated aggregation, which in turn led to more microplastic fibers being incorporated into newly formed aggregates. The concentration of microplastic fibers in aggregates was previously observed to have increased with the addition of one type of organic matter addition (Zhang and Zhang, 2020). In addition, the increased incorporation of microplastic fibers into soil aggregates might produce fracture points, therefore reducing the stability of the coarse, sandy soil that we used here. Given that the effects of organic matter on aggregation varied among different types of organic matter, the amount of increased incorporation of fibers into aggregates might depend on the type of added organic matter; this helps explain the phenomenon that microplastic fibers generally exerted more negative effects on WSA in soil to which a type of organic matter was added that favored soil aggregation.

In our study, microplastic fibers had no effects on WSA in soil without added organic matter. The sandy loam soil we used had limited intrinsic potential to form aggregates, thus there must have been only relatively few microplastic fibers that were incorporated into aggregates, resulting in no effects of microplastic fibers in soil without added organic matter. We assumed organic matter stimulated microbial activity, accelerating the formation of aggregates and the integration of fibers into those aggregates. A similar result was found in a previous study (Lehmann et al., 2019b): microplastic fiber had

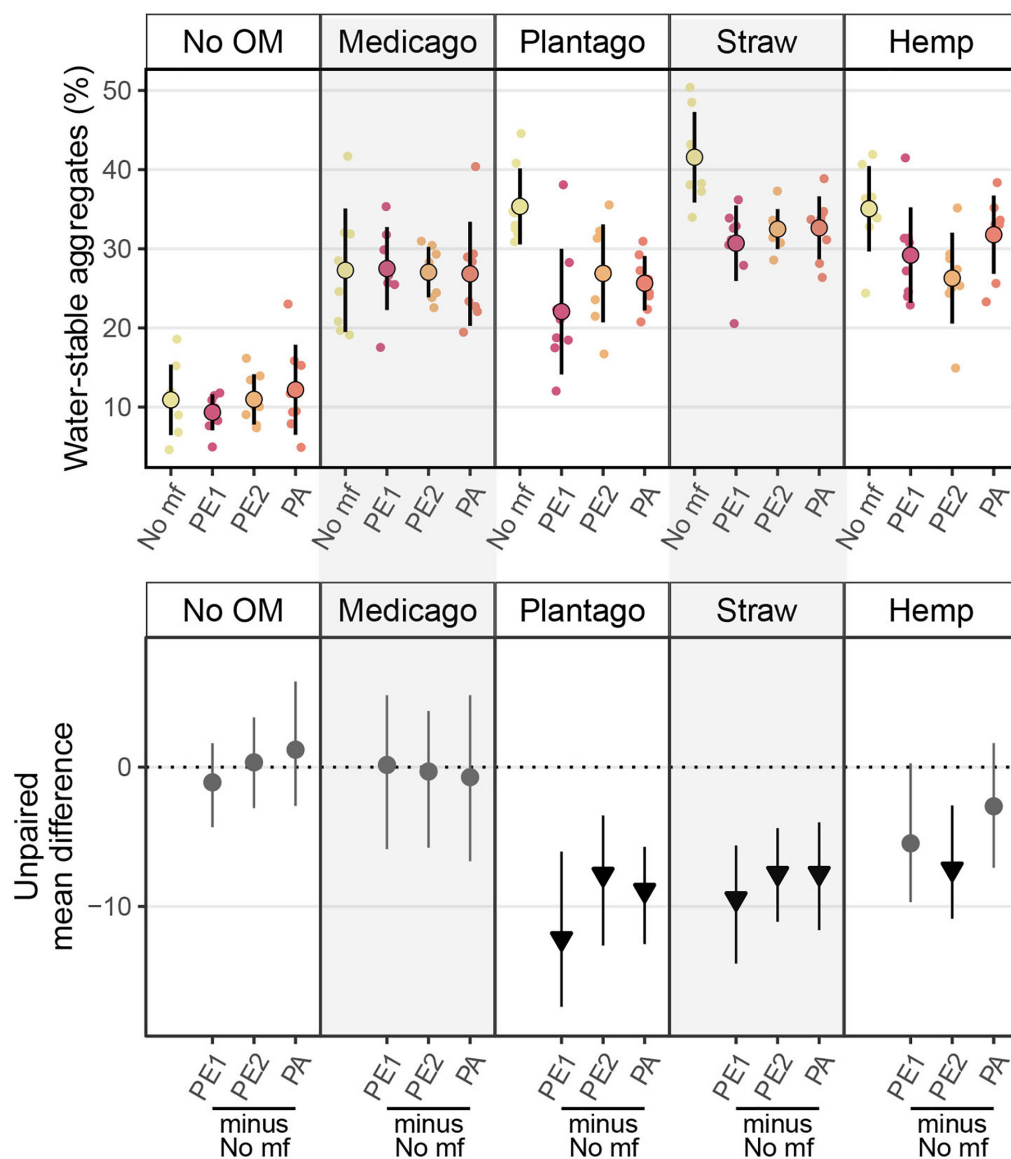


FIGURE 1 | Effects of microplastic fibers on soil water stable aggregates (WSA in %) with the addition of different types of organic matter. The upper panel shows the raw data of water stable aggregates, data distributions are aligned with corresponding mean and standard deviation ($n = 8$ for each treatment). The lower panel shows the unpaired mean differences of the microplastic fiber addition and control under different organic matter addition. Circles and triangles represent the effect size mean (unpaired mean; effect magnitude) and the vertical lines the corresponding confidence intervals (effect precision). Negative (arrow head down) effect sizes and corresponding CIs of treatment compared to control are depicted in black while neutral effects (circle) are colored in gray; neutral effects occur when the CIs overlap the dashed zero line (line of no effect). “No OM” represents no organic matter addition, “No mf” represents no microplastic fiber addition.

TABLE 1 | ANOVA results for the effects of organic matter, microplastic fibers, and the interaction of these factors on the stability of soil aggregates (WSA), β -Glucosidase (BG) activity, cellobiohydrolase (CB) activity, phosphatase (Phos) activity, and N-acetyl-glucosaminidase (NAG) activity.

Treatment	DF	WSA		BG activity		CB activity		Phos activity		NAG activity	
		<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Organic matter	4	141.29	<0.0001	74.83	<0.0001	95.98	<0.0001	71.84	<0.0001	584.18	<0.0001
Microplastic fiber	3	11.38	<0.0001	4.78	<0.01	3.20	<0.05	2.88	<0.05	1.82	0.15
Microplastic fiber: Organic matter	12	2.55	<0.01	0.61	0.83	0.93	0.52	0.60	0.84	0.98	0.47

$p < 0.05$ was considered significant and marked in bold. Degrees of freedom (DF), *F*- and *p*-value for each variable are presented.

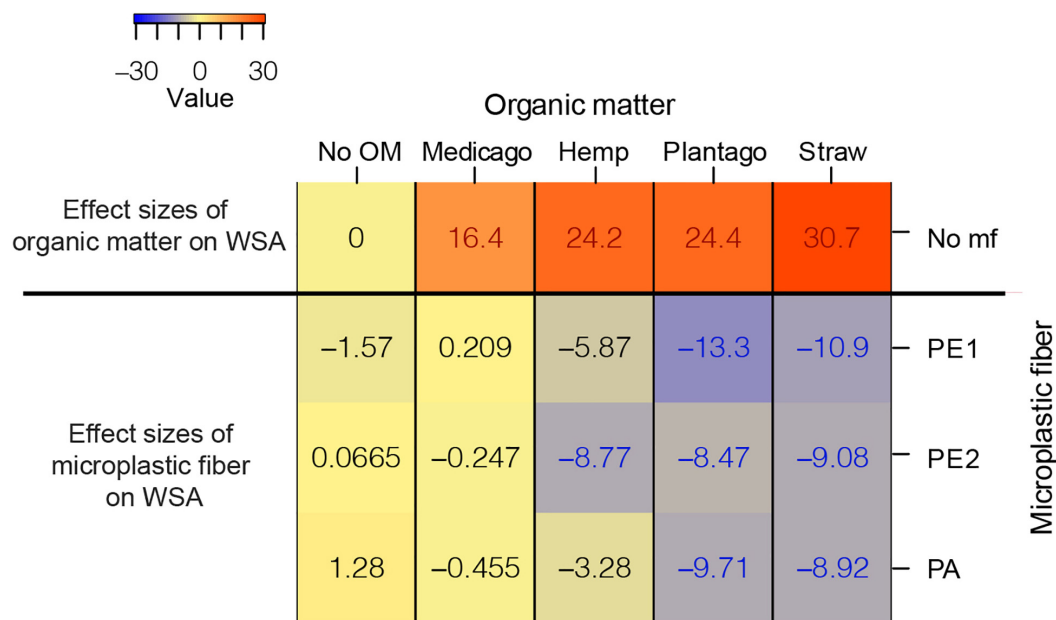


FIGURE 2 | The heatmap of the effect sizes of microplastic fiber and organic matter on WSA. The effect sizes of organic matter on WSA were the unpaired mean differences of organic matter addition alone and control ($WSA_{\text{organic matter}} - WSA_{\text{No OM}}$). The effect sizes of microplastic fiber are the unpaired mean differences of the microplastic fiber addition and control with the addition of the different types of organic matter. ($WSA_{\text{organic matter_microplastic fiber}} - WSA_{\text{organic matter_Norm}}$) The blue text represents the statistically significant negative effect of microplastic fiber on WSA, the red text represents the statistically significant positive effect on WSA. “No OM” represents no organic matter addition, “No mf” represents no microplastic fiber addition.

no effects on aggregate stability in sterile soil, while decreased aggregate stability in soil with a microbial community, indicating that microorganisms mediate effects of microplastic fibers on soil aggregate stability (Forster, 1990; Lehmann et al., 2017). A contrary result was found in a study using clayey non-sterile soil, in which polyester fiber increased macroaggregates (Zhang et al., 2019b). Soil mineralogy and clay content play important roles in the formation and stability of aggregates (Seta and Karathanasis, 1996), microplastic fibers may have different effects on soil aggregates in soils with different textures. Further studies are needed to explore the interaction between microplastic and soil minerals.

Our WSA results also reveal that predictions based on just the type of microplastic fibers alone would have failed: effects are dependent on the type of organic matter available for microbial processing—and thus on the rate of soil aggregate formation, and as such our study has uncovered an important aspect of context dependency of microplastic effects in soils. However, there is a limitation to our study. The temporal effects of organic matter input on soil aggregation are not covered in our study design. *Medicago* had the lowest C:N ratio, thus had the fastest decomposition rate. Therefore, we expected the greatest increase of WSA in soil amended with *Medicago* alone, and also the greatest loss in WSA caused by microplastic fibers in soil amended with *Medicago*. Nevertheless, neither was observed in our study. We assumed the *Medicago* as a rapidly degraded organic matter exhibited the maximum aggregate stability before we harvested the experiment, then the aggregate began to break down as the binding agents were

decomposed (Abiven et al., 2008). Our findings could be relevant for agroecosystems: wheat straw is widely used as an organic amendment in agriculture due to the benefits it offers, such as increasing sequestration of soil organic carbon and improving soil structure (Han et al., 2018; Zhao et al., 2019). However, as agricultural fields are prone to microplastic contamination, our study suggests that this can lead to unforeseen effects of straw additions on soil properties; an assertion that should now be explicitly tested in the field.

Effects of Microplastic Fibers on Enzymatic Activities

Contrary to our hypothesis, microplastic fibers did not increase enzyme activities, but had even negative effects on enzyme activities with the addition of specific types of organic matter. The results depended on the microplastic type, but the pattern was not consistent across the organic matter treatments. PE2 decreased enzyme activities more frequently than PE1 and PAN, which might be attributed to the highest particle numbers of PE2 in each experimental unit. Moreover, those negative effects were more likely to appear in the presence of straw. This again emphasizes the context dependency of microplastic impacts on the soil environment. Such negative effects of microplastics are also found in other studies, as microplastics reduce soil nutrient levels (Yu et al., 2020). We do not know what caused this negative response in our study, but microplastic fibers might have caused shifts in the microbial community via changing soil physical properties (de Souza Machado et al., 2018b), releasing

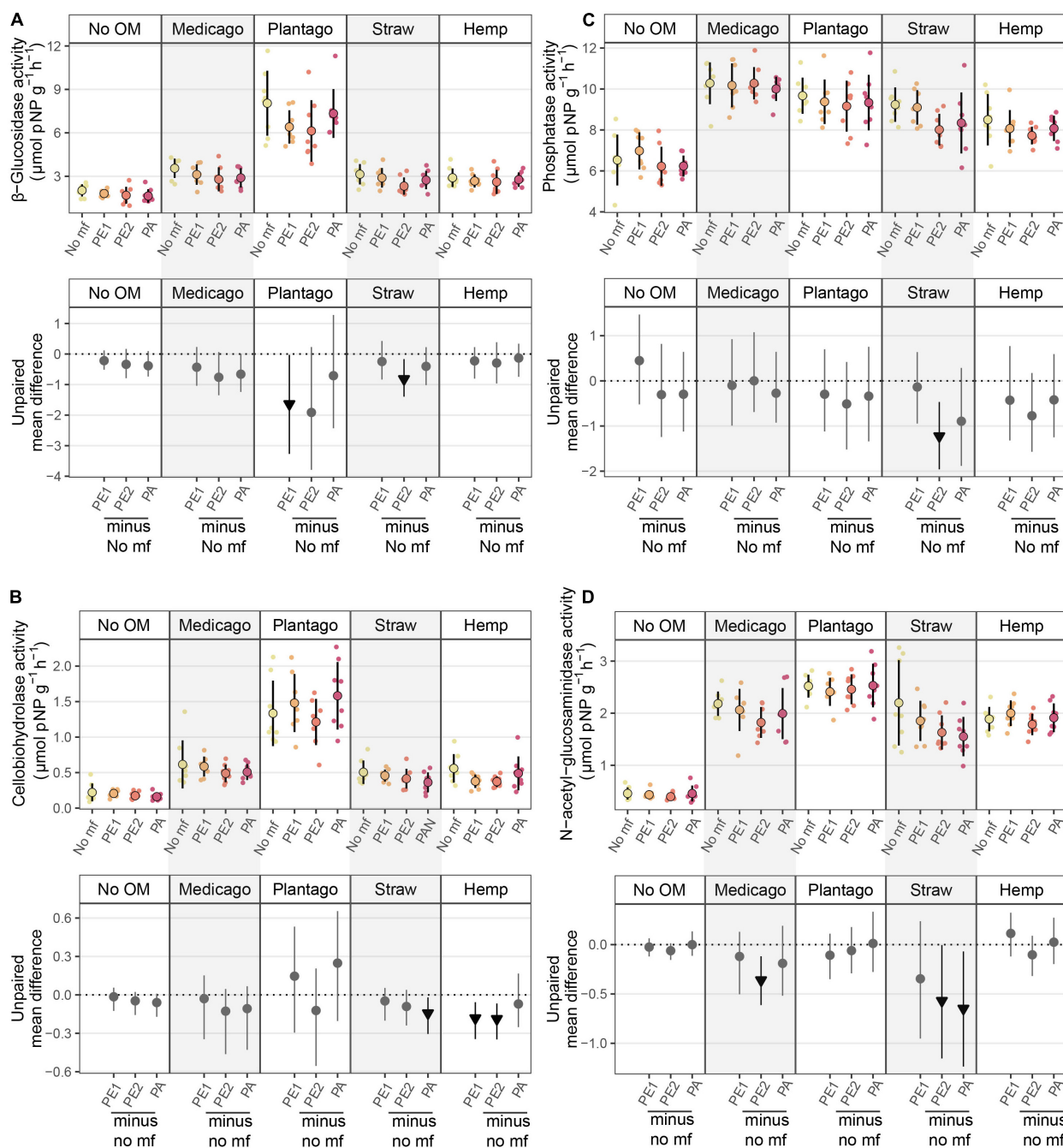


FIGURE 3 | Effects of microplastic fibers on β -Glucosidase activity (A), cellobiohydrolase activity (B), phosphatase activity (C), and N-acetyl-glucosaminidase activity (D) in the presence of different types of organic matter. The upper panel shows the raw data of enzyme activity, data distributions are aligned with corresponding mean and standard deviation ($n = 8$ for each treatment). The lower panel shows the unpaired mean differences of the microplastic fiber addition and control with different types of organic matter addition. Circles and triangles represent the effect size mean (unpaired mean; effect magnitude) and the vertical lines the corresponding confidence intervals (effect precision). Negative (arrow head down) effect sizes and corresponding CIs of treatment compared to control are depicted in black while neutral effects (circle) are shown in gray; neutral effects occur when the CIs overlap the dashed zero line (line of no effect). "No OM" represents no organic matter addition, "No mf" represents no microplastic fiber addition.

additives (Widén et al., 2004; Hahladakis et al., 2018) that were not water-soluble (fibers were washed before being mixed in the soil) that migrated into the soil (Kim et al., 2020), or serving as microbial habitats (Huang et al., 2019; Zhang et al., 2019c); the

enzymatic activities could have changed as a consequence of such community shifts.

Microplastic fibers had negative effects on enzyme activities in the presence of organic matter, which could lead to decreased

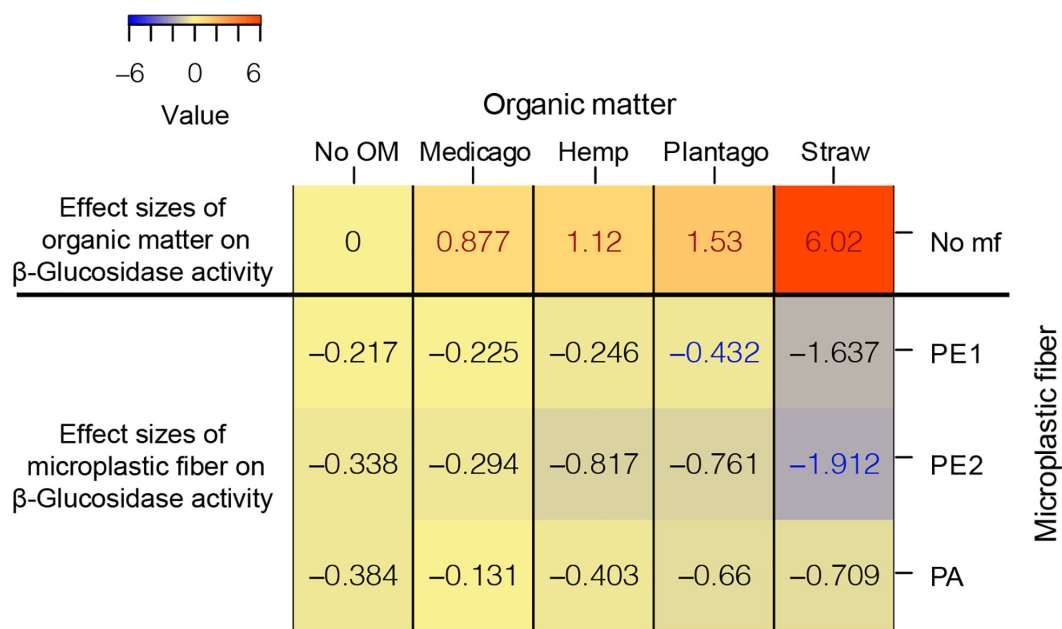


FIGURE 4 | The heatmap of the effect sizes of microplastic fiber and organic matter on β -Glucosidase activity. The effect sizes of organic matter on β -Glucosidase activity were the unpaired mean differences of organic matter addition alone and control (β -Glucosidase activity_{organic matter} – β -Glucosidase activity_{No OM}). The effect sizes of microplastic fiber are the unpaired mean differences of the microplastic fiber addition and control with the addition of the different types of organic matter (β -Glucosidase activity_{organic matter_microplastic fiber} – β -Glucosidase activity_{organic matter_No mf}). The blue text represents the statistically significant negative effect of microplastic fiber on β -Glucosidase activity, the red text represents the statistically significant positive effect on β -Glucosidase activity. "No OM" represents no organic matter addition, "No mf" represents no microplastic fiber addition.

nutrient uptake into microbes and mineralization rates. The restricted nutrient transfer from organic matter to microbes could change the microbial community in the long term, potentially resulting in altered soil functions and processes (Waldrop et al., 2000). Additionally, assuming an accumulation of microplastic fibers in the future, decreased enzyme activities could lead to a reduction of plant available nutrients and thus ultimately diminish agricultural productivity or crop quality (Paz-Ferreiro et al., 2014).

Moreover, the decreased nutrient uptake could lead to decreased microbial biomass and metabolites, which are important for accumulating stabilized C in soil (Cotrufo et al., 2013; Cenini et al., 2015). Nevertheless, decreased enzyme activities accompanied by decreased transformation of organic matter could also lead to less C loss, the eventual fate of added C could not be predicted from our study due to the scope of our work here. Further studies should target long-term C dynamics of added organic matter, to enable prediction of the potential effects of microplastic fibers on C sequestration.

Though soils received different types of microplastic fibers, which varied in diameters, particle numbers per experimental unit, and probably varied in their additives, we did not find a consistent pattern of the different microplastic fibers across our response variables. In terms of WSA, as a previous study found microplastic fibers decreased WSA with increasing concentrations of microplastic fibers (de Souza Machado et al., 2018b), we assume the decreases in WSA already reached the

upper limit response of soil to microplastic fibers in our study, resulting in no obvious different effects on WSA among the types of microplastic fibers.

In terms of enzyme activities, we found the decrease in enzyme activities appeared randomly among microplastic fibers. We assumed microplastics needed a longer time to reveal their effects on the microbial community and thus on microbial function, such as accumulation of additives released from aged plastic over time (Bandow et al., 2017).

Microplastic fibers we produced had higher average length than that microplastic fibers found in the environment, with most studies finding fibers of shorter than 1 mm (Cai et al., 2017; Zhou et al., 2018; Corradini et al., 2019). For our experimental work, it was impractical to manually cut fibers that small (but see Schmiedgruber et al., 2019; Frehland et al., 2020).

CONCLUSION

In our study, microplastic fibers affected soil aggregation by interfering with the formation of stable aggregates, with effects dependent on the type of added organic matter. It seems that greater soil aggregation activity leads to increased opportunities for microplastic to interact with this biological process; this is very likely due to microplastic fibers becoming integrated into aggregates to an increasing degree, leading to subsequent destabilization of these structures by as yet unknown mechanisms. Effects of microplastic additions on soil processes

have been variable, in part likely due to the plastic material itself, but our study points to soil properties, in particular soil organic matter, as another important variable contributing to the context dependency of such effects in terrestrial ecosystems.

DATA AVAILABILITY STATEMENT

All data for this paper have been deposited under <https://doi.org/10.6084/m9.figshare.14270807.v3>.

AUTHOR CONTRIBUTIONS

YL and EL designed the research. YL performed the research, and conceived and performed the data analyses. YL, AL, GY, EL, and MR wrote the manuscript and contributed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Projection of the Precipitation Extremes in Thailand Under Climate Change Scenario RCP8.5

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This study explores the predicted extreme precipitation during 2020–2029 in Thailand, using the output of the nested regional climate model (NRCM) with the Representative Concentration Pathway (RCP) 8.5 emission scenario. The research used five different extreme precipitation indices, i.e., annual precipitation total (PRCPTOT), the annual number of rainy days (RD), simple daily intensity index (SDII), consecutive dry days (CDDs), and consecutive wet days (CWD). The PRCPTOT was generally located in eastern Thailand, with significant declining trends, while the increasing trend was found in northern Thailand. The quantity of the PRCPTOT varies marginally from 100 to 200 mm per decade. The annual RD was influenced mostly by the negative trends in Thailand. A remarkable trend is a rising of annual SDII, with major statistical increases ranging from 5 to 20%. A widespread decline in CWD was found in most regions. The statistically significant pattern of CWD has been geographically concentrated in the northern, southern, and eastern regions of Thailand. Southern Thailand continues to decline by –10 to –30%. A drastic decline has been observed in the south of Thailand. Projected interannual precipitation variability shows that December–January–February (DJF) variability will be greater than either annual or June–July–August (JJA) variability in most years, with the exception of 2022, 2025, and 2026, in which the JJA variability will be greater than both the annual variability and the DJF variability.

Keywords: precipitation, extreme events, climate change, Thailand, climate extreme

INTRODUCTION

Precipitation is an important factor in the hydrological cycle. It is one of the most important variables affecting life and human well-being because precipitation change can cause consequences to both the environment and the human society (Pielke and Downton, 2000; Trenberth et al., 2003; Barretta and Santos, 2014; Guan et al., 2014). During the last decades, it has become apparent that, during anthropogenic climate warming, the intensity and the frequency of extreme precipitation events increase, consecutively (Allan and Soden, 2008; Min et al., 2011; Coumou and Rahmstorf, 2012; Donat et al., 2013). Recently, the research focusing on extreme precipitation events has increased in the tropics and subtropics. For example, Endo et al. (2009) expressed that rainfall severity has increased over time. Goswami et al. found that, in the monsoon seasons from 1951 to 2000, the intensity and strength of the extremes in the precipitation events in India increased. An

analysis of daily rainfall data across the Philippines between 1951 and 2010 demonstrated that the frequency and the severity of the extreme rainfall events substantially increased (Cinco et al., 2014). Also, the information from regional workshops shows that the patterns in precipitation extremes vary regionally and are not uniform across Southeast Asia to Arab regions (Choi et al., 2009; Caesar et al., 2011; Donat et al., 2014).

In Southeast Asia, the future of the precipitation extremes was predicted using the output from CORDEX-SEA simulation with 25-km grid spacing under emission scenarios of RCP4.5 and 8.5 at the end of the twenty-first century. Their results indicate that changes in extreme indices are more pronounced and cover a wider area under RCP8.5 than RCP4.5, with the most significant change in consecutive dry days (CDD) (Tangang et al., 2020). However, in order to support decision-making in relation to agriculture, water resources, and disasters in the near future, the study of precipitation extremes, using the fine resolution of climate change output, is required. In this study, we used the output from the simulation of the nested regional climate model (NRCM) with 10-km grid spacing under climate change scenario RCP8.5 for input data in the precipitation extreme indices, including annual precipitation total (PRCPTOT), the annual number of rainy days (RD), simple daily intensity index (SDII), consecutive dry days (CWD), and CDD, for studying the precipitation extremes in Thailand during the period 2020–2029.

MATERIALS AND METHODS

To analyze the precipitation extreme, the output from the simulation of the NRCM was used as input data in five extreme precipitation indices that were suggested by the World Meteorological Organization (WMO)–Commission for Climatology (WMO–CCI), the World Climate Research Programme (WCRP), The Climate and Ocean: Variability, Predictability and Change (CLIVAR), and The Expert Team on Climate Change Detection and Indices (ETCCDI) projects, namely, annual PRCPTOT, the annual RDs, SDII, CDD, and CWD (Zhang et al., 2011).

General Information of Nested Regional Climate Model Configurations

The data used in the simulation of the NRCM are based on Amnuaylojaroen and Chanvichit, 2019 and Amnuaylojaroen et al., 2021. The output from the NRCM, with resolution of 10-km grid spacing under the RCP8.5 scenario, was used for both time periods of 1990–1999 and 2020–2029. The NRCM is based

on the Weather Research and Forecasting (WRF) model and has been forced by the Community Climate System Model version 4 (CCSM4) for meteorological initial and boundary conditions (Gent et al., 2011; Done et al., 2013; Bruyère et al., 2017). In the model configuration, the interaction between land and ocean estimating oceanic parameters using the Price–Weller–Pinkel (PWP) model (Price et al., 1986). The PWP model is an oceanic mixing layer model that accounts for the oceanic mixing layer convection change and oceanic shear stability. It was created in the HYbrid Coordinate Ocean Model (HYCOM) (Chassignet et al., 2007) model for vertical mixing. Other model configurations were set up similar to the one set up by Amnuaylojaroen et al. (2018). The feedback and the evolution of the aerosol in the atmosphere were carried out through the Rapid Radiative Transfer Model for General Circulation (RRTMG). In this model, feedback is taken from aerosols on meteorological processes under the Thompson scheme (Thompson et al., 2004). Grell-3 and the Noah Land Surface Model were responsible for sub-grid scale calculation and the interaction between land and atmosphere in the model (Chen and Dudhia, 2001). Grid nudging was also applied precisely to provide large-scale meteorology (Stauffer and Seaman, 1990).

Performance of Nested Regional Climate Model

The good performance of the NRCM was shown by statistical analysis, i.e., index of agreement (IOA), mean bias (MB), SD, and R-square (R^2), in a study by Amnuaylojaroen and Chanvichit (2019). The statistical calculations between the model data and the observed data (TMD, GPCP, and APHRODITE) are averaged over 1990–1999 from 44 station locations of TMD annually and for the rainy season and the dry season, as shown in **Table 1**. The model has a high ability to capture precipitation that is indicated by R-squared values, ranging from 0.94 to 0.97. Similar to R-squared, IOA values were in the range of 0.60–0.68 in the annual precipitation. In the dry season and in the rainy season, the modeled precipitation was underestimated as compared with observations with an MB ranging from –3 to –2 mm. The SD of the residuals of the model is 2–3.

RESULTS AND DISCUSSION

The results of spatial distribution of the yearly PRCPTOT showed that there were both wetting and drying patterns in Thailand during 2020–2029 (**Figure 1A**). The quantity of PRCPTOT slightly changes in the ranges of 100–200 mm per decade, as

TABLE 1 | Statistics comparing the modeled precipitation and observation [adapted from Amnuaylojaroen and Chanvichit (2019)].

Data	R^2			Mean bias			SD			IOA		
	Annual	Rainy	Dry	Annual	Rainy	Dry	Annual	Rainy	Dry	Annual	Rainy	Dry
APHODITE	0.95	0.92	0.97	–1.96	–2.62	–1.30	2.00	2.09	1.40	0.68	0.67	0.92
TMD	0.97	0.96	0.98	–2.68	–3.56	–1.80	2.54	2.76	1.69	0.63	0.50	0.84
GPCP	0.94	0.89	0.99	–3.51	–4.73	–2.27	3.06	3.41	1.85	0.60	0.36	0.77

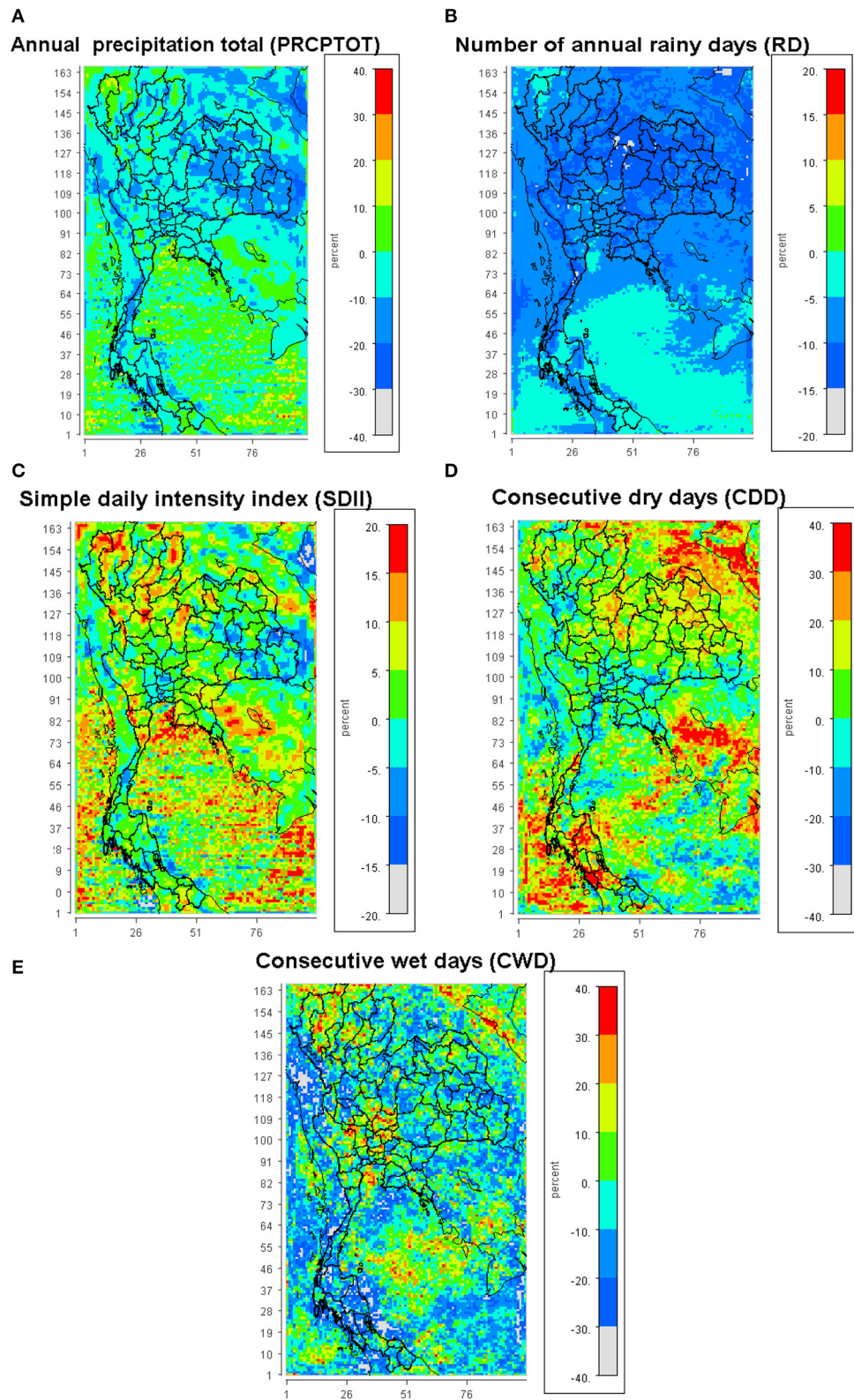
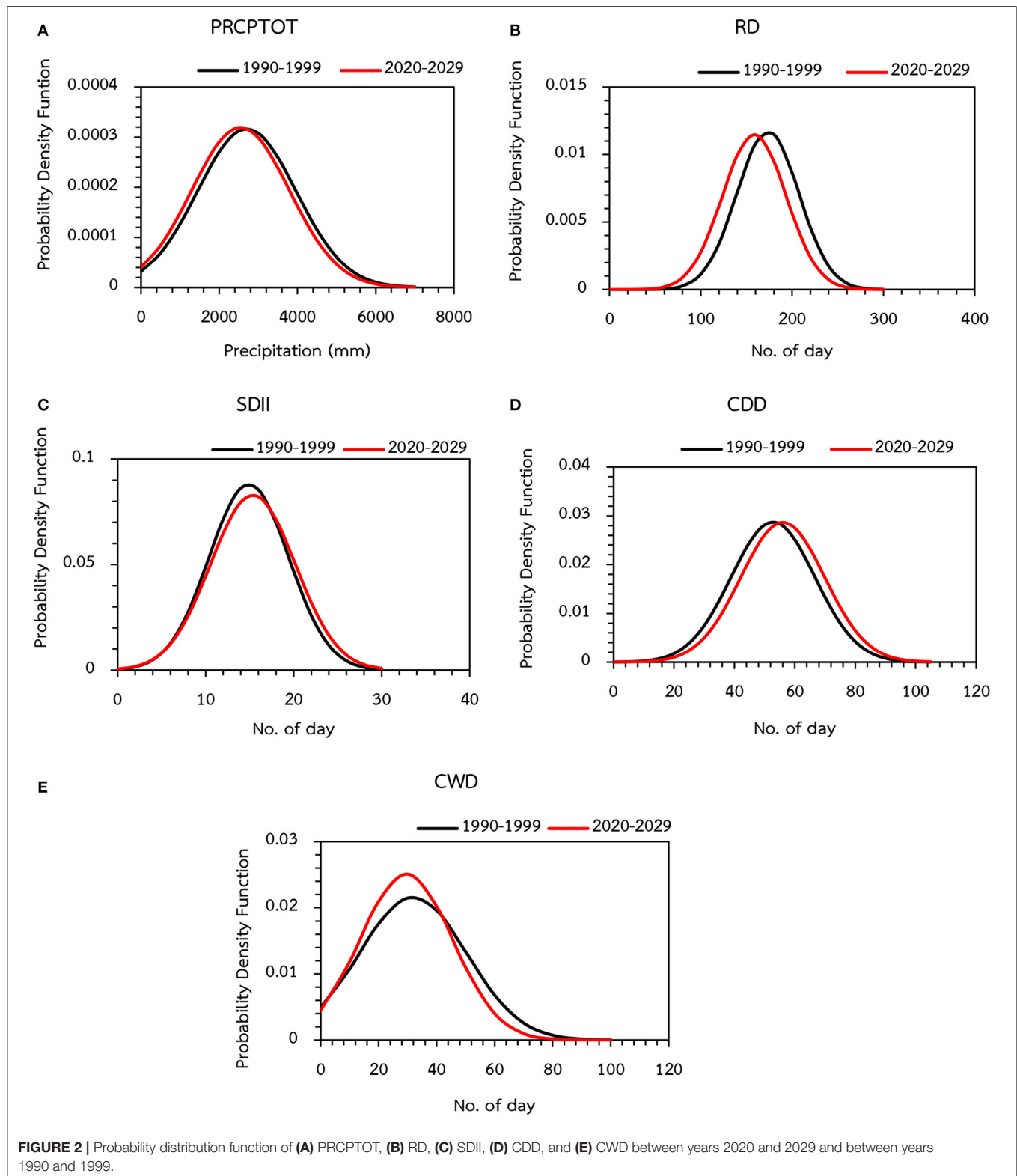
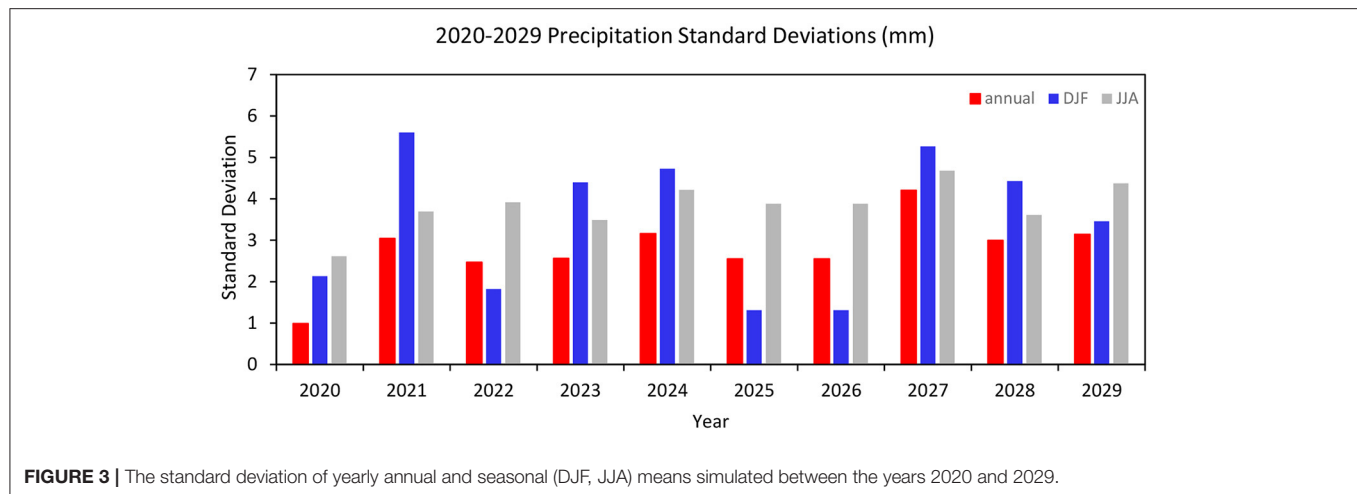


FIGURE 1 | Spatial distribution of percentage differences in (A) PRCPTOT, (B) RD, (C) SDII, (D) CDD, and (E) CWD between years 2020 and 2029 and between years 1990 and 1999 in Thailand.



shown in **Figure 2A**. The annual RD (**Figure 1B**) affected most of Thailand with negative patterns. A remarkable pattern that is noticeable in **Figure 1C** is the domination of increasing annual

SDII in Thailand, with significant statistical rises, ranging from 5 to 20%. The conclusion of this study reasonably indicates that the substantial rise in SDII across most of Thailand



represents a decrease in RD instead of an increase in the overall rainfall. Indices of extreme precipitation events were analyzed, using CWD throughout Thailand. The analysis of indices indicates a widespread reduction of CWD in most areas. In the central, southern, and eastern regions of the country, the statistically significant trend of CWD was geographically localized. Southern Thailand continues to decrease by -10 to -30% . A dramatic decrease was found in the south of Thailand. Compared to CWD, CDD normally displayed the opposite signal (**Figures 1D,E**). The CDD showed a general drying trend, with statistically significant trends in two areas, including the eastern and southern regions of Thailand. Although the spatial distribution of increasing CDD pattern was robust in most of the country, no statistically significant regional relation was found. During the years 2020–2029, the plot of the probability density function of five indices (**Figures 2A–E**) will, in general, not be significantly different compared to the present day (1990–1999). For example, PRCPTOT, SDII, and CDD had smaller change; in contrast, there were some indices, such as RD (**Figure 2B**) and CWD (**Figure 2E**), that showed great difference compared to the present-day simulation. In **Figure 2B**, the substantial decreasing trends in annual RD varied from 10 to 20 days per decade. The annual RD decreased dramatically by 5–15% when considering the entirety of Thailand, while the CWD generally decreased in ranges of 2–4 days per decade.

The ability of a regional climate model to capture realistic interannual variability in the future is shown in **Figure 3**. We use the SD of yearly annual and seasonal (DJF, JJA) means simulated between the years 2020 and 2029 to show the magnitude of the interannual variability of precipitation of the model. Using the year 2020 as a baseline, we found that only the year 2029 is adequate for capturing the relative magnitude of annual, DJF, and JJA variability. The DJF variability is greater than either the annual or JJA variability in the years 2021, 2023, 2024, 2027, and 2028; whereas, the JJA variability is greater than both the annual variability and the DJF variability in the years 2022, 2025, and 2026.

Mostly, the result of this study was similar to those of the previous studies in the other regions of Asia (Choi et al., 2009;

Endo et al., 2009; Caesar et al., 2011; Limsakul and Singhruck, 2016). However, compared to the previous regional studies, this study, which is based on the regional climate model, could bring some difference. For example, by comparing the ensemble mean of projected change by the regional climate model, with 25-km grid spacing in Southeast Asia (Supari et al., 2020), their results showed decreasing the PRCPTOT in northern Thailand, but the PRCTOT was generally located in eastern Thailand, with substantial decreasing patterns, while the rising pattern was in northern Thailand in this study. In general, increasing resolution may better capture small-scale processes and features (downscaling effect), but increased GCM resolution may also improve the representation of large-scale atmospheric circulation (upscaling effect) (Iles et al., 2020). Particularly, precipitation extremes are sensitive to resolution over complex orography. In addition, some studies suggest that country-scale higher-resolution precipitation datasets still give heavier precipitation extremes, which may agree best with the 0.11° simulations. For example, Prein et al. (2016) and Torma et al. (2015) found that various aspects (biases, frequency–intensity distributions, spatial patterns) of the mean and extreme precipitation improved in EURO668 CORDEX at 0.11° compared with at 0.44° . Prein et al. (2016) ascribe this mostly to the better representation of orography at higher resolution and the ability to capture the larger scales of convection.

As mentioned in the previous research, (i.e., Caesar et al., 2011; Nguyen et al., 2014; Villafuerte et al., 2014), the El Niño–Southern Oscillation (ENSO) has a strong impact on the year-to-year precipitation variability in Southeast Asia. In addition, Limsakul and Singhruck discussed that the La Niña and El Niño years have more precipitation and less extreme events, and *vice versa*, during the negative years of Pacific Decadal Oscillation (PDO) in Thailand. It is, primarily, warming of the Walker circulation in the eastern Pacific Ocean that has been suggested to influence the moisture and the cell movement in the Indo-Pacific region, hence the incidence of El Niño (Juneng and Tangang, 2005; Singhrattana et al., 2005; Nguyen et al., 2014). The ENSO events were recorded to disrupt the monsoon vertical atmospheric circulation in Thailand and caused decreased

precipitation (Wang et al., 2003; Juneng and Tangang, 2005; Singhrattana et al., 2005). A rise in the formation of tropical cyclones may well be connected to increases in the number of days with heavy precipitation in the area, which is a potential factor in the shift in storm tracks in the east Indian Ocean, and maybe a factor contributing to the increased number of days with tropical cyclones (Caesar et al., 2011). Krishnamurthy and Krishnamurthy (2014) found that the PDO phase is essential for the Walker circulation patterns in the Pacific and Indian Oceans. One of the mechanisms by which PDO influences the monsoon rains is the Hadley circulation in the monsoon region. Changes in the Walker circulation over the Pacific and Indian Oceans also follow the PDO. The PDO, which causes the monsoon rainfall, is being linked to the Hadley circulation; hence, the mechanism by which it impacts the monsoon rainfall is limited to the monsoon region (Krishnamurthy and Krishnamurthy, 2014). However, the limitation of this study is that it is not represented well in terms of climate change. With a 10-year integration period, it is difficult to express the interannual variability, but it is noteworthy that people can do adaptation in the future. To extend the knowledge and better expression of the precipitation variability in Thailand and Southeast Asia, further study of the mechanisms underlying the ENSO and PDO linkage is required. Moreover, climate adaptation, a mitigation plan in the future, a regional climate simulation, and a long-term precipitation-variation analysis should be performed in Thailand.

CONCLUSION

This study predicts the future extreme precipitation in Thailand during the years 2020 and 2029 relative to the reference period 1990–1999 under the RCP8.5 scenario. The output from the NRCM was used as input into five indices, i.e., PRCPTOT, RD, SDII, CWD, and CDD. The evaluation of the model,

with ground-based measurement and APHRODTE and GCPD data, represents well the precipitation in Thailand. The analysis of the indices expressed that precipitation is becoming less frequent in the entirety of Thailand, and the frequency of the CWD has decreased significantly; precipitation has also become more intensive. The indexes calculating the amount of heavy precipitation caused by wet daytime events and the maximum 1-day precipitation showed a negative trend in wetter conditions. The annual PRCPTOT, RD, SDII, and CWD illustrated spatially consistent patterns with important trends in the regional average annual series.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

TA: conceived and designed the experiments, performed the experiments, analyzed and interpreted the data, contributed reagents, materials, analysis tools, or data, and wrote the paper.

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Assessing the Moisture Transports Associated With Nocturnal Low-Level Jets in Continental South America

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Given the crucial role of low-level circulation in convective events, this study presents a climatological characterization of the moisture sources and sinks associated with the occurrence of nocturnal low-level jets (NLLJs) in South America. Six selected NLLJ cores are identified according to the jet index that considers a vertical wind speed shear of the lower troposphere at 00:00 local time (LT). The Lagrangian FLEXible PARTicle (FLEXPART) model was used to provide the outputs for tracking atmospheric air masses to determine the moisture sources and sinks for the NLLJ cores (Argentina, Venezuela, and the regions of Brazil: south—Brazil-S, southeast—Brazil-SE, north—Brazil-N, and northeast—Brazil-NE). The analysis is based on 37 years (1980–2016) of the ERA-Interim reanalysis. We found that the NLLJ index is stronger in the warm periods of a year (austral spring and summer) for the six selected regions. The NLLJ frequency is also higher in the warm months of the year, except in Brazil-NE where it is very frequent in all months. In Brazil-NE, the NLLJ also persists for 8 or more days, while the other NLLJs frequently persist for 1–2 days. The NLLJs occupy a broad low-level layer (from 1000 to 700 hPa) and exhibit a mean speed between 7 and 12 ms⁻¹, which peaks mostly at 900 hPa. The moisture transport for each NLLJ shows that in addition to the intense local moisture sources, the NLLJs in Argentina and Brazil-S receive moisture from the tropical-subtropical South Atlantic Ocean and the Amazon basin, while the tropical-subtropical South Atlantic Ocean is the main moisture source for the NLLJ in Brazil-SE. Both moisture sources and sinks are stronger in the austral summer and fall. The NLLJ in Brazil receives moisture from the tropical South Atlantic (TSA) Ocean, which has weak seasonality. The moisture sources for the NLLJs in Brazil-N and Venezuela come from the tropical North Atlantic (TNA) Ocean in the austral summer and fall, while the TSA Ocean appears as an additional moisture source in the austral winter. This research contributes to improving our understanding of the NLLJs and their role in transporting moisture and controlling precipitation over the continent according to the seasons of a year, helping to improve seasonal climate forecasting.

Keywords: nocturnal low-level jet, South America, moisture transport, precipitation, Lagrangian model

INTRODUCTION

A nocturnal low-level jet (NLLJ) is defined as a maximum of wind velocity in the first few kilometers of lower troposphere occurring at nighttime (Means, 1952). NLLJ is classified as an intense airflow observed in several regions of the globe, presenting sub-synoptic dimensions and a vertical depth from 0.5 to 0.9 km (Bluestein, 1992).

The dynamics of NLLJs have been described by various theories in the literature. Although each theory attempts to explain some particular characteristics, a single or full theory describing NLLJs has not yet been developed. One of the possible explanations for the NLLJ formation is through the inertial oscillation in the convective planetary boundary layer (PBL) where nocturnal inversion, turbulence, and large wind shear play an important role (Blackadar, 1957). This oscillation results in the nocturnal wind vector rotating around the geostrophic wind vector. According to Stull (1988), other mechanisms that may explain the NLLJ development are the topographic effects (mechanical and thermal) and the effects associated with the heterogeneous surface (sea, land, lakes, forests, plantations, cities, etc.) resulting in thermal gradients (baroclinicity). NLLJs are often observed in continental North America, Australia, Europe, North Africa, South America, and other parts of the globe (Blackadar, 1957; Hoecker, 1963; Bonner, 1968; Parish et al., 1988; Stensrud, 1996; Zhong et al., 1996; Whiteman et al., 1997; Marengo et al., 2004; Song et al., 2005; Vera et al., 2006; Baas et al., 2009; Oliveira et al., 2018; Montini et al., 2019).

Nocturnal low-level jets play an extremely important role by transporting heat and humidity at low levels from the tropics to the poles. In addition, the peak of its cycle occurs at night, with maximum velocities observed between 900 and 850 hPa (Stensrud, 1996). For example, they may be important for the initiation of the deep convection and severe weather phenomena where their strong vertical wind shear can be dangerous for aviation near airports (Newton, 1950; Saulo et al., 2007; dos Santos et al., 2008; Montini et al., 2019).

In South America, NLLJs are responsible for influencing the weather and climate in the whole continent, and they can be observed with greater frequency and intensity in the warmer months of a year (Nicolini et al., 2004; Saulo et al., 2004; Marengo et al., 2004; Vera et al., 2006; Montini et al., 2019). The most studied NLLJ in the continent named South America low-level Jet (SALLJ), occurs on the eastern side of the Andes Mountains occupying a large meridional extension. In general, SALLJs were mostly identified by using Bonner's (1968) criterion, which is based on a minimum value of wind speed at the level of the jet and vertical speed shear. Using these criteria, Nicolini et al. (2004) identified different types of SALLJs on the east of the Andes Mountains: the Chaco low-level jet (CJE) occurring southward of 25°S; the non-Chaco LLJ (NCJE) acting to the north of 25°S; and the LLJ over the north-west of Argentina (LLJA) and also occurring southward of 25°S. The CJE and LLJA differ in the wind direction and associated synoptic system, with the CJE showing north-northwesterly winds and northeasterly predominating for LLJA.

Rife et al. (2010) proposed a more general intensity index to identify NLLJs considering a vertical shear of wind speed and stronger winds at local nighttime. This index, named as the NLLJ index, does not impose any minimum value for the shear or wind speed, as occurs with Bonner's (1968) criterion. The fact of not using fixed wind speed thresholds reduces the subjectivity in the identification of NLLJs. Moreover, Rife et al. (2010) base the characterization of NLLJ events on an intensity index. Algarra et al. (2019) have applied this NLLJ index to an ERA-Interim reanalysis in sigma-pressure levels for all the globe for the months of January and July. Specifically, for South America, the NLLJ index was able to identify the nocturnal jets occurring in both months in Maracaibo, Llanos, Guyana, northeastern Brazil (Rio Grande do Norte and Piauí), and also in subtropical latitudes on the east of the Andes Mountains (Algarra et al., 2019).

Most of the studied SALLJs, identified at some points in eastern of Bolivia and Paraguay, is the NCJE, due to its role in transporting moisture from the tropical to subtropical South America (Marengo et al., 2004; Vera et al., 2006; Algarra et al., 2019; Montini et al., 2019). The moisture transport in SALLJ organizes deep convective systems in the form of a mesoscale convective complex and also contributes to the extreme precipitation events associated with cyclones over the La Plata basin (Velasco and Fritsch, 1987; Nicolini and Saulo, 2006; Salio et al., 2007; Cavalcanti et al., 2009; Reboita et al., 2010; Reboita et al., 2015; Carril et al., 2016; De Jesus et al., 2016), giving this region the highest occurrence of thunderstorms in the continent (Morales Rodriguez, 2019). In South America, moisture transport has been studied by using the Eulerian approach, i.e., the vertically integrated moisture flux (Arraut et al., 2012; Satyamurty et al., 2013; Zemp et al., 2014; Montini et al., 2019) or the Lagrangian one that computes the change of moisture following the air particles (Dirmeyer et al., 2009; Durán-Quesada et al., 2010; Van der Ent et al., 2010; Gimeno et al., 2012; Martinez and Dominguez, 2014; Zemp et al., 2014; Algarra et al., 2019).

Given the important control of NLLJ over the weather and climate events, the objective of this study is to obtain a complete seasonal climatology of the NLLJs in South America and their associated moisture sources and sinks. Therefore, we applied the NLLJ index proposed by Rife et al. (2010) to identify the jets and to analyze their characteristics. To obtain the moisture sources and sinks for a 37-year period (1980–2016), the outputs from the Lagrangian FLEXible PARTicle (FLEXPART) model (Stohl and James, 2005) were used. This study is of paramount importance since it will provide guidance for weather forecasting by identifying the regions where NLLJs are more frequent. In addition, the identification of the moisture sources and sinks for each NLLJ can help to understand extreme climate events such as drought or flood.

The study is organized as follows. Section "Data and Methodology" describes the data and methodology for the identification of NLLJ and Lagrangian algorithm for the moisture sources and sinks. Section "Results" discusses a climatology of the NLLJs and associated moisture sources and sinks, while Section "Discussion and Conclusion" presents the main conclusions.

DATA AND METHODOLOGY

Data

The climatology of NLLJs and associated moisture sources are obtained by using the ERA-Interim reanalysis data set from the European Centre for Weather and Forecasting (ECMWF; Dee et al., 2011). The FLEXPART tracking model uses the ERA-Interim data [three dimensional (3D) wind components and specific humidity] interpolated for a horizontal grid with $1^\circ \times 1^\circ$ of latitude by longitude and considers all 61 levels in the vertical available in the reanalysis (from the surface to 0.1 hPa). We identify the NLLJ using ERA-Interim data sets in a horizontal grid with $1.5^\circ \times 1.5^\circ$ longitude by latitude at different pressure levels. For all analyzes, we use the ERA-Interim data for every 6 h [00:00, 06:00, 12:00, and 18:00 Coordinated Universal Time (UTC)] from 1980 to 2016 (37 years). More details on the ECMWF-Interim are available at <https://apps.ecmwf.int/datasets/data/interim-full-daily/levtype=sfc/>. Montini et al. (2019) discussed the better performance of the ERA-Interim compared with other reanalyses to represent a vertical structure of the South American low-level jet. In addition, Anselmo et al. (2020) found minor differences between the ERA-Interim and a modern and finer resolution reanalysis (MERRA-2) to reproduce a vertical structure of the low-level jet in the Amazon. They showed that the MERRA-2 low-level jet has a time of occurrence and vertical structure (with maximum wind speeds at 950 hPa) similar to local sounding observations, although it underestimates the wind intensity. These comparisons with local observations indicating reliable features of low-level jets in the ERA-Interim reanalysis motivated its use in the present study.

For NLLJ days we also evaluated the relationship between the moisture sinks and rainfall using rainfall from the ERA-Interim and Climate Prediction Centre (CPC) data set (Xie et al., 2010). For continental areas of the globe, CPC is an intermediary resolution ($0.5^\circ \times 0.5^\circ$ of latitude by longitude) analysis of daily rainfall based only on rain gauge observations. It is available at <https://psl.noaa.gov/data/gridded/data.cpc.globalprecip.html>.

Methodology

NLLJ Identification

The identification of the NLLJ follows the method proposed by Rife et al. (2010), which checks the time evolution of a vertical structure of horizontal wind (zonal and meridional components, respectively, u and v) according to the expression:

$$NLLJ = \lambda \Phi \sqrt{[(U_{00}^{L1} - U_{00}^{L2}) - (U_{12}^{L1} - U_{12}^{L2})]^2 + [(V_{00}^{L1} - V_{00}^{L2}) - (V_{12}^{L1} - V_{12}^{L2})]^2} \quad (1)$$

where the subindices 00 and 12 correspond, respectively, to 00:00 (midnight) and 12:00 (noon) local time (LT); L1 and L2 are, respectively, the near-surface (originally 500 m AGL) and middle (originally 4000 m AGL) levels; λ and Φ are two binary numbers that compare, respectively, the time evolution of wind speed and its vertical shear. Lambda is given by:

$$\lambda = 0, \text{ if } W_{00}^{L1} \leq W_{12}^{L1} \quad \text{or} \quad \lambda = 1, \text{ if } W_{00}^{L1} > W_{12}^{L1} \quad (2)$$

i.e., λ compares the near-surface wind speed (W) at local midnight and local noon. According to Equation (2), $\lambda = 1$

corresponds to the near-surface wind speed at local midnight greater than at local noon, otherwise $\lambda = 0$. As lambda, Φ assumes the values 0 or 1 according to the criteria:

$$\Phi = 0, W_{00}^{L1} \leq W_{00}^{L2} \quad \text{or} \quad \Phi = 1, W_{00}^{L1} > W_{00}^{L2} \quad (3)$$

i.e., Φ compares the near-surface (L1) and mid-level (L2) wind speeds at local midnight; $\Phi = 1$ requires a near-surface wind velocity greater than that at mid-levels, otherwise $\Phi = 0$. In this way, Φ ensures the existence of a vertical structure of the jet at local midnight.

For the present study, the NLLJ index [Equation (2)] is adapted by using vertical levels in an isobaric vertical coordinate rather than height as proposed by Rife et al. (2010) or the sigma levels as in Algarra et al. (2019). The two pressure levels selected to calculate the NLLJ index are 900 and 650 hPa, corresponding approximately to 1000 and 4000 m above sea level, respectively. These two levels are more appropriate in order to define a vertical structure of the jet at midnight than the other two-level combinations tested over the region (1000–650, 1000–700, 1000–750, 1000–850, 1000–900, 900–850, 900–700, 900–650, 850–650, and 850–700 hPa; not shown). With these combinations, it was possible to verify that the NLLJ cores for the six regions are more evident (more intense) using the levels of 900–650 hPa. In this way, the levels that best represent the NLLJ indices in SA on a spatial scale and speed intensity are 900–650 hPa. The wind components at 00:00 LT are obtained as an average of 00:00 and 06:00 UTC (~21:00 and 03:00 LT in most parts of the continent), while 12:00 LT is given by an average of 12:00 and 18:00 UTC (~09:00 and 15:00 LT in most parts of the continent). Using these adaptations, the NLLJ index is calculated for each grid point by using the data from the ERA-Interim in South America, which allows the regions with NLLJ activity to be identified. The application of Equation (2) results in a daily NLLJ index that is used to obtain the seasonal climatology of the NLLJ and the associated moisture sources and sinks for the selected areas with a great frequency of occurrence of NLLJs.

The FLEXPART Model and Identification of Moisture Sources and Sinks

The FLEXPART dispersion model based on a Lagrangian method was developed by Stohl et al. (1995; 1998; Stohl and James, 2005) and has been widely used to identify the moisture sources for many regions and atmospheric systems (Gimeno et al., 2010, 2012, 2013). The method calculates the changes in the water vapor content (i.e., evaporation minus precipitation) for each particle of the air parcels along its trajectory from a source area to a target region, or *vice versa*, following the parcels forward or backward in time, respectively (Stohl and James, 2004; Sodemann et al., 2008; Sodemann and Stohl, 2009; Gimeno et al., 2010; Drumond et al., 2014; Gimeno et al., 2016; Durán-Quesada et al., 2017). The first step in the FLEXPART moisture screening is to divide the atmosphere into a large number of “particles” (2 million in this experiment) with constant mass homogeneously distributed according to the atmosphere mass distribution. The FLEXPART calculates the trajectories of the particles using a 3D

wind field (among other variables and several parameterizations). The variations of the moisture at each point of the trajectory are calculated by considering the changes in the specific humidity (q). For each particle, the net rate of change in water vapor content at each time step (6 h) is calculated as:

$$(e - p) = m (dq/dt) \quad (4)$$

where m , the mass of the particle, is a function of the density of the air, which remains constant throughout the simulation, and where e and p represent an increase or a decrease in the rates of moisture, which are seen as evaporation or precipitation, respectively. The difference in $(e - p)$ represents an increase or a decrease in the humidity of each particle along its trajectory. The sum of the values $(e - p)$ for all the tracked particles (k) that reside in a specific atmospheric column (A) is:

$$E - P = \frac{\sum_{k=1}^i (e - p)}{A} \quad (4)$$

where i is the total number of particles in the column, E is the total evaporation, and P is the total precipitation per unit area (A), and the difference $(E - P)$ represents the net surface freshwater flux.

In order to determine the moisture sources for a particular NLLJ region, the particles modeled by FLEXPART are first followed in a backward mode to identify those regions where evaporation is greater than precipitation $(E - P) > 0$ values. To identify the areas where moisture flux contributes to the precipitation, the moisture sinks [those areas with $(E - P) < 0$ values] and the particles leaving the NLLJ region were tracked forward. From a global point of view, the choice of using 10 days has been frequently used in the studies of the global water budget. Some studies discuss the ideal time for tracking moisture particles. Läderach and Sodemann (2016), using an offline tracking methodology, obtained a shorter time scale of 4–5 days for the average time of the moisture residence in the atmosphere. van der Ent and Tuinenburg (2017) used a reanalysis and modeling to derive the average global residence time of 8–10 days (that is closer to the classic values for the water vapor residence time in the atmosphere; Numaguti, 1999). The moisture computation results using Lagrangian tracks are susceptible to the time of integration definition. In this way, Nieto and Gimeno (2018, 2019) provided an annual and a monthly database of an optimal integration time for the Lagrangian studies of atmospheric moisture sources and sinks. Therefore, we used for both backward and forward tracking modes the time limitation to the results of the averaged values from Gimeno (2014), Nieto and Gimeno (2018) for all the NLLJ selected regions. Our results related to the sources and sinks of moisture associated with the NLLJ using these times are comparable to those by Algarra et al. (2019).

Analysis of NLLJs and Moisture Transport

Composite analysis is a useful technique to understand a particular weather-type event providing the information of a more recurrent atmospheric pattern. The daily time series of the NLLJ index at the center of the NLLJs occurrence regions is separated into days with and without NLLJ for all 37 years

(1980–2016). This data allows us to calculate the climatology (seasonality, persistence, vertical profiles, etc.) of the NLLJ events as well as to identify the associated moisture sources and sinks of NLLJs through the FLEXPART model. In addition, we calculate for some variables (precipitation and winds) the composite anomaly, i.e., the difference between the days with and without NLLJ events.

RESULTS

Seasonal Climatology of the NLLJ Index

Figure 1 shows the seasonal climatology for the period 1980–2016 of the NLLJ index and the wind at 900 hPa. In all South America and adjacent oceans, the NLLJ index (in velocity units, m/s) is stronger in the warm periods of a year, i.e., austral spring [September–October–November (SON)] and summer [December–January–February (DJF)]. A relatively intense NLLJ index occurs throughout the year over northeastern Brazil and northern Argentina, while in other regions it only intensifies in specific seasons as in northern Brazil in DJF, and Venezuela in DJF–March–April–May (MAM). By comparing the seasons, **Figure 1** presents a greater number of cores with an intense NLLJ index in the austral spring–summer. In northeastern Brazil, an intense NLLJ index is associated with climatological easterly low-level winds over the year, while in northern Argentina the winds are mostly from the north-northeast. In the north of Brazil and Venezuela, a more intense NLLJ index occurs and immersed in the climatological northeasterly trade flow. **Figures 1A,C** resemble that of Rife et al. (2010), but our indices, with a maximum of $\sim 3.5 \text{ m s}^{-1}$, are weaker (as occurs also in Algarra et al., 2019) than $\sim 6\text{--}8 \text{ m s}^{-1}$ in their study. In Rife et al. (2010), the possibility of a stronger NLLJ index exists because they used finer resolution data (25 km) with high time-frequency (for every 1 h) and only January and July for the climatology.

The NLLJ index in **Figure 1** highlights the seasons with a high frequency of occurrence and secondary regions, such as southeastern Brazil, with an NLLJ, that have not been previously documented using the other criteria or in the specific months of a year (Marengo et al., 2004; Rife et al., 2010; Algarra et al., 2019). This is the case of the NLLJ in northeastern Brazil that is stronger in the austral spring (SON) than in the summer (DJF), as previously reported in the literature (Rife et al., 2010; Algarra et al., 2019). In this season, a relatively intense NLLJ index advances into southeastern Brazil right to western São Paulo and Minas Gerais ($\sim 52^\circ\text{W}$ – 20°S ; **Figure 1D**).

An intense NLLJ index east of the Andes Mountains, from Bolivia to northern Argentina, was analyzed previously and received different denominations in terms of a function of the location and its main characteristics (Nicolini et al., 2004; Salio et al., 2007). Considering the Bonner (1968) criterion, Nicolini et al. (2004) classified the low-level jet to the east of the Andes as the CJE occurring southward of 25°S , the NCJE developing northward of 25°S , and LLJA the low-level Argentina jet. Both CJE and NCJE are associated with the eastern Andes poleward northerly northwesterly flow extending from the equator to the subtropics of South America but differ in the latitude of the

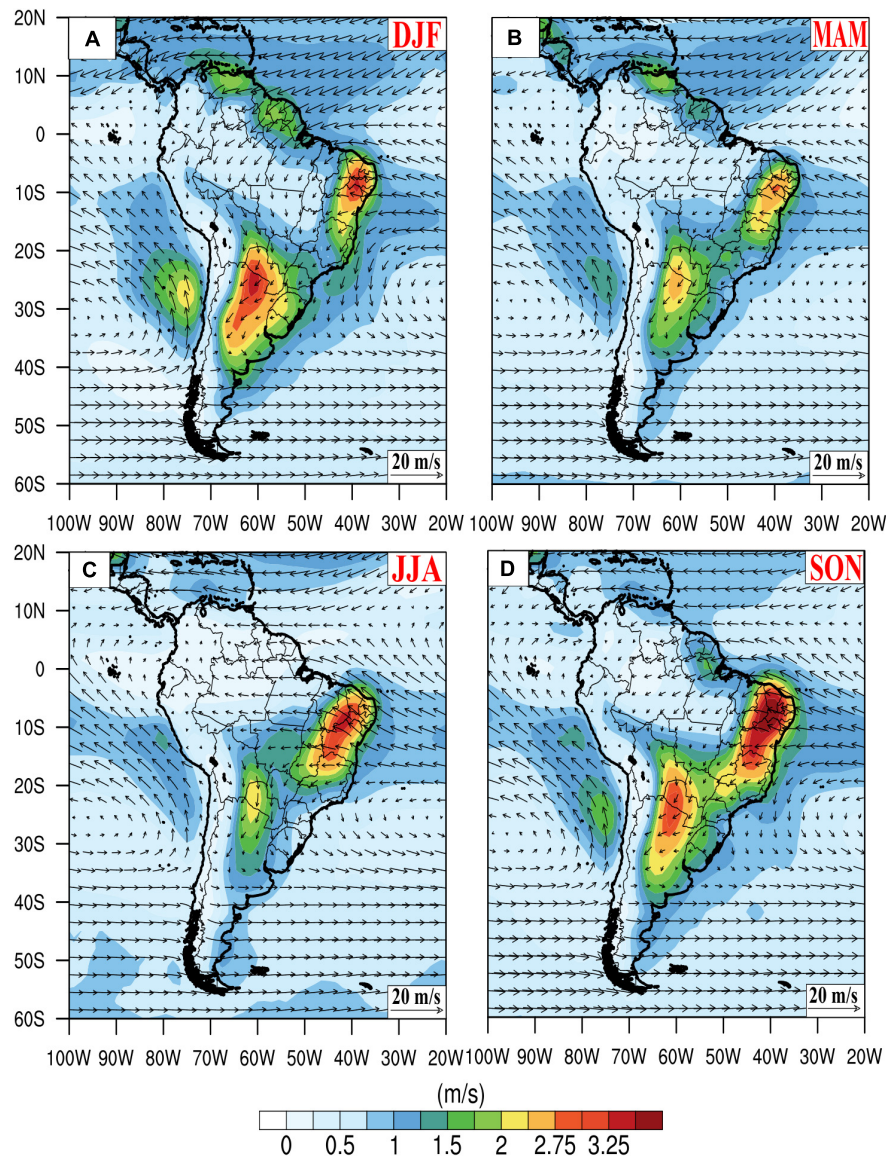


FIGURE 1 | Seasonal climatology (1980–2016) of the nocturnal low-level jet (NLLJ) index (shaded in m s^{-1}), and wind (vectors with scale in the bottom right in m s^{-1}) at 900 hPa for: **(A)** December–January–February (DJF); **(B)** March–April–May (MAM); **(C)** June–July–August (JJA); **(D)** September–October–November (SON).

jet core; LLJA is characterized by prevailing northeasterly flow occurring in the western sector of a postfrontal anticyclone (Nicolini et al., 2004, 2006).

Considering the classification of Nicolini et al. (2004, 2006), the most studied LLJ east of the Andes (Marengo et al., 2004; Saulo et al., 2007; Oliveira et al., 2018; Jones, 2019; Montini et al., 2019), occurring between 15°S and 25°S in the neighborhood of Santa Cruz de la Sierra ($\sim 18^{\circ}\text{S}$, Bolivia), would be classified as NCJE. According to **Figure 1**, our results provide the most intense NLLJ index core in northern Argentina ($\sim 26^{\circ}\text{S}$ – 61°W), a latitude that characterizes it as LLJ Argentina (LLJA) according to the classification of Nicolini et al. (2004).

The NLLJ index in **Figure 1** also presents relatively strong values associated with southerly/southeasterly winds along the

western coast of South America over the South Pacific Ocean, with a core of the NLLJ index migrating from the coast of Chile ($\sim 28^{\circ}\text{S}$) in the austral summer to the Peruvian ($\sim 12^{\circ}\text{S}$) coast in the winter. This intense NLLJ index basically follows the north-south displacement of the subtropical anticyclone and is known as the Peru-Chile coastal low-level jet (Lima et al., 2018). We have not selected this coastal NLLJ for further analysis because we are only interested in understanding the moisture transport for the continental areas.

For each season, **Figure 2** presents the relative frequency of the NLLJ index. The NLLJ is more frequent during the austral summer, reaching values from 60% in northern Argentina to above 90% in the northeast-north of South America (**Figure 2A**). Specifically, over northeastern Brazil, the NLLJ occurs more than

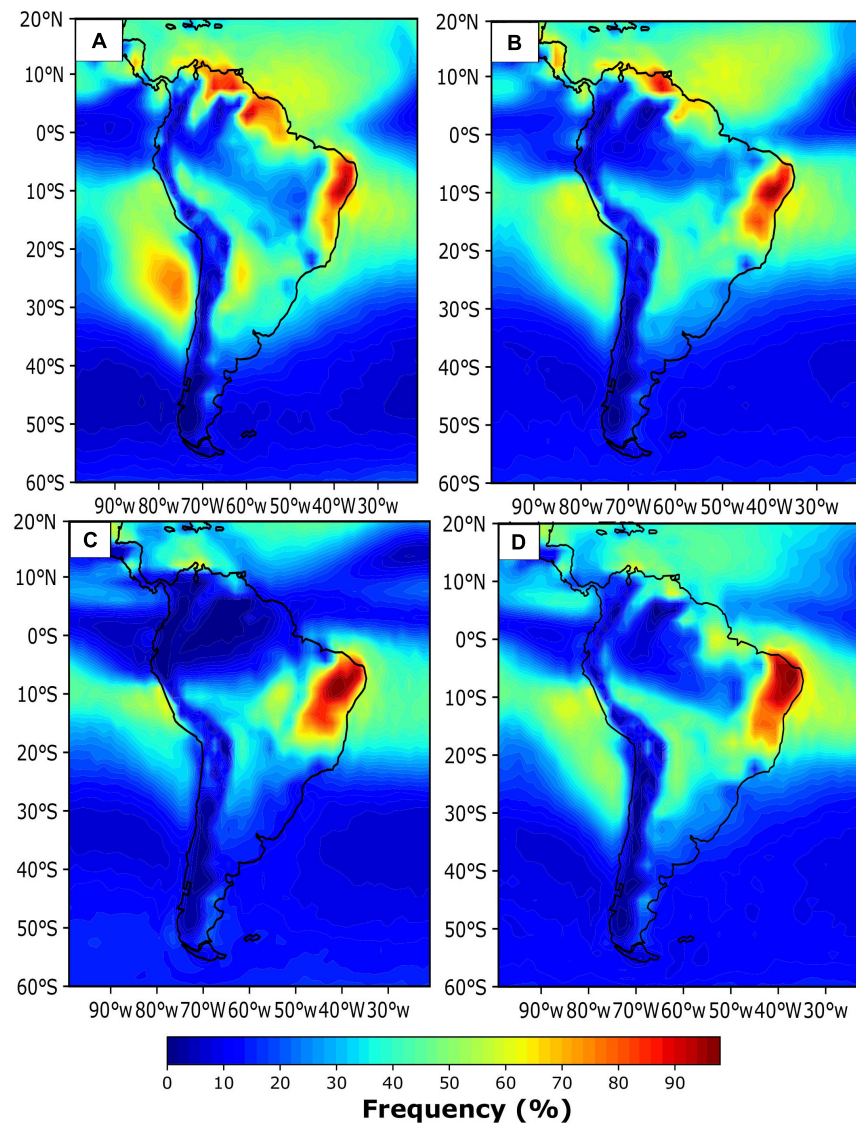


FIGURE 2 | Seasonal climatology (1980–2016) of the relative frequency (%) in shaded) of the NLLJ occurrence for: (A) DJF; (B) MAM; (C) JJA; and (D) SON.

80% of the days in all seasons. In other regions, the frequency of the NLLJ depends on the season of the year: in Venezuela, it is greater in the austral summer-autumn; in north Brazil, the coast of Chile–Peru, northern Argentina–Paraguay in the austral summer. Therefore, in South America, there is a preference for the development of the NLLJs during the warmer months of a year, except in the northeast of Brazil where the frequency is high all year round.

Figures 1, 2 allow us to identify the regions of South America in which the NLLJ index is more intense and frequently occurs throughout the year. Based on these two features, **Figure 3** shows the four maximum NLLJ cores denominated herein as: Venezuela, Argentina, northeastern Brazil (Brazil-NE), and northern Brazil (Brazil-N). In addition, **Figure 3** also presents two secondary NLLJ cores, one over southern Brazil (Brazil-S) and the other over southeastern Brazil (Brazil-SE), due to their

importance for the South America austral summer precipitation. They are located at two points of the continent normally associated with the anomalous precipitation in a pattern known as “seesaw” (Doyle and Barros, 2002; Carvalho et al., 2011). The six cores shown in **Figure 3** are used to understand the NLLJ climatological features while the surrounding boxes [with 5 by 5 degrees of latitude by longitude, except for Venezuela (5 by 4.5) and Brazil-N (4 by 4) because the areas occupied by these NLLJs are a little smaller] define the areas to calculate the associated moisture sources and sinks through the FLEXPART model.

Climate Features of the NLLJ Cores

The mean annual cycles of the NLLJ frequency in each of the six selected regions of the NLLJ occurrence are shown in **Figure 4**. Comparing all six regions, the highest NLLJ occurrences throughout the year are registered in Brazil-NE, and

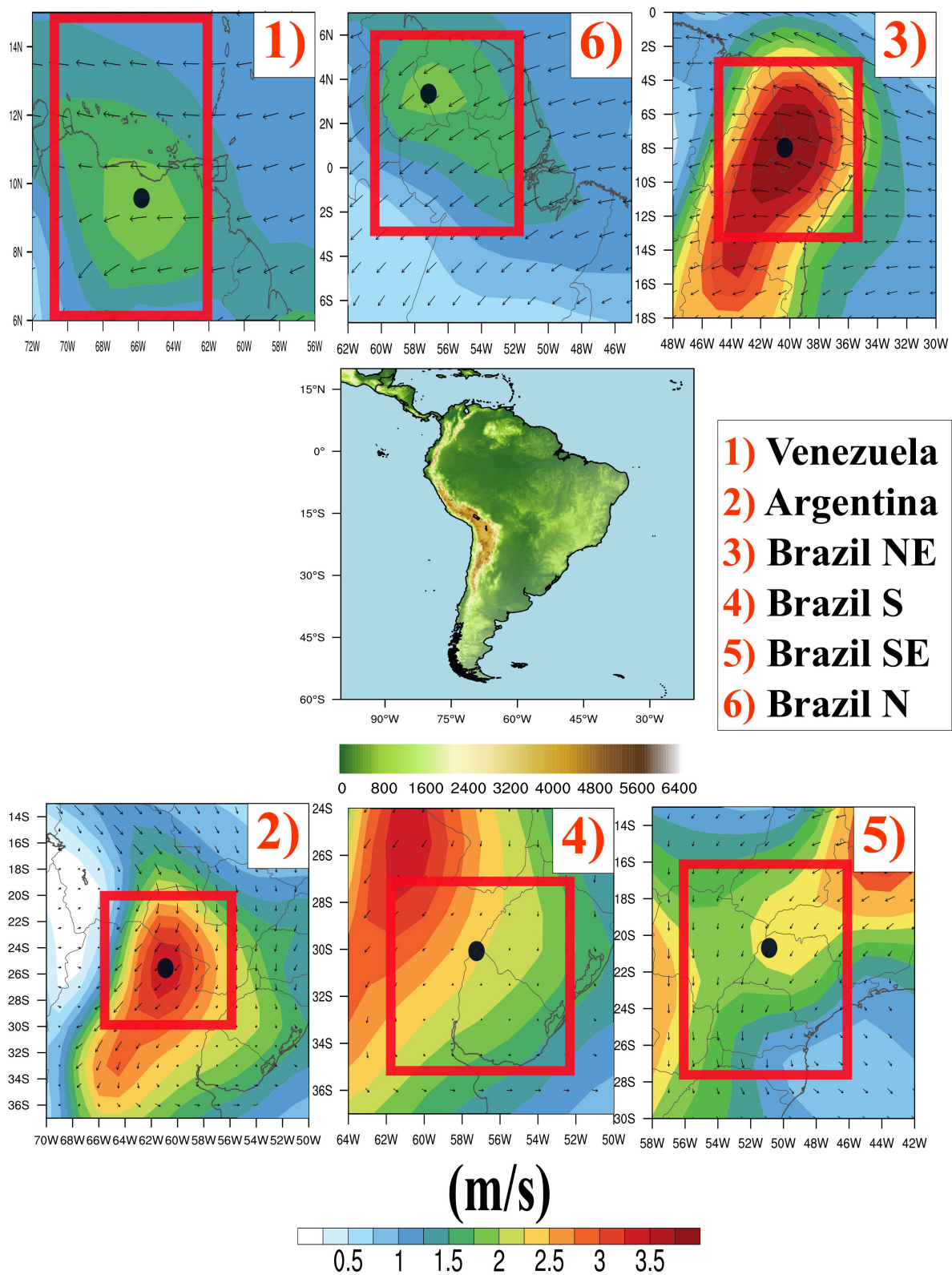


FIGURE 3 | The numbers in the central map identify the locations of the six NLLJ cores for the regions named: **(1)** Venezuela, **(2)** Argentina, **(3)** Brazil-NE (northeastern Brazil), **(4)** Brazil-S (southern Brazil), **(5)** Brazil-SE (southeastern Brazil), and **(6)** Brazil-N (northern Brazil). Central panel includes the topography (in m) of South America. The small panels present the 00:00 local time (LT) mean wind (vectors and in shaded the intensity in m s^{-1}) at 900 hPa for the NLLJ days during DJF for Venezuela, Argentina, Brazil-S, Brazil-N; SON for Brazil-NE and Brazil-S. The black dot in each panel represents the NLLJ center.

from December to April for the NLLJs in Brazil-N and Venezuela. We identify three main patterns of NLLJ mean annual cycles: (a) Venezuela and Brazil-N presenting a stronger seasonality with a very high and low frequency, respectively, in December–April and June–September; (b) Brazil-NE with a small amplitude, i.e., the jet occurs with the similar frequency over the months throughout the year, with a slightly higher (lower) frequency in July–November (February–April); (c) Argentina, Brazil-S, and Brazil-SE with an intermediary amplitude, being slightly more and less frequent, respectively, in the austral summer and winter.

For each season, the relative frequency of the persistence (number of consecutive days with the NLLJ index) of the NLLJ for the six cores is shown in **Figure 5**. For most of the seasons and regions, the NLLJ persists for 1–2 days with a greater frequency. One exception occurs in Brazil-NE where the NLLJ is over 8 days with a higher frequency, except in the austral autumn, when the frequency is similar for all classes of persistence. The different behavior of persistence of the NLLJ in Brazil-NE in the autumn may be associated with the establishment of the rainy season and the associated change in the circulation (De Souza et al., 2005). According to **Figure 5**, the longest persistence of NLLJ is registered for Venezuela in the austral summer–autumn and Brazil-N in the summer (Martin and Schumacher, 2011). This may be associated with the establishment of the intertropical convergence zone in these seasons, with the persistent northeasterly trade winds over the north of SA, including Venezuela and Brazil-N (Poveda et al., 2006, 2014).

Figure 5 also shows that over 70% of the NLLJ persists for only 1 day in Argentina and Brazil-S during the austral winter, which may be associated with the postfrontal characteristic of these jets and will be shown in the subsection “Moisture transport associated to NLLJs.”

The NLLJ index in the core of each region (black dotted points in **Figure 3**) is used to calculate the mean vertical profiles of the wind speed for days with and without NLLJ at 12:00 and 00:00 LT. **Figure 6** shows the NLLJs having a broad low-level layer from 1000 to 700 hPa, with a mean speed between 7 m s^{-1} (Brazil-SE in MAM) and 12 m s^{-1} (Brazil-NE and Argentina),

and mostly peaking at 900 hPa. Exceptions in relation to the level of maximum wind speed occur for the NLLJ in Argentina in June–July–August (JJA) and the NLLJ in Brazil in SON, where it appears at 850 hPa. The most intense NLLJ cores, with a mean wind speed of $\sim 11\text{--}12 \text{ m s}^{-1}$, are observed over Argentina and Brazil-NE during JJA and SON. The stronger jets are also associated with a stronger vertical wind shear above the jet core as already mentioned by Bonner (1968). For the days without NLLJ, some regions in specific seasons (Argentina in SON–DJF, Brazil-NE in JJA–SON) still have a wind speed with a vertical structure similar to a jet, but the vertical wind shear criterion does not allow it to be classified as a jet. For the local midnight (00:00 LT), the mean wind direction at 900 hPa during the NLLJ are from: (a) the east for Venezuela and Brazil-NE; the northeast for Argentina, Brazil-S, and Brazil-N; the north-northeast for Brazil-SE (see insets in **Figure 3**).

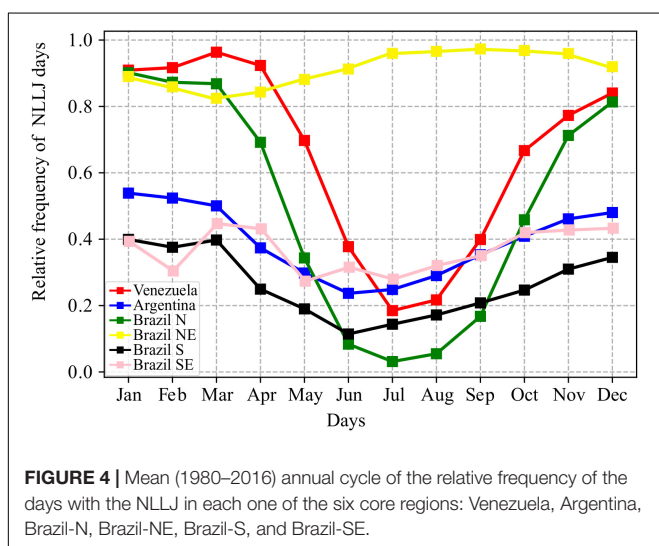
In Rife et al. (2010), the wind velocity and vertical speed shear at 00:00 LT are stronger than those at 12:00 LT, as shown in **Figure 6**, but, in some regions and seasons, a vertical structure of the jet with relatively intense speeds is still observed at 12:00 LT. The differences in most of the vertical profiles of the wind speed from 00:00 to 12:00 LT make clear the nocturnal mechanism described by Blackadar (1957), i.e., the absence of large eddies leads to a decoupling between the parts of the near-surface layer, resulting in a super geostrophic flow. According to **Figure 6**, this mechanism is more evident in NLLJs in Argentina, Brazil-S, Brazil-SE, and Brazil-NE than in Brazil-N and Venezuela. As in this study, an LLJ in the Orinoco basin located (9.5°N and 64.2°W) closer to our (jet core) Venezuela point also presents a maximum jet speed at night ($\sim 00:00$ LST) between 950 and 800 hPa (Jiménez-Sánchez et al., 2019). For this LLJ, in addition to the mentioned mechanism, Jiménez-Sánchez et al. (2019) also proposed that the wind acceleration is explained by a combination of sea breeze penetration over the Orinoco River delta and Unare, and downsloping winds along the Coastal Cordillera River.

Some differences are found between the NLLJs identified in this study and those previously identified by Bonner (1968) or other criteria. The main difference is the level where the wind speed peaks, which is 900 hPa in the present study and 850 hPa in the other studies (Marengo et al., 2004; Jones, 2019; Montini et al., 2019). Using the ERA-Interim in a sigma vertical coordinate for the global identification of NLLJs, Algarra et al. (2019) found that the NLLJ cores are located from 400 to 700 m above the surface, which is similar to our results.

Moisture Transport Associated to NLLJs

Previous analysis has argued that the low-level jets carry moisture from the tropical North Atlantic (TNA) and tropical South Atlantic (TSA) Oceans or continental regions, as is the case of the Amazon basin due to strong tropical forest evapotranspiration, to subtropical latitudes of South America (Marengo et al., 2004; Drumond et al., 2008; Knippertz et al., 2013; Landsberg et al., 2014; Durán-Quesada et al., 2012, 2017; Rocha et al., 2017; Algarra et al., 2019).

The Lagrangian FLEXPART tracking are integrated along 10 days, which are close to the mean values of the optimal time of integration for the selected six regions calculated using the



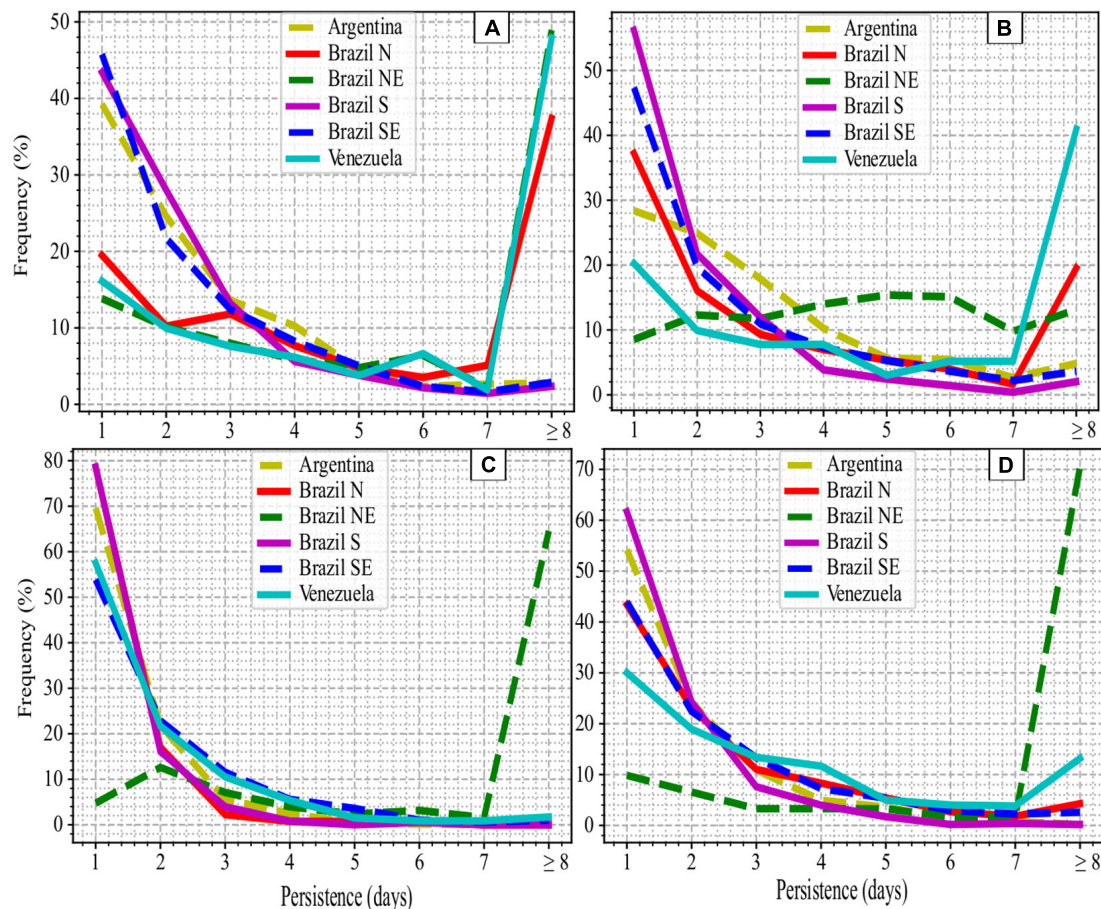


FIGURE 5 | Seasonal climatology (1980–2016) of the relative frequency of the persistence (days) of the NLLJ events for each region for: **(A)** DJF, **(B)** MAM, **(C)** JJA, and **(D)** SON. The category eight includes values equal to or greater than 8 days.

annual database for the Lagrangian optimal times of integration available in Nieto and Gimeno (2018, 2019) [for Venezuela and Brazil-S (DJF), Brazil-NE and Brazil-SE (SON) the optimal time results in 10 days, for Argentina (DJF) in 8 days, and for Brazil-N (DJF) in 12 days]. These time integrations agree, for example, with Drumond et al. (2008) that found higher contributions of TSA occurring at least in 8 days (behind) for the La Plata basin, and with the time used in Algarra et al. (2019). Only considering the NLLJ days, the methodology applied in a backward mode allows identifying the moisture source ($E - P > 0$ values), while a forward integration provides the sinks of moisture sinks ($E - P < 0$ values). The climatology (1980–2016) of the moisture sources and moisture sinks for each of the selected six regions are shown for DJF and JJA in **Figures 7, 8**. In the **Supplementary Figures 1, 2**, we present similar figures for the intermediary seasons MAM and SON. These figures also depict the seasonal composites of the wind at 900 hPa, the most frequent level of maximum speed for the NLLJ days at 00:00 LT (see **Figure 5**).

By comparing all regions, **Figure 7** and **Supplementary Figure 1** show the moisture sources occupying a broader and smaller area for the NLLJ days, respectively, in Brazil-S and Argentina.

According to **Figure 7A**, in DJF the NLLJ days in Argentina take the contributions of moisture transported by low-level northwesterly winds from the Amazon basin and by northeasterly winds from the TSA to the region. However, a very strong local moisture source is located over southeastern South America, more specifically over northeastern Argentina, Paraguay, and southwestern Brazil. Another remote moisture source of an intermediary magnitude ($\sim 6\text{--}10$ mm/day) is located over the Andes Mountains, occupying a latitudinal band of $25\text{--}45^\circ\text{S}$. For summer, Drumond et al. (2008) also detected all these sources when investigating the climatology of the moisture sources for the La Plata Basin. As expected, for only NLLJ days the moisture sources (**Figure 7A**) are stronger than in the climatology of Drumond et al. (2008), while they are slightly weaker than for the NLLJ days in January (Algarra et al., 2019). As in **Figure 7A**, Drumond et al. (2008) also highlighted a great contribution of local sources to the moisture in the La Plata Basin. The moisture origin pattern observed in DJF (**Figure 7A**) also occurs in the intermediary seasons MAM and SON (**Supplementary Figures 1A–G**) where the NLLJ winds are predominantly from the northeast. Some important changes are noted in DJF (**Figure 7B**), when the

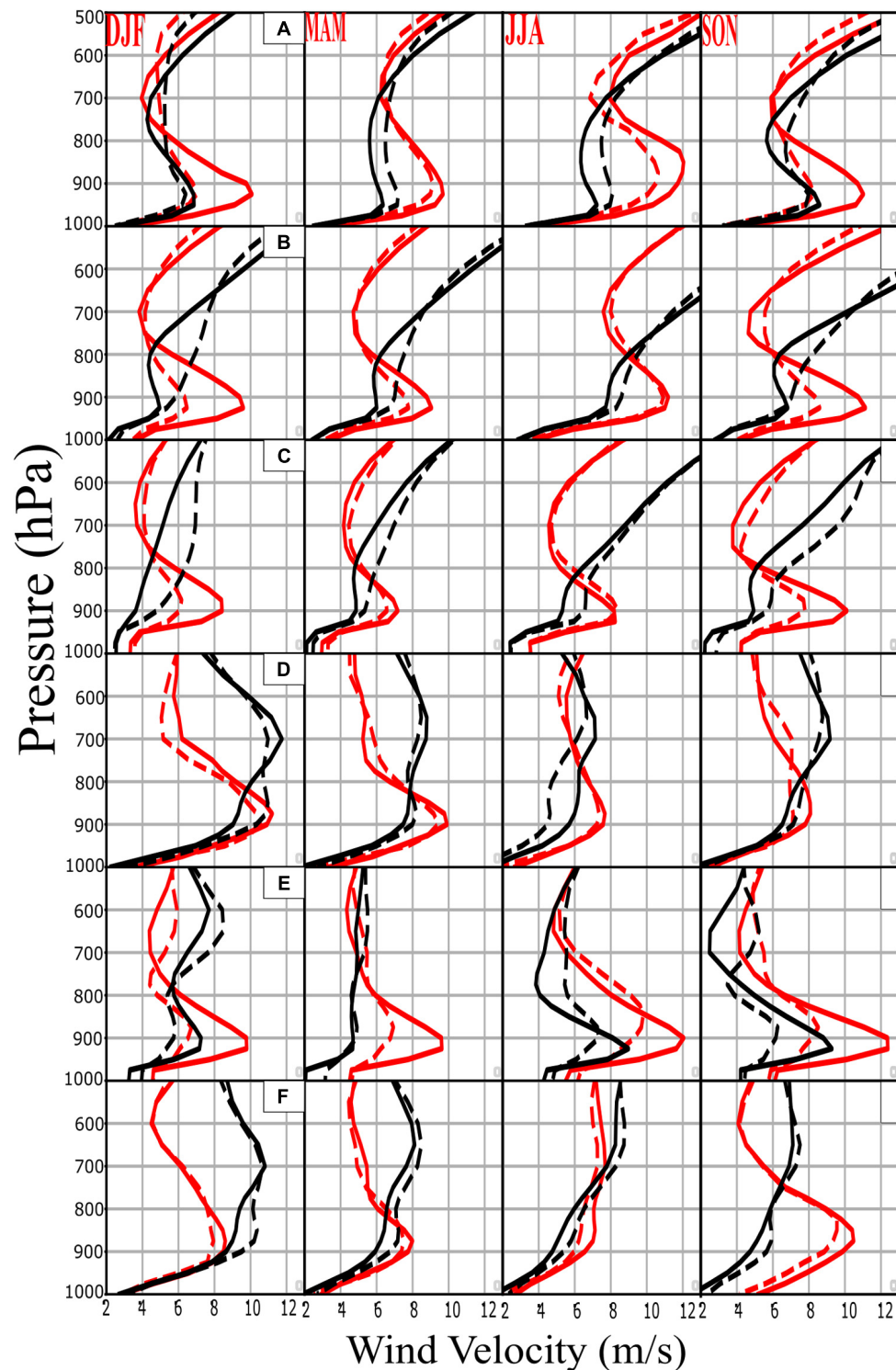


FIGURE 6 | Seasonal climatology (1980–2016) of the vertical profile of wind intensity (m s^{-1}) for the days with (red) and without (black) NLLJ at 00:00 LT (continuous lines) and 12:00 LT (dotted lines) for the regions: **(A)** Argentina, **(B)** Brazil-S, **(C)** Brazil-SE, **(D)** Brazil-N, **(E)** Brazil-NE, and **(F)** Venezuela.

moisture sources originate in the South Atlantic Ocean and local sources, and the winds at 900 hPa are from north on the NLLJ days.

For Brazil-S, the moisture sources are more intense in DJF than JJA on the days with NLLJ (**Figure 7B** and **Supplementary Figures 1B,H**). They are the main contribution of the local

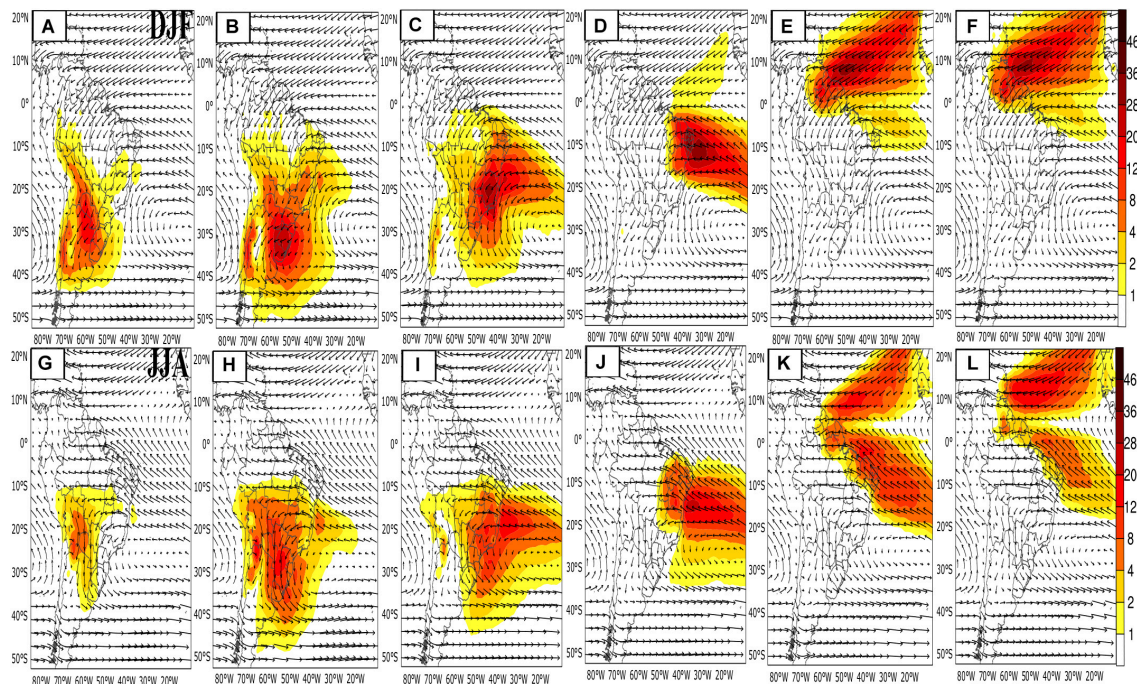


FIGURE 7 | DJF (top) and JJA (bottom) climatology (1980–2016) of the moisture sources (10-days integrated $E - P > 0$ in mm day^{-1}) and 900 hPa wind (vectors; m s^{-1}) at 00:00 LT for the NLLJ days in: (A–G) Argentina, (B–H) Brazil-S, (C–I) Brazil-SE, (D–J) Brazil-NE, (E–K) Brazil-N, and (F–L) Venezuela. The moisture sources ($E - P > 0$, i.e., evapotranspiration greater than precipitation) are provided by backward trajectories (red colors intensity scale in the right side).

source, while a less intense remote contribution is carried out by northeasterly winds from TSA and northwesterly winds from the Amazon basin to the region during most of the year (Figure 7B and Supplementary Figures 1B,H). The NLLJ days in JJA have TSA as their main source of humidity, which is associated with easterly northeasterly mean winds (Figure 7H and Supplementary Figure 1H). Particularly in the austral summer (DJF), there is an additional moisture source being carried out from the southern Amazon basin to Brazil-SE (Figure 7A).

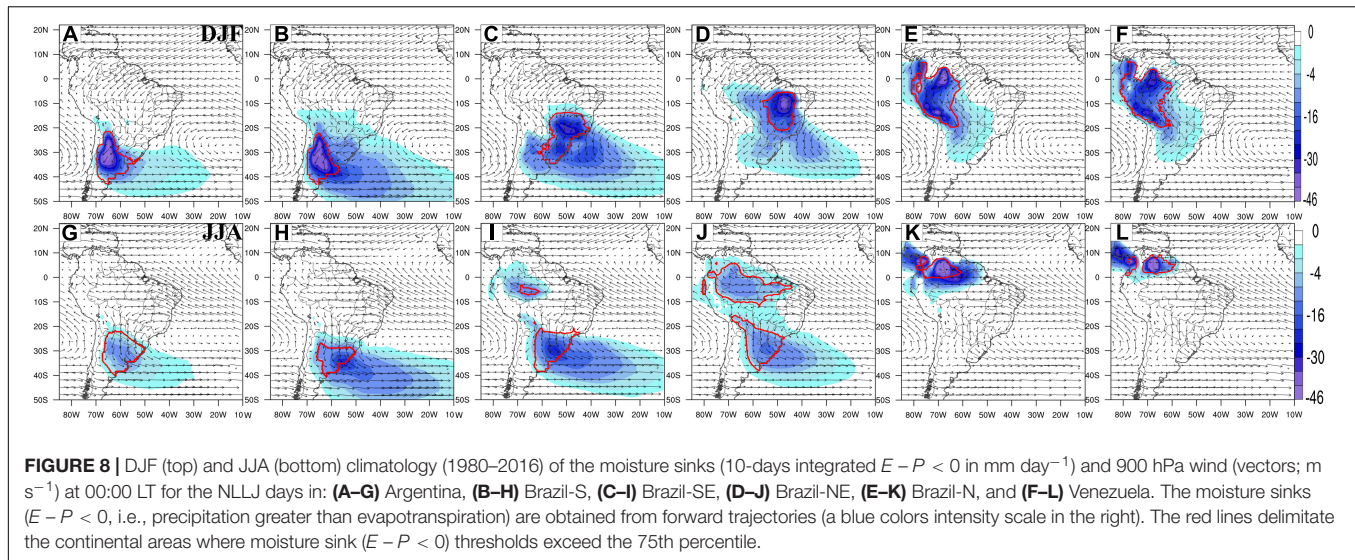
The days with the NLLJ in Brazil-SE have TSA as a main source of humidity, in particular there is an additional source of moisture being carried from the south of the Amazon basin to Brazil-SE (Figure 7C and Supplementary Figures 1C,I). Specifically, for DJF, the changes in circulation imposed a northern NLLJ, and the humidity comes from TSA and local sources (Figure 7C). Except in SON (Supplementary Figure 1I), the north-south extension of the TSA moisture source for the NLLJ in Brazil-SE occupies a broad latitudinal band (Martín-Gómez et al., 2016), from the equator to subtropical latitudes (35°S).

Tropical South Atlantic is also the most important moisture source for the NLLJ days in Brazil, being stronger and concentrated in narrow southern bands in DJF and JJA (Figures 7D,J and Supplementary Figures 1D,J). The direction of mean winds is from the southeast for the NLLJ days for most of the year, except in DJF, when it changes to easterly. A small amount of humidity comes from the TNA to the Brazil-NE

during the NLLJ days in the austral autumn (Supplementary Figure 1D), which corresponds to the rainy season in most parts of the northeast of Brazil. In this season, a stronger TSA moisture source core is over the sea away from the continent.

The spatial patterns of the moisture sources have similarities for the NLLJ days in Brazil-N and Venezuela (Figures 7E,F and Supplementary Figures 1E,F). The NLLJ days in Brazil-N are characterized by an intensification of the northeasterly and southeasterly trade winds transporting moisture, respectively, from TNA ($5^{\circ}\text{--}15^{\circ}\text{N}$ and $55^{\circ}\text{--}35^{\circ}\text{W}$) and TSA to the region during DJF and JJA (Figures 7E–K). In NLLJ locations, the mean wind at 900 hPa is from the east. The contribution of TNA continues intensification of the NLLJ days in Venezuela and Brazil-N during MAM (Supplementary Figures 1E,F), while it practically disappears in SON (Supplementary Figures 1K,L), when the main moisture source is over the TSA with predominant easterly winds at NLLJ locations.

The seasonal climatology in Figure 8 and Supplementary Figure 2 shows the maximum and minimum areas of the moisture sinks occupied by the NLLJ days. The NLLJ days in Argentina have moisture sinks with a stronger intensity in DJF (Figure 8A). For all seasons, the moisture sources are located downstream (to the west) of the low-level jet core, i.e., over the center-north of Argentina, Uruguay, and extending to the east over the South Atlantic Ocean. In MAM, the moisture sinks occupy a broader area over the ocean (Supplementary Figure 2A). A general weakening of the moisture sinks is noted for the NLLJ days in JJA (Figure 8G),



which intensify again in SON (**Supplementary Figure 2G**) and reach a stronger intensity in DJF (**Figure 8A**). The moisture sinks for the NLLJs in Brazil-S present a spatial pattern and seasonality similar to those for the NLLJ days in Argentina but occupy a broader area of midlatitudes and advance north toward the south of Brazil (**Figures 8B,H** and **Supplementary Figures 2B,H**).

For Brazil-SE, the moisture sinks spread over a wider area, from the central part of Brazil to northeastern Argentina, south Brazil, and the adjacent ocean during the NLLJ days in DJF (**Figure 8C**). In JJA the moisture sink is concentrated downstream of the NLLJ region, affecting a smaller area from eastern Argentina to south Brazil, advancing over the sea (**Figure 8I**). In addition, there is a remote impact of the NLLJ days in Brazil-SE that can be seen in a weak and small area of the moisture sink over the northwest Amazon in JJA, which would be explained by the deceleration of the easterly winds crossing the tropical sector of the continent (**Figure 8F**). Moisture sinks continue to intensify and occupy a broader area in MAM (**Supplementary Figure 2C**), but weaken in SON (**Supplementary Figure 2I**).

The moisture sinks for the NLLJ days in Brazil-NE covers a broad area of the continent throughout the year (**Figures 8D,G** and **Supplementary Figures 2D,G**). The spatial pattern is similar in DJF and MAM, where stronger moisture sinks occur near and downstream of the NLLJ point (**Figure 8D** and **Supplementary Figure 2D**). For JJA, there is a general weakening of the moisture sinks and two main cores occur away from the NLLJ area, i.e., one over the north of the continent, and the other over the southeast of South America (**Figure 8J**). The moisture sinks in MAM and SON are stronger than those in JJA (**Supplementary Figures 2D,J** and **Figure 8D**).

The NLLJ days in Brazil-N and Venezuela have associated moisture sinks located downstream and occupying a broad area from the Amazon basin to the center-west of the continent in DJF (**Figures 8E,F**) and SON (**Supplementary Figures 2E,F**) when nocturnal jets are more frequent. The source receives moisture from the NLLJ and contributes to the precipitation downstream

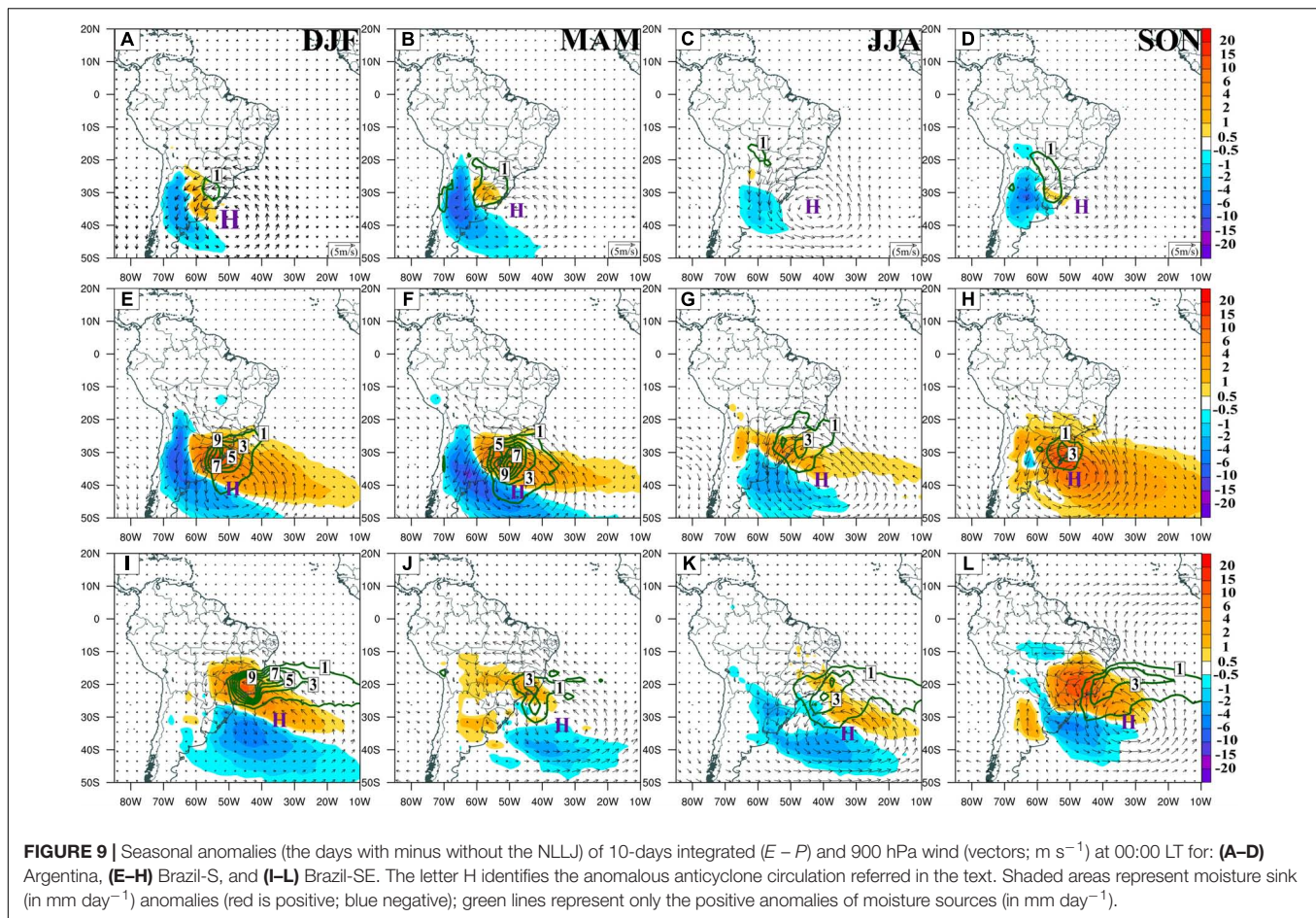
over the Amazon basin and the center-west of the continent. In DJF, the stronger (reaching until 45 mm/day) moisture sinks occur over the Andes Mountains for both NLLJ days in Brazil-N and Venezuela (**Figures 8E,F**). DJF moisture sources and sinks for the NLLJ days in Venezuela and Brazil-N have similar patterns to those obtained only for January by Algarra et al. (2019). In JJA, when the frequency of the NLLJ decreases, the moisture sinks continue to intensify, but they occupy a smaller area near the NLLJ points in Brazil-N and Venezuela (**Figures 8K,L**). In SON, there are large differences between the moisture sinks for Venezuela and Brazil-N, i.e., they occupy a small area near the NLLJ point for Venezuela and a larger area for Brazil-N (**Supplementary Figures 2K,L**).

NLLJs: Anomalous Circulation Patterns and Moisture Transport

The synoptic climatology and anomalous moisture sources/sinks for the six regions of NLLJs are discussed in this section. The objective is to understand the meteorological systems influencing the moisture sources/sinks associated with the nocturnal low-level cores.

Figures 9, 10 present the seasonal anomalies (difference between the days with and without NLLJ) of the moisture sources/sinks along with the anomalies of 900 hPa winds at 00:00 LT. In these figures, the positive (indicating decreased precipitation) and negative (indicating increased precipitation) anomalies of the moisture sink (precipitation, $E - P < 0$) are shaded; for the moisture sources ($E - P > 0$) only positive anomalies (above climate values) are shown in continuous lines.

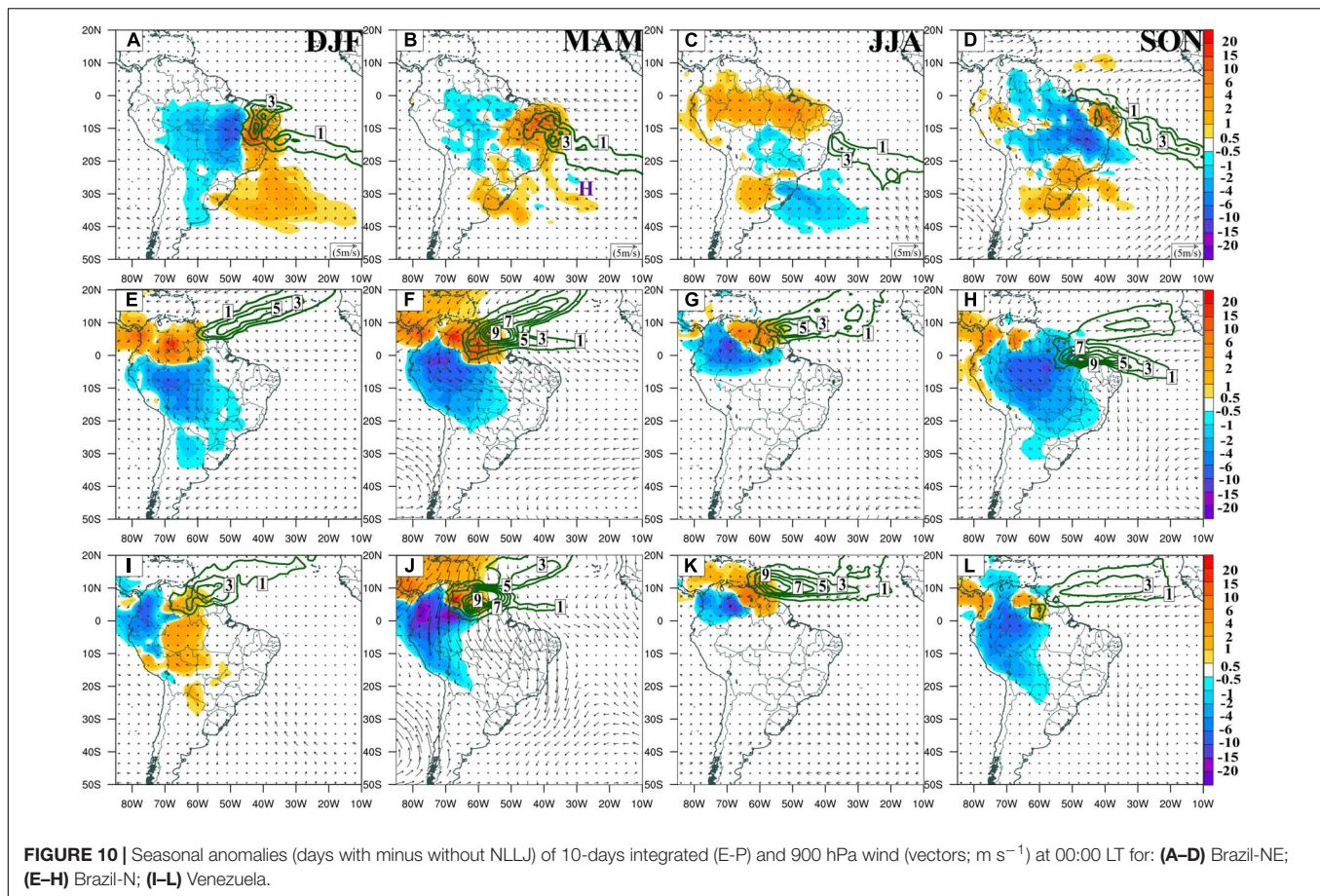
In DJF and MAM, the NLLJ days in Argentina and Brazil-S are characterized by a negative anomaly of the moisture sink (intensified precipitation) covering the center-west of Argentina, while a positive anomaly (decreased precipitation) is observed over the south of Brazil and Paraguay (**Figures 9A,B,E,F**). Both positive (less precipitation) and negative (more precipitation) anomalous moisture sinks are stronger for the NLLJ days in



Brazil-S than Argentina. Observing **Figures 9A,B,E,F**, one can see that the NLLJs in these regions at the nighttime 900 hPa winds, there is the establishment of an anomalous anticyclone centered over the South Atlantic ($\sim 38\text{--}40^\circ\text{S}$ and $48\text{--}50^\circ\text{W}$), which intensifies the northerly/northeasterly winds at the jet cores, as described by Nicolini et al. (2004) for the LLJ Argentina. For the DJF and MAM seasons, the positive anomalies of the moisture sources (continues green lines) are also stronger for the NLLJ in Brazil-S than the Argentina, which is also probably related to the anomalous anticyclone described before and produces a more intense easterly wind at 900 hPa (**Figures 9A,D**). The stronger winds reinforce both the moisture transport from the sea to the NLLJ core in Brazil-S and the local moisture source. The anomalous low-level anticyclonic circulation centered on $\sim 35^\circ\text{S}$ and $40\text{--}45^\circ\text{W}$ is also one common feature in the JJA/SON seasons for the NLLJ days in Argentina and Brazil-S (**Figures 9C–H**), but the moisture sink anomalies (positive and negative) are weaker in comparison to DJF/MAM. In addition, there are some differences in a spatial pattern of anomalies between the NLLJ days in Argentina and Brazil-S, i.e., in SON the NLLJ days in Argentina (**Figures 9C,D**) are characterized by increased precipitation (negative moisture sink anomaly), while for Brazil-S (**Figures 9G,H**) the suppression of precipitation (positive moisture sink anomaly) predominates. Moisture source positive

anomalies are a clear characteristic of the NLLJ days in Argentina and Brazil-S, as well as in JJA/SON (**Figures 9C,D,G,H**). The anomalous low-level circulation patterns for Argentina and Brazil-S have similarities to those described by Nicolini et al. (2004, 2006), as in Argentina LLJ, i.e., the low-level jet is from north-northeasterly and occurs in the western sector of a postfrontal anticyclone. Our study shows that for most of the year these NLLJs have their main source of moisture over the TSA ocean (green lines in **Figures 10A–H**) and contribute to an increase/decrease in precipitation in the west/east dipole mode covering northern central Argentina and southern Brazil (shaded in **Figures 10A–H**). This is in line with the previous analyses in this region (Silva et al., 2009; Martinez and Dominguez, 2014).

The NLLJ days in Brazil-SE are characterized by positive moisture sink anomalies (suppression of precipitation) over the east of southeastern Brazil during most of the year, while the moisture sources are intensified over the subtropical (centered on 20°S) South Atlantic Ocean (**Figures 9I,L**). At the same time, except in MAM, there is increased rainfall (negative moisture sink anomaly) over the south of Brazil where the low-level winds slow down, configuring a dipole pattern of precipitation between southeastern (increased) and southern (decreased) Brazil during the NLLJs in Brazil-SE. In low levels, the anomalous moisture sinks/sources are associated with the presence of an anticyclone



over the South Atlantic Ocean (centered on $\sim 30^\circ\text{S}$ and $30\text{--}35^\circ\text{W}$), which accelerates the northerly/northeasterly wind in the region of the NLLJ in Brazil-SE.

The NLLJ days in Brazil-NE are associated with the intensification of the easterly/southeasterly winds over the northeast of Brazil, with the consequent intensification of moisture sources over TSA and some sectors of northeastern Brazil (Figures 10A–D). The acceleration of low-level winds is stronger in SON and weaker in JJA. According to Figures 10A–D, for in Brazil-NE, the NLLJ days have a great influence over the local and remote moisture sinks. Although the frequency of NLLJ days increases a little during the year (Figure 4), the moisture sink anomalies exhibit different patterns according to the season of a year (Figures 10A–D). For DJF and MAM, there is suppression of rainfall over the northeast of Brazil and enhanced rainfall downstream of the NLLJ core (Figures 10A,B). For JJA, the local impact is weaker, but the remote effect is greater and results in a large area of the Amazon basin having decreased rainfall during the NLLJ days (Figure 10C). Another different anomalous pattern occurs in SON (Figure 10D). In this season, the moisture sinks exhibit decreased precipitation only in the northern sector of northeastern Brazil, and a broad area of enhanced rainfall is located to the south and east of the NLLJ core.

The intensification of southeasterly trade winds for Brazil-SE and Brazil-NE for the NLLJ days, mainly during the

austral spring, favors greater evaporation from the sea to the atmosphere, thereby increasing the availability of water vapor in the atmosphere, and, in consequence, the sources of moisture (lines in Figures 9I,L, 10A,D), which is transported to the remote regions (Drumond et al., 2014).

The NLLJ days in Venezuela and Brazil-N have similar patterns of the negative anomaly of the moisture sink, i.e., the precipitation exceeding evapotranspiration downstream of the jet cores covering the central-western Amazon basin, including Peru and Colombia, during MAM and SON (Figures 10F,H,J,L). In these seasons, small areas with a positive moisture sink (less precipitation) are established near the NLLJ cores. Comparatively, enhanced precipitation associated with the NLLJ is stronger in MAM than SON for Brazil-N and the Venezuela jets. In this season, the anomalous moisture sources (green lines) are also more intense than in the rest of the year. During JJA, compared to other seasons, a decrease in the areas of the positive and negative moisture sinks can be seen (Figures 10G,K). Different spatial pattern anomalies of the moisture sink emerge in DJF for the NLLJs in Brazil-N and Venezuela (Figures 10E,I). For Brazil-N, there is a north-south dipole with suppression of precipitation in the northwest of South America and an increase over the Amazon basin extending to a central part of the continent (Figure 10E). For Venezuela, the dipole is east-west oriented with increased rainfall occupying Colombia

and Peru, while a deficit of rainfall is noted in the center-west of the Amazon basin (**Figure 10I**). This difference could be explained by the stronger northeasterly winds occurring in the NLLJ days in Brazil-N than those in Venezuela. Although the NLLJs in Venezuela and Brazil-N present a higher frequency in DJF, we note that the anomalous moisture sources are stronger (more than 5 mm day^{-1}) in MAM and JJA, associated with the intensification of north-northeasterly winds in TNA (**Figures 10F,G,J,K**). Specifically, in SON, the NLLJ days in Brazil-N also present a strong moisture source anomaly, but this is associated with the intensification of the easterly winds in TSA (**Figure 10H**).

For the NLLJ days, the composites of the winds at 250 hPa have a great similarity with the climatology presented by Reboita et al. (2010). For example, in DJF, there is the presence of the Bolivian High over Bolivia followed downstream by a trough over the northeast of Brazil, while for SON, westerly winds extend from extratropical to tropical latitudes ($\sim 12^\circ\text{S}$), and there is the presence of a high pressure over the northwestern of Amazon basin (figure not shown). As a result, we can discuss the upper-level circulation only in terms of anomalies, i.e., the differences between the days with NLLJ and without NLLJ. Thus, **Figure 11** presents the anomalies of 250 hPa winds and precipitation (CPC). The anomalies of precipitation are related to the moisture sinks since more negative (positive) values of moisture sinks are associated with an intensification (weakening) of precipitation.

For the NLLJ days in Argentina, an anomalous anticyclone at 250 hPa is located over Uruguay, followed downstream by an anomalous low pressure in the east of southeastern Brazil during DJF and SON (**Figures 11A,D**). These circulation patterns are associated with above-normal precipitation over the center-north of Argentina, which coincides with the areas of intensification of the moisture sinks (**Figures 11A,D**). Immediately downstream over Uruguay and southern Brazil, the suppression of the precipitation in agreement with the positive anomaly of moisture sinks is noted. For MAM and JJA, the anomalous anticyclone at upper levels is located to the southeast of that in DJF and SON (in eastern Uruguay), and the common signal in both seasons is a negative anomaly of precipitation over southern Brazil (**Figures 11B,C**).

For each season, we defined the area limited by the 75th percentile of the moisture sink (negative values in **Figure 9**) to integrate the moisture sink and precipitation in the NLLJs. We then calculated the time correlation between these time series. For NLLJs in Argentina, the moisture sinks and precipitation are statistically significantly correlated (negative correlations indicate an increase in rainfall when the moisture sinks are more negative), as shown in **Table 1**. The correlations are greater (in modules) when the moisture sinks are compared to the ERA-Interim than with CPC. This is an expected result considering that the moisture sinks are obtained directly from the ERA-Interim, and a water budget is expected to exist in the reanalysis data. This balance does not occur in CPC since it only interpolates rain gauge observations without considering any other dynamic or thermodynamic processes in the atmosphere.

For all seasons, the NLLJ days in Brazil-S are characterized by an anomalous upper-level high pressure with similarities

(**Figures 9E–H**) to that of Argentina (**Figures 9A–D**), but its center is slightly shifted to the south. The most evident consequence of the NLLJ in Brazil-S is the strong suppression of rainfall over southern Brazil and Uruguay and an increase in central-northern Argentina. Some remote impacts are noted also over central-eastern Brazil, with positive anomalies of rainfall, except in JJA (**Figure 11G**). The correlations between the areas (75% percentile) of moisture sink (**Figures 8D–H**) and rainfall (**Figures 11D–H**) anomalies are also statistically significant but smaller in a module than in the NLLJ days in Argentina (**Table 1**).

Except in winter, the rainfall anomalies associated with the NLLJ days in Argentina and Brazil-S (**Figures 11A–H**) present a dipole pattern, in the form of a northwest-southeast band, with the rainfall suppression over most of southeastern South America (southern Brazil, northeastern Argentina, and Paraguay) and a rainfall increase from the Amazon to southeastern Brazil, a pattern normally referred to as seesaw (Carvalho et al., 2011).

When the NLLJ in Brazil-SE occurs, there is also the presence of one anomalous anticyclone at upper levels located eastward of southern Brazil, with an anomalous low pressure (or trough) downstream over southeastern Brazil (**Figures 9I–M**). Associated with this anomalous circulation, another dipole pattern in the precipitation anomalies emerges during the NLLJ days in Brazil-SE, i.e., less precipitation in the center-east of Brazil and more precipitation over the south of Brazil (**Figures 11I–L**). In this case, the areas of negative moisture sink anomalies (percentile 90%) are relatively less correlated with rainfall in comparison to Brazil-S and Argentina, but they are still statistically significant in some seasons (**Table 1**). These smaller correlations may be associated with the moisture sink anomalies occupying larger areas over the ocean than in the continent (**Figures 9I–L**), while the opposite occurs for precipitation anomalies (**Figures 11I–L**). A greater increase in precipitation over southern Brazil in the austral spring (**Figure 11L**) during the NLLJs in Brazil-SE may be associated with a higher occurrence of cold fronts crossing the south/southeast of Brazil, reinforcing the formation of clouds and precipitation over the region (Rodrigues et al., 2004; De Jesus et al., 2016).

Upper-level circulation anomalies for the NLLJs in Brazil show the presence of low pressure over the northeast of Brazil in DJF, which migrates to the southeast during MAM and JJA and to the south in SON (**Figures 11M–P**). This low pressure induces subsidence with the consequent suppression of precipitation during the NLLJs over a wide area, which exceeds that of the NLLJ core and advances from the northeast to the southeast of Brazil in DJF and MAM. The negative anomaly of rainfall occupies a greater area in comparison to the positive moisture sink (less precipitation in **Figures 10A–D**). When in JJA and SON, the anomalous upper-level low pressure moves away (to the south), the negative precipitation anomaly occupies a smaller area. The time correlations between the moisture sink and precipitation anomalies are greater in SON, decreasing in the other seasons (**Table 1**).

According to **Figures 11Q–T**, the NLLJ days in Brazil-N and Venezuela have similar upper-level circulation (and precipitation) anomalies, mainly in DJF and MAM, when these regions present higher frequencies of NLLJs (**Figure 4**). At

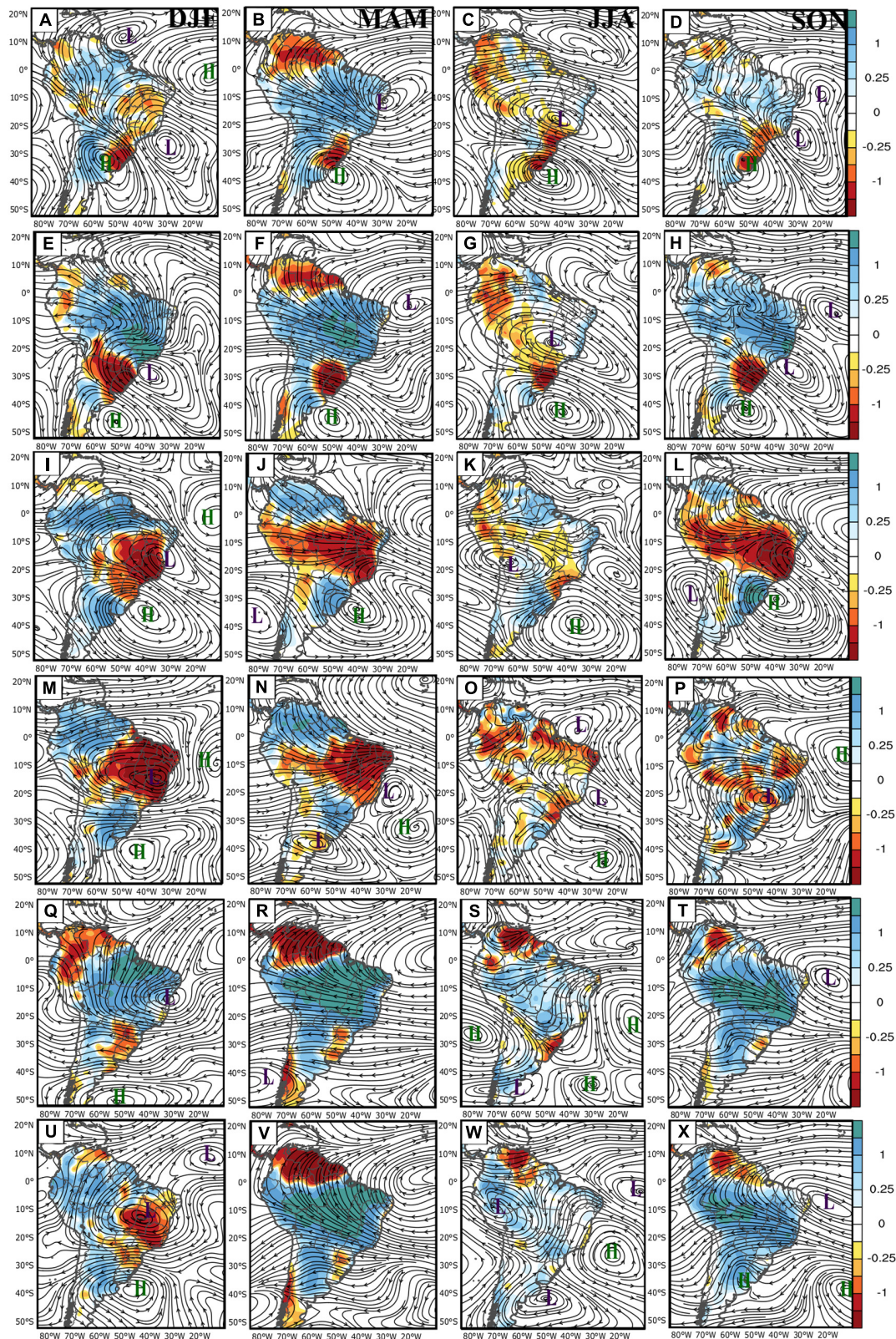


FIGURE 11 | Seasonal anomalies (differences between the days with and without NLLJs) of daily precipitation (mm day^{-1}) and streamlines at 250 hPa at 00:00 LT for: (A–D) Argentina, (E–H) Brazil-S, (I–L) Brazil-SE, (M–P) Brazil-NE, (Q–T) Brazil-N, and (U–X) Venezuela. Shaded areas indicate the anomalies of precipitation (red colors are negative and blue colors are positive).

TABLE 1 | Correlation coefficients between the moisture sink areas (the areas are highlighted with red lines in **Figure 8**) and the Era-Interim and Climate Prediction Centre (CPC; values in parenthesis) precipitation during the nocturnal low-level jet (NLLJ) days.

	DJF	MAM	JJA	SON
Argentina	-0.56 (-0.45)	-0.79 (-0.70)	-0.67 (-0.30)	-0.79 (-0.74)
Brazil-S	-0.37 (-0.42)	-0.54 (-0.40)	-0.37 (-0.30)	-0.48 (-0.49)
Brazil-SE	-0.14 (0.04)	-0.35 (-0.47)	0.12 -0.12	0.18 (-0.26)
Brazil-NE	-0.33 (0.17)	-0.35 (-0.36)	-0.48 (-0.50)	-0.77 (-0.57)
Brazil-N	-0.63 (-0.29)	-0.71 (-0.29)	-0.04 (-0.21)	-0.26 (-0.11)
Venezuela	-0.64 (-0.48)	-0.21 (-0.12)	-0.47 (-0.29)	-0.65 (-0.05)

Statistically significant correlations at 95% confidence level are in bold.

tropical latitudes (15°S to 15°N), the circulation anomalies show a weakening and an intensification of easterly winds, respectively, to the north and south of the equator, except in DJF when a cross-equatorial southerly southwesterly anomaly (**Figures 11Q–T**) can be noted. In addition, these NLLJs are associated with the suppression of convection near the jet cores in agreement with a positive anomaly of the moisture sink (**Figures 10E–H**) and excessive precipitation downstream of the cores. In most seasons, there is a statistically significant correlation between the moisture sinks and precipitation for the NLLJ days in Brazil-N and Venezuela (**Table 1**).

DISCUSSION AND CONCLUSION

We used 37 years (1980–2016) of the ERA-Interim reanalysis to identify the regions of occurrence of NLLJs and associated moisture sources and sinks in South America. The NLLJ identification considered an index based on the vertical wind speed shear at 00:00 LT compared to 12:00 LT proposed by Rife et al. (2010), while the moisture sources/sinks are obtained by the Lagrangian FLEXPART model (Stohl et al., 1995).

In South America, the NLLJ index is stronger in the warm periods of the year, i.e., austral spring and summer. Relatively intense NLLJs occur throughout the year over northeastern Brazil and northern Argentina, while in the other regions they only intensify in specific seasons (northern Brazil in DJF and Venezuela in DJF-MAM). The NLLJ index permits the identification of the seasons with a high frequency of occurrence and secondary regions, such as southeastern Brazil, where this kind of jet has not been previously documented. Based on the NLLJ index, we selected six main regions of the NLLJ occurrence: Argentina, southern Brazil (Brazil-S), southeastern Brazil (Brazil-SE), northeastern Brazil (Brazil-NE), northern Brazil (Brazil-N), and Venezuela. The vertical profiles of wind speed show these NLLJs acting between 900 and 650 hPa layers, with mean speeds between 7 and 12 m s⁻¹ peaking mainly at 900 hPa level. Therefore, the NLLJs analyzed here have maximum velocity at lower levels than previously documented for the SALLJ, with a peak velocity at 850 hPa (Marengo et al., 2004; Montini et al., 2019).

For most of the selected regions, NLLJs are more frequent in the austral spring and summer, except for Brazil-NE, where the NLLJ is very frequent during all months of a year, with a small increase in frequency in the austral spring. The NLLJs in Argentina, Brazil-S, Brazil-N, and Venezuela are more frequent in the summer. In terms of persistence, the NLLJs for most seasons and regions persist with a higher frequency for 1 or 2 days. The exception is a great occurrence of the long-lived NLLJs (up to 8 days) in Brazil-NE for most of the year, except in austral winter. The greater persistence of the NLLJ in Brazil-NE is explained by the link between the jet and the persistence and intense easterly/southeasterly trade winds in the northwestern sector of subtropical South Atlantic anticyclone.

The track in a backward and forward mode of the particle trajectories from the outputs of the Lagrangian FLEXPART model over the six NLLJ core regions allows identifying the origin and the sink of moisture, respectively. The NLLJs in Argentina and Brazil-S are characterized by the predominance of northerly and northeasterly low-level winds, respectively, in the austral winter and other seasons. The main moisture sources for these NLLJs are from the tropical/subtropical South Atlantic Ocean and Amazon basin, which are stronger in the austral summer-autumn and weaker in the winter. For both NLLJs, the local moisture sources also are intense over the year. Northeasterly low-level wind characterizes the NLLJ in Brazil-SE throughout the year, having the tropical-subtropical South Atlantic Ocean as its main remote moisture source. Local moisture sources are also found to contribute to the moisture during the Brazil-SE NLLJ episodes. For the subtropical NLLJs (Argentina, Brazil-S, and Brazil-SE), the moisture transported could precipitate (their moisture sinks) over the center-north of Argentina, southern Brazil, part of southeastern Brazil, and the subtropical South Atlantic Ocean. As for the moisture sources, subtropical NLLJs have relatively stronger moisture sinks during the austral summer and fall.

Throughout the year, the NLLJ in Brazil-NE receives moisture from the TSA Ocean through the intensification of the low-level easterly/southeasterly trade winds. As this jet is very frequent throughout the year, weak variations in the intensity of the associated moisture sources may also occur. The moisture transported by this NLLJ will precipitate downstream over remote and wide areas of the tropical and subtropical South America. The moisture sources for the tropical NLLJs (Brazil-N and Venezuela) come from the tropical North Atlantic Ocean in the austral summer and fall. In the austral winter and spring, the TSA Ocean appears to be an additional source of moisture for both NLLJs. The Brazil-N and Venezuela NLLJ moisture sinks (precipitation) are located downstream from the jet core, occupying a large (small) area of western South America in austral summer-fall (winter-spring).

Low- and high-level circulation anomalies for the subtropical NLLJs (Argentina, Brazil-S, and Brazil-SE) show an equivalent barotropic vertical structure with the presence of an anomalous anticyclone located to the east-southeast of the jet cores. In low levels, the jet is characterized by the intensification of north/northeasterly winds on the western side of the anomalous

anticyclone, which generate precipitation (negative anomaly of moisture sinks) downstream of the NLLJ cores. A dipole pattern is found with increases and decreases, respectively, in the precipitation over southern and southeastern Brazil during the establishment of the NLLJ in Brazil-SE. In addition, for tropical NLLJs, the core region is characterized by the suppression (positive moisture sink anomaly) of the precipitation with enhanced precipitation occurring downstream. In addition, the moisture sink associated with the NLLJ is positively statistically significantly correlated with precipitation for most of the seasons.

The methodology of the NLLJ identification allowed us to describe some new and less explored regions of activity of the NLLJs (Brazil-S, Brazil-SE, Brazil-NE, and Argentina) as well as the associated moisture transport over South America. Thus, research contributes to improving our understanding of the NLLJs and their role in transporting moisture and controlling precipitation over the continent according to the season of a year. However, the role of the NLLJs and their relationship with the interannual variability of the moisture sources and sinks need to be further explored. A better understanding of the NLLJ modulation on the climate variability may help to improve the seasonal to sub-seasonal climate forecasting in the region. A more detailed study in this direction is underway. Another point in the future will be the use of new ERA5 reanalysis data, which are available with finer resolution, from hour to hour, and also with more vertical levels.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

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DB processed and analyzed the data and wrote the manuscript. IA, RN, and LG contributed with data processing and analysis of the Flexpart model. TA and RR in the process of analyzing the results of nocturnal low-level jets in continental South America. All authors discussed the results and worked together on the manuscript.

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The Volatilome: A Vital Piece of the Complete Soil Metabolome

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Soils harbor complex biological processes intertwined with metabolic inputs from microbes and plants. Measuring the soil metabolome can reveal active metabolic pathways, providing insight into the presence of specific organisms and ecological interactions. A subset of the metabolome is volatile; however, current soil studies rarely consider volatile organic compounds (VOCs), contributing to biases in sample processing and metabolomic analytical techniques. Therefore, we hypothesize that overall, the volatility of detected compounds measured using current metabolomic analytical techniques will be lower than undetected compounds, a reflection of missed VOCs. To illustrate this, we examined a peatland metabolomic dataset collected using three common metabolomic analytical techniques: nuclear magnetic resonance (NMR), gas chromatography-mass spectroscopy (GC-MS), and fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). We mapped the compounds to three metabolic pathways (monoterpenoid biosynthesis, diterpenoid biosynthesis, and polycyclic aromatic hydrocarbon degradation), chosen for their activity in peatland ecosystems and involvement of VOCs. We estimated the volatility of the compounds by calculating relative volatility indices (RVIs), and as hypothesized, the average RVI of undetected compounds within each of our focal pathways was higher than detected compounds ($p < 0.001$). Moreover, higher RVI compounds were absent even in sub-pathways where lower RVI compounds were observed. Our findings suggest that typical soil metabolomic analytical techniques may overlook VOCs and leave missing links in metabolic pathways. To more completely represent the volatile fraction of the soil metabolome, we suggest that environmental scientists take into consideration these biases when designing and interpreting their data and/or add direct online measurement methods that capture the integral role of VOCs in soil systems.

Keywords: soil metabolome, volatilome, volatile organic compounds, soil microbial processes, GC-MS, FT-ICR-MS, PTR-TOF-MS, metabolomics

Abbreviations: Mono Bio, monoterpenoid biosynthesis; Di Bio, diterpenoid biosynthesis; PAH Deg, polycyclic aromatic hydrocarbon degradation; RVI, relative volatility index; NMR, nuclear magnetic resonance; GC-CI-MS, gas chromatography coupled to chemical ionization mass spectrometry; GC-EI-MS, gas chromatography electron-ionization mass spectrometry; LC-EI-MS, liquid chromatography electron-ionization mass spectrometry; FTICR-MS, fourier-transform ion cyclotron resonance mass spectrometry; PTR-TOF-MS, proton-transfer-reaction time-of-flight mass spectrometry.

INTRODUCTION

As a complex and heterogeneous ecosystem, soil harbors a myriad of biological processes that are challenging to uncover. Two major contributors to biological soil processes are microbial communities and plant roots. Soil microbial communities are engines of chemical interconversion—microbes produce and consume chemical substrates for metabolism, generating metabolites as waste by-products. Tightly interwoven with the soil microbial network, plant roots also emit metabolites as exudates (Bertin et al., 2003; Vives-Peris et al., 2020). These diverse metabolites form the metabolome, which can be interrogated to provide insight into belowground biological processes. Volatile metabolites, constituting the volatilome (Amann et al., 2014), represent a part of the comprehensive metabolome. Volatile organic compounds (VOCs) have high vapor pressures causing them to enter the gaseous phase depending on environmental conditions. While atmospheric chemists routinely measure VOCs directly using specialized techniques, VOCs are rarely considered in the burgeoning collection of soil microbial metabolomic studies. *Could removing bias in sample processing and including more direct measurements of VOCs help fill in a missing, important part of the soil metabolome?*

Challenges inherent to most approaches evaluating soil organic matter composition and soil metabolomics make it difficult to simultaneously detect VOCs. First, soil metabolomic field studies start with collecting soil samples to bring back to the lab for sample processing and metabolic measurements. These ex-situ methods overlook VOCs that escape to the atmosphere either prior to sample collection or during disruptive soil sampling (Hewitt, 1996). This artifact may even affect VOC-resolving techniques, such as gas chromatography-mass spectrometry (GC-MS), where VOC loss to the atmosphere prior to sample analysis is a possibility (Eriksson et al., 2001). Second, some metabolomics measurement techniques require an initial liquid chromatography (LC) step, as is the case with LC-mass spectrometry (LC-MS) to characterize compound structure. This process immediately limits the detectable VOCs to those dissolved in the liquid phase. The solvent used to prepare samples in the lab for LC and other direct injection methods can also bias the extracted metabolites from soil samples; methanol preferentially extracts semipolar to nonpolar molecules, whereas water extracts polar molecules (Hollywood et al., 2006). Furthermore, soluble VOCs may partition between the gas and liquid phases making quantification unreliable, while insoluble VOCs will go undetected. Finally, inherent biases, including target size of compounds and ionization mode (positive vs negative), exist for each of the most widely used metabolomic analysis techniques (Table 1). Some of these challenges to measuring the soil volatilome may be addressed by adapting online measurement methods from atmospheric chemistry that directly measure VOCs in the gas phase such as proton-transfer-reaction time of flight MS (PTR-TOF-MS). Increasingly, the soil volatilome is being characterized in its own right using these approaches, either by measuring gases at the soil-atmosphere surface (e.g., using soil incubation chambers

[Asensio et al., 2007]), and recently with belowground diffusive probes to measure VOCs in situ (Gil-Loaiza et al., 2020).

VOCs also play important ecological roles in soil and influence atmospheric chemistry. Unlike their non-volatile counterparts, VOCs readily exchange between the soil and atmosphere. In the atmosphere, VOCs are active in photochemical reactions and secondary aerosol formation, and thereby affect air quality (Chameides et al., 1988; Park et al., 2013; Ghirardo et al., 2020), climate (Müller et al., 2017), and precipitation dynamics (Zhao et al., 2016). In the soil, VOCs are important signaling molecules that drive microbe-microbe, plant-microbe, and plant-plant interactions (Penuelas et al., 2014). Specifically, like non-volatile metabolites, microbial VOCs can promote plant growth (Tahir et al., 2017; Tyagi et al., 2018) and plant-root VOCs can either deter or attract microbes (Bitas et al., 2013). But unlike non-volatile metabolites, VOCs diffuse through soil more readily, extending their zone of influence. For example, while the rhizosphere zone influenced by root exudates may be restricted to millimeter-scales for non-volatile compounds, VOCs may diffuse centimeters or farther from roots, thereby extending the reach of the effective rhizosphere (de la Porte et al., 2020). These examples emphasize some of the unique roles of VOCs in the soil and signify that capturing VOCs within the complete soil metabolome is important for resolving belowground processes and aboveground interactions.

Despite the important roles of soil VOCs, we argue that VOCs have often been overlooked in soil metabolomic studies. These studies typically map metabolites to metabolic pathways to guide expectations for specific biological processes. However, metabolite volatility is rarely considered, even in cases where standard metabolomic analytical techniques capture VOCs. In fact, the volatility of many soil organic compounds is unknown, confounding our current understanding of whether VOCs are underrepresented. Despite a lack of measurements of the volatility of organic compounds, tools that predict volatility from molecular functional groups (Hilal et al., 2007; Nannoolal et al., 2008; Pankow and Asher, 2008) have been widely adopted by the atmospheric chemistry community.

Here, we integrate disciplinary approaches to predict metabolite volatility in three VOC-containing metabolic pathways. We show that compounds with high volatility are disproportionately undetected in a peatland metabolomic dataset derived from three techniques (GC-MS, Fourier-transform ion cyclotron resonance MS [FTICR-MS] by direct injection, and nuclear magnetic resonance [NMR]). The peatland ecosystem is ideal for evaluating the representation of VOCs in metabolomics because we expect high quantities of VOCs (Seewald et al., 2010) as fermentation products of anaerobic metabolism in water-logged, anoxic conditions. This approach establishes a baseline understanding of the soil volatilome and the implications of its underrepresentation in current soil metabolomics studies.

CONCEPT

To predict the volatility of metabolites along metabolic pathways, we adapted tools used to estimate VOC partitioning

TABLE 1 | Analytical capabilities and biases of common soil metabolomic analytical techniques.

Method	Measurement and chemical interpretation	Biases					Recommendations for targeting VOCs and other biases
		<i>In situ</i> measurement?	Sample extraction?	Chemical mass range	Ionization?	Limited to database?	
Sample Extraction	- Water (polar compounds). - Methanol (non-polar compounds). - Folch sequential methanol, chloroform, and water (non-polar and polar compounds in bi-phasic layers.)* (Only the top aqueous layer for this study).						- Folch extraction biased against terpenes. - Recommend using solvent that maximizes VOC extraction (Rowan, 2011), or solvent-free method (e.g., SPME).
NMR*	- Measures interaction of nuclear spins under magnetic fields.* - Extracted samples dried and reconstituted in water.* - Obtain structural isomers/chemical structures and compound concentrations.*	no	yes	<100 Da	no	yes	- Targets primary metabolites only, including small VOCs. - Low sensitivity.
GC-EI-MS*	- Measures retention times and m/z. - Samples dried and GC derivatized.* - Obtain retention time index, m/z charge, and fragmentation pattern.*	no	yes	1–variable Da	yes	yes	- Choose column/instrument design that will maximize VOC detection. - Can collect samples in absorptive cartridges to trap VOCs.
LC-EI-MS	- Measures retention times and m/z on liquid samples. - Obtain retention time index, m/z charge, and fragmentation pattern.	no	yes	1–variable Da	yes	yes	- Not recommended for VOCs
FT-ICR-MS*	- Measures m/z of liquid phase (ESI*) and gas phase (APPI). - Obtain exact m/z, then perform formula assignment.*	no	yes	200–1,200 Da	yes	no	- Affected by ionization efficiency (i.e., carboxylic acid ionizes better than sugars). - Mass may match multiple isomers.
PTR-TOF-MS	- Measures m/z of gas-phase molecules. - Obtain exact m/z with elemental identification (e.g., C _x H _y O _z) of gas-phase molecules	yes	no	19–500 Da	yes	no	- Good for VOCs. - Some fragmentation and low transmission for low volatility analytes.
GC-CI-MS (e.g., GC-PTR-TOF-MS)	- Measures retention times and m/z of gas-phase molecules. - Direct injection - Obtain structural identification (e.g., benzene) by combining elemental identification with retention.	yes	no	19–500 Da	yes	yes	- Good for VOCs. - Volatility range is dependent on the chromatography column.

*Methods used in peatland dataset presented in this paper. NMR: Varian Direct Drive 600 MHz spectrometer. GC-EI-MS: HP-5MS column and Agilent GC 7980A coupled with single quadrupole MSD 5975C. FT-ICR-MS: 12 Tesla Bruker FTICR mass spectrometer.

NMR, nuclear magnetic resonance; GC-EI-MS, gas-chromatography electron-ionization mass spectrometry; SPME, solid phase microextraction; LC-EI-MS, liquid-chromatography electron-ionization mass spectrometry; APPI, atmospheric pressure photo ionization; ESI, electron spray ionization; m/z, mass-to-charge ratio; FTICR-MS, fourier-transform ion cyclotron resonance mass spectrometry; PTR-TOF-MS, proton-transfer-reaction time-of-flight mass spectrometry; GC-CI-MS, Gas-chromatography coupled to chemical ionization mass spectrometry.

between the gaseous and condensed aerosol phases under the assumption of standard conditions (i.e., temperature and pressure) and dry sorbent material. Detailed discussions of properties of and factors affecting volatility have been well

described elsewhere (Hilal et al., 2003; Nannoolal et al., 2008; Compennolle et al., 2011; Tang et al., 2019). We calculated metabolite vapor pressure (P; atm) using SIMPOL.1 (Pankow and Asher, 2008), which accounts for the impact of functional

groups. Specifically, we estimated vapor pressure using the following equation:

$$\log_{10} P = b_0 + \sum_k v_k b_k.$$

for functional groups $k = 1, 2, 3, \dots$, where b_0 is a constant, b_k is the functional group contribution term for group k , and v is the number of groups of type k in the compound. For example, hydroxyl ($-\text{OH}$), carboxylic acid ($-\text{C}[\text{O}]\text{OH}$), ketone ($\text{O} = \text{O}$), carbon double bond ($\text{C} = \text{C}$), and aromatic ring functional groups each decrease P to a different degree. The method did not specify the impact of phosphate groups, which are common in metabolic pathways, so we used nitrate as a proxy with the assumption that these functional groups have a similar relative contribution to P (Nannoolal et al., 2008).

The volatility of VOCs in a given environment can be expressed by the tendency to partition to the gas vs condensed phase. In the atmosphere, VOC partitioning (ξ) to the gas phase increases with vapor pressure (C^*), which is by convention converted using the ideal gas law to mass-based saturation vapor concentration ($\mu\text{g m}^{-3}$) that accounts for molecular mass ($\log_{10} C^* = \log_{10}(PM/RT)$, where M is molecular mass, R the universal gas constant, and T temperature). Under clean atmospheric conditions, thresholds for nonvolatile, intermediate volatility, and volatile are on the order $C^* = 0.01 \mu\text{g m}^{-3}$, $1 \mu\text{g m}^{-3}$, and $100 \mu\text{g m}^{-3}$, respectively, or more conveniently on a log scale: $\log_{10} C^* = -2, 0$, and 2 (Donahue et al., 2006). VOC partitioning also depends on the total availability of condensed-phase organic molecules (e.g., total aerosol; C_{Total}). The same VOC will appear less 'volatile' in a polluted atmosphere with greater partitioning on high aerosol loadings [$\xi_i = 1/(1+C_i^*/C_{\text{Total}})$]. Soil contains large quantities of organic matter, but theories linking soil C_{Total} to thresholds for volatility in the subsurface have not been established. Here, we therefore report a relative volatility index (RVI) using $\log_{10} C^*$ ($\text{RVI} = \log_{10} C^*$) as the volatility scale, with the understanding that gas phase partitioning will be dependent upon the environment in soil pores (temperature, moisture, pressure) or at the soil-atmosphere interface. While the RVI does not give an absolute indication of whether a compound is volatile in the soil, it can be used to compare compound volatilities relative to one another.

To provide a reference framework for assessing the extent of detected volatiles, we used metabolic pathway maps that visualize metabolic reactions and their intermediates. Metabolic pathway maps, such as those in the KEGG metabolic database (Kanehisa and Goto, 2000), help soil scientists visualize active soil processes. To illustrate our concept, we assessed a peatland soil metabolomic dataset (generated using typical soil metabolomic analytical techniques that first extracted metabolites from soil (Folch et al., 1957) for analysis by FTICR-MS, ^1H NMR, and GC-MS (Wilson et al., 2021) (methods included in **Table 1**). While FTICR-MS provides compound masses that can be used to predict formulae, ^1H NMR, and GC-MS provides m/z values that must be matched to compounds in reference databases. For

all metabolite analysis, previously reported methods, standards for peak picking, compound databases, and formula assignment were used (e.g., Weljie et al., 2006; Hiller et al., 2009; Kind et al., 2009; Tolici et al., 2017; Tfaily et al., 2018).

All metabolomic data were combined, matched to KEGG compound IDs when possible, and mapped to pathways using the KEGG Pathway Mapper (Kanehisa and Sato, 2020). We note that some formulas matched to more than one isomer or compound, and all matches were included in this analysis. We selected three VOC-containing pathways that we expect to be present in peatland ecosystems: (1) monoterpene biosynthesis (Mono Bio), describing the formation of monoterpenes which are highly volatile; (2) diterpene biosynthesis (Di Bio), describing the formation of diterpenes which include many non-volatile compounds with a few exceptions; and (3) polycyclic aromatic hydrocarbon degradation (PAH Deg), describing the breakdown of hydrocarbons and including many semi-volatile compounds. Along these pathways, we calculated average RVIs for detected and undetected compounds in the peatland dataset and tested for significant differences using non-parametric Mann Whitney U -test.

OVERALL PATTERNS

In all our focal pathways, most compounds were detected using FTICR-MS, with only phthalate detected by GC-MS and catechol by NMR (both from the PAH Deg pathway). All these pathways contain mostly secondary metabolites, compounds that tend to exceed the detectable target range of NMR (**Table 1**). NMR is well suited to measure smaller molecules that are in higher abundance, particularly primary metabolites, some of which are VOCs (i.e., catechol, methanol, and acetone). Indeed, the average RVI of NMR-detected compounds across all three pathways was $+2.1 \pm 7.2$. Therefore, NMR may be a good option for detection of small, primary VOC metabolites, however, the technique suffers from biases that may preclude detection of many VOCs (**Table 1**). On the other hand, based on the RVIs of the compounds in our focal pathways, the GC-MS and FTICR-MS standard soil metabolomic analysis techniques were better suited to detect compounds with lower volatility.

Biosynthesis Pathways

Diterpene Biosynthesis

Diterpenoids are a class of molecular compounds containing four joined isoprene (C_5H_8) units that include momilactones, oryzalexins, gibberellins, and kaurenes. The capacity to biosynthesize diterpenoids is present in plants, fungi, and select bacteria (Gutiérrez-Mañero et al., 2001; Zi et al., 2014; Tang et al., 2015). The roles of diterpenoids in plants are diverse and include pathogen defense, plant growth effectors, signaling, and abiotic stress responses (Lu et al., 2018; Murphy and Zerbe, 2020). Fungi and bacteria also produce diterpenoids as antimicrobial agents and plant growth promoters (de Boer and de Vries-van Leeuwen, 2012; Zhao et al., 2018).

We elaborated on the Di Bio pathway because a high proportion of metabolites were detected in the peatland dataset (40.8%; **Figure 1A**), strongly suggesting that this metabolism was active in the peatland. Previous studies in peatland ecosystems examining VOC emissions in situ (PTR-TOF) also detected diterpenes (Li et al., 2020), signifying the presence of volatile diterpenes in peatlands. In our peatland dataset, the average RVI of undetected compounds was significantly higher than those detected ($RVI_{undetected} = -0.97 \pm 5.7$ vs $RVI_{detected} = -4.6 \pm 2.8$, $p < 0.0001$; **Figure 1A**). After removing five undetected outliers with extremely low RVIs (-14 , -16 , -19 , -21 , and -26), $RVI_{undetected} = +0.04 \pm 3.4$. This indicates a preference for detection of compounds with lower volatilities by standard metabolomic methods.

A closer examination of the gibberellin biosynthesis sub pathway within the Di Bio pathway shows that almost all of the gibberellins were detected in the peatland dataset; however, there were a series of undetected intermediate compounds on the pathway to their production (**Figure 1B**). Gibberellins are plant hormones that promote growth and root elongation (Tanimoto, 2005), and even some plant growth promoting bacteria can also produce gibberellins (Gutiérrez-Mañero et al., 2001; Bottini et al., 2004). Within this sub pathway, five compounds between *ent*-Copalyl-PP and GA12-aldehyde were undetected (**Figure 1B**) and, furthermore, had higher average RVIs than the detected compounds ($RVI_{undetected} = +0.34 \pm 2.2$ vs $RVI_{detected} = -5.4 \pm 2.1$, $p < 0.0001$). To support the SIMPOL.1 prediction that these undetected kaurene compounds had high volatility, previous research has found kaurene to be volatile and emitted by plant flowers, stems, leaves, and roots (Yáñez-Serrano et al., 2018). The capacity to synthesize kaurene compounds within this sub pathway is shared between plants, fungi, and bacteria (Salazar-Cerezo et al., 2018). Therefore, kaurene should be produced regardless of source, but may remain undetected due to its higher volatility.

Monoterpenoid Biosynthesis

Monoterpenoids are molecules with two joined isoprene units that include α -pinene, linalool, camphor, and iridoids. Plants are the primary producers of monoterpenoids, and there are very few reports of bacteria or fungi capable of this synthesis (Penuelas et al., 2014). It is well established that plants emit monoterpenes from flowers to attract pollinators (Barragán-Fonseca et al., 2020) and from their roots to attract beneficial microbes and small eukaryotes like nematodes (Ali et al., 2011). This release of monoterpenes also deters pathogens using their antimicrobial and anti-fungicidal attributes (Lee et al., 2016; Reis et al., 2016).

We focused on the Mono Bio pathway map because, unlike diterpenes, most monoterpenes are volatile, and we therefore expected fewer compounds from this pathway to be present in our peatland dataset. Indeed, we detected only 20% of the compounds in the Mono Bio pathway (**Figure 1A**). As expected, the overall RVIs were higher in the Mono Bio than the Di Bio pathway, signifying a higher overall volatility of the monoterpene compounds ($+2.7$ vs. -1.6 , respectively). Similar to the Di Bio pathway, the average volatility of the compounds detected were

significantly lower than those undetected ($RVI_{undetected} = +5.4$ vs. $RVI_{detected} = -1.3$, $p < 0.001$).

The sub pathway to iridoid compound biosynthesis contained a majority of the detected compounds including loganin, secologanin, and laganate (**Figure 1C**). These detected iridoids had a lower volatility than the seven missing compound intermediates stemming from Gereanyl-PP ($RVI_{undetected} = +2.79$ vs. $RVI_{detected} = -8.94$, $p < 0.001$; **Figure 1C**). This represents another example of intermediate metabolites that should be present in the soil but are absent from the dataset and have a significantly higher volatility.

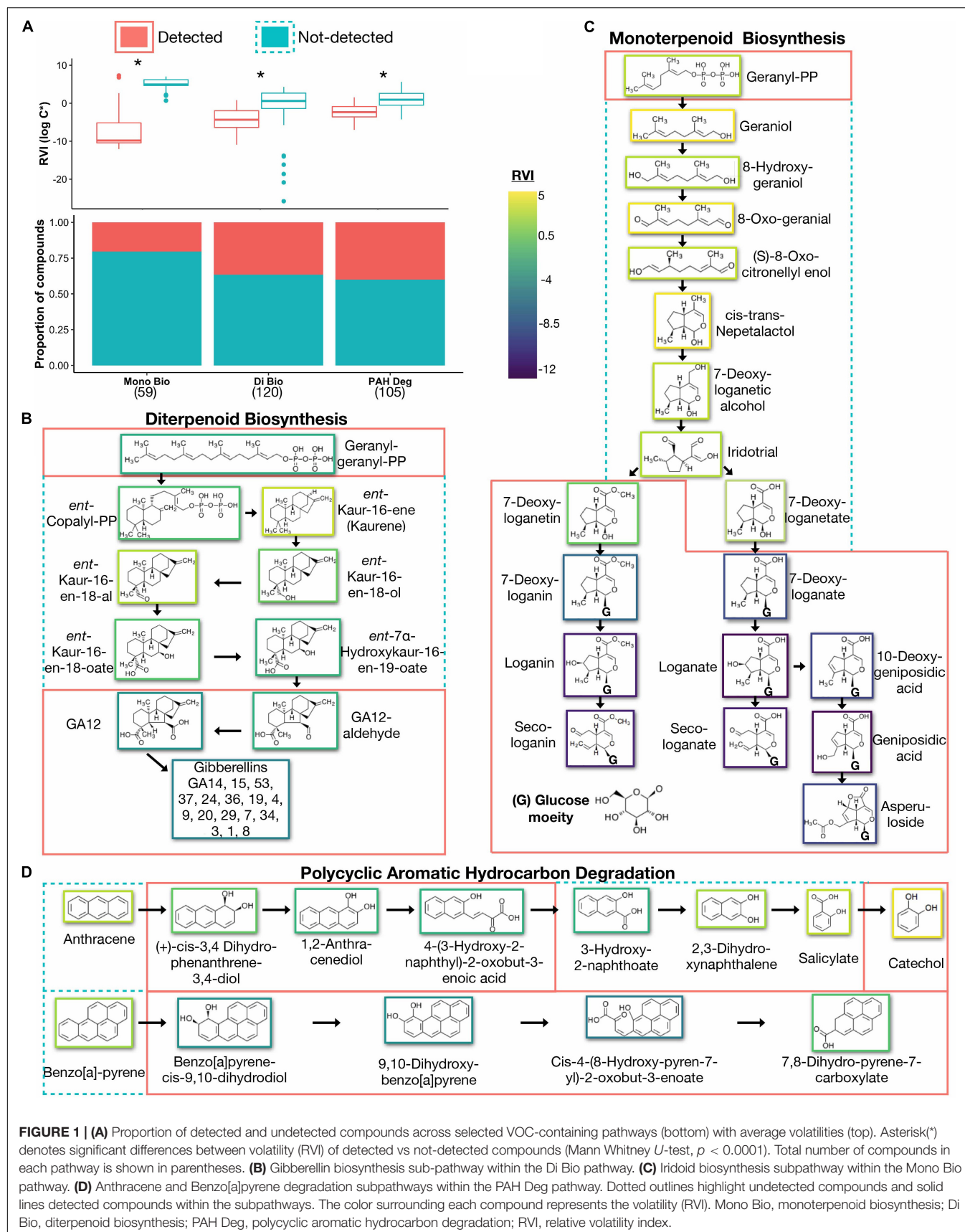
Degradation Pathway

Polycyclic Aromatic Hydrocarbon Degradation

Polycyclic aromatic hydrocarbons (PAHs), a class of chemicals with two or more benzene rings fused together, occur naturally in coal, oil, and gasoline or can be produced through the incomplete combustion of these biomasses. PAHs in soils are often from anthropogenic sources as fossil fuel combustion creates atmospheric emissions that deposit on land (Malawska et al., 2006), but natural PAHs can also form, for example, from microbial decomposition of plant residues in aeration-exposed peatlands that go through seasonal thaws (Gabov et al., 2020). Soils, particularly peatlands, are the main reservoir for PAHs in the environment (Wilcke and Amelung, 2000) where PAHs are persistent and difficult to break-down due to their hydrophobic properties causing them to strongly bind to soil particles (Gabov et al., 2020). Some bacteria (Déziel et al., 1996; Ghosal et al., 2016) and fungi (Hammel, 1995; Kadri et al., 2017) are capable of PAH degradation, however, in peatlands, PAHs accumulate at fast rates due to low degradation rates in the highly anaerobic conditions with high organic content. Yet, some peatlands are capable of higher degradation rates; for example, *Ledum* peatlands can degrade PAHs at a greater rate than *Sphagnum* peatlands (Wang et al., 2019).

We focused on the PAH Deg pathway because, as expected, our peatland dataset contained a high number of compounds this pathway (40%, **Figure 1A**). Furthermore, PAHs and their degradation products are semi-volatile (Ghosal et al., 2016), therefore, have the potential to be missed using standard metabolomic techniques. Consistent with our above findings, the average volatility of the detected compounds was significantly lower than those that were undetected ($RVI_{undetected} = +0.88 \pm 2.4$ vs $RVI_{detected} = -2.2 \pm 2.1$, $p < 0.001$).

Compounds from sub-pathways within the PAH Deg pathway were detected in the peatland dataset to different degrees. From the benzo[a]pyrene degradation sub-pathway, almost all compounds were detected, except for benzo[a]pyrene itself. Benzo[a]pyrene is the largest PAH with six rings, and the compounds from this sub pathway had the lowest volatilities overall (average RVI = -3.5 ± 2.3). In contrast, compounds from the other three sub-pathways for pyrene, anthracene, phenanthrene, and fluorene have more patchy detection, but also higher RVIs on average. For example, anthracene, a PAH with



three rings, and its degradation products have on average RVI of $+0.20 \pm 2.6$ (for comparison of Benzo[a]pyrene and anthracene degradation see **Figure 1D**).

CONCLUSION

Here, we provide compelling evidence that typical soil metabolomic analytical techniques miss some soil VOCs and therefore underestimate the role of the volatilome in the total soil metabolome and make it difficult to conclude which pathways are active. We showed that compounds undetected in a peatland dataset had significantly higher estimated volatilities than those detected within the context of three important VOC-containing metabolic pathway. There are several reasons that a compound could be undetected, including low steady state concentrations, chemical instability, short lifetime, and fast metabolism. While these other processes could be affecting compound detectability, we argue that non-volatile compounds are just as susceptible as volatile compounds, and, therefore, do not affect our conclusions. Given the plethora of known and currently unknown metabolic pathways in soil, these results only begin to unearth the potential for a missing volatilome in current soil metabolomic research projects.

Already, researchers often use more than one measurement method because no single technique can capture all metabolites in a sample. While existing techniques can be tuned to target specific compounds, there is currently no global method that can provide molecular identification of all chemicals in a system at high time resolution. Each technique is specialized to target different sizes and classes of compounds and comes with its own biases depending on how samples were processed and data analyzed (**Table 1**). Furthermore, biases inherent in sample collection, extraction, and measurement can compound, therefore, the methodology for each step should be carefully considered. Adjustments in sample processing (i.e., collecting samples in air-tight containers or capturing VOCs in adsorptive cartridges) and selection of analysis methods could help gear soil metabolomic measurement techniques to capture more VOCs. Additionally, in situ VOC measuring techniques, such as PTR-TOF-MS or GC-CI-MS (**Table 1**), could be added to the soil metabolomic repertoire to directly target volatile

metabolites. Moreover, online VOC analytical techniques yield faster measurements than FT-ICR-MS, GC/LC-EI-MS, and NMR, with near continuous (1Hz) data for uninterrupted analysis by PTR-TOF-MS, and longer sample measurement time for GC-CI-MS, but with greater structural information. New efforts to integrate VOC measuring techniques to capture and characterize the complete soil metabolome will provide a deeper understanding of the complex biological processes occurring belowground.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: SPRUCE long-term repository (doi: <https://doi.org/10.25581/spruce.083/1647173>).

AUTHOR CONTRIBUTIONS

LM, LH, JK, and MT conceived of the concept and approach. LH and LM wrote the manuscript. MT provided peatland dataset. LH curated the data and chose metabolic pathways. KG and LH calculated compound RVIs. JK contributed his expertise in volatility predictive models. LH produced the figure. All authors contributed to editing the manuscript.

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Pesticides and Soil Invertebrates: A Hazard Assessment

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Agricultural pesticide use and its associated environmental harms is widespread throughout much of the world. Efforts to mitigate this harm have largely been focused on reducing pesticide contamination of the water and air, as runoff and pesticide drift are the most significant sources of offsite pesticide movement. Yet pesticide contamination of the soil can also result in environmental harm. Pesticides are often applied directly to soil as drenches and granules and increasingly in the form of seed coatings, making it important to understand how pesticides impact soil ecosystems. Soils contain an abundance of biologically diverse organisms that perform many important functions such as nutrient cycling, soil structure maintenance, carbon transformation, and the regulation of pests and diseases. Many terrestrial invertebrates have declined in recent decades. Habitat loss and agrichemical pollution due to agricultural intensification have been identified as major driving factors. Here, we review nearly 400 studies on the effects of pesticides on non-target invertebrates that have egg, larval, or immature development in the soil. This review encompasses 275 unique species, taxa or combined taxa of soil organisms and 284 different pesticide active ingredients or unique mixtures of active ingredients. We identified and extracted relevant data in relation to the following endpoints: mortality, abundance, biomass, behavior, reproduction, biochemical biomarkers, growth, richness and diversity, and structural changes. This resulted in an analysis of over 2,800 separate “tested parameters,” measured as a change in a specific endpoint following exposure of a specific organism to a specific pesticide. We found that 70.5% of tested parameters showed negative effects, whereas 1.4% and 28.1% of tested parameters showed positive or no significant effects from pesticide exposure, respectively. In addition, we discuss general effect trends among pesticide classes, taxa, and endpoints, as well as data gaps. Our review indicates that pesticides of all types pose a clear hazard to soil invertebrates. Negative effects are evident in both lab and field studies, across all studied pesticide classes, and in a wide variety of soil organisms and endpoints. The prevalence of negative effects in our results underscores the need for soil organisms to be represented in any risk analysis of a pesticide that has the potential to contaminate soil, and for any significant risk to be mitigated in a way that will specifically reduce harm to soil organisms and to the many important ecosystem services they provide.

Keywords: pesticide, soil, regulation, EPA, invertebrate, terrestrial

INTRODUCTION

Soils are arguably the most complex and biodiverse ecosystems on earth, containing nearly a quarter of the planet's diversity (Ram, 2019). A handful of soil contains an estimated 10 – 100 million organisms belonging to over 5,000 taxa (Ramirez et al., 2015), only a small percentage of which have been described (Adams and Wall, 2000). A typical functional soil community is comprised of hundreds to thousands of species of macroinvertebrates and nematodes as well as a vast abundance of microorganisms, including hundreds of fungal and thousands of bacterial species (Bardgett and van der Putten, 2014; Ram, 2019; Singh et al., 2019).

Soil invertebrates perform a variety of different ecosystem services essential for agricultural sustainability. Soil biodiversity enables self-perpetuating ecosystem functions that fuel specialized processes such as soil structure maintenance, nutrient cycling, carbon transformations, and the regulation of pests and diseases (Balvanera et al., 2006; Perrings et al., 2006; Kibblewhite et al., 2008; Chagnon et al., 2015). Burrowing activity by soil organisms modifies soil porosity by increasing aeration, water infiltration and retention, and reducing compaction (Pisa et al., 2015; Ram, 2019). Earthworms alone can construct up to 8,900 km of channels per hectare, decreasing soil erosion by 50% via increased soil porosity and water infiltration (Blouin et al., 2013; Gaupp-Berghausen et al., 2015). Nutrients travel through multiple soil layers by means of foragers, tunnelers, and ground-nesting insects including beetles, ground-nesting bees, ants, and termites (Stork and Eggleton, 1992; Willis Chan et al., 2019), and detritivores like nematodes, springtails, earthworms, millipedes, and woodlice, transform decaying material and minerals into usable forms, cycle nutrients, and increase soil fertility (Stork and Eggleton, 1992; Kibblewhite et al., 2008; Ram, 2019). For example, nematodes and mites enable nitrogen mineralization by feeding on fungal roots and stimulating and regulating microbial activity (Stork and Eggleton, 1992). Dead invertebrates decay and add nitrogen to the soil (Stork and Eggleton, 1992). Soil invertebrates also form up to half of all soil aggregates by breaking down litter and releasing organically rich casts and feces (Stork and Eggleton, 1992). The formation of these large soil aggregates allows for greater soil carbon sequestration, thus these ecosystem engineers play a role in offsetting fossil fuel emissions and combating climate change (Lal, 2004a,b; Lavelle et al., 2006; Dirzo et al., 2014).

Many soil invertebrates also play a role in controlling agricultural pests. Nematodes and mites are used in targeting disease-related bacteria in crops (Stork and Eggleton, 1992; Kibblewhite et al., 2008; Ram, 2019). Predators and parasitoids, such as beetles and parasitic wasps prey on arthropods that interfere with crop production (Stork and Eggleton, 1992; Gill et al., 2016), and herbivorous soil insects can eat the seeds of undesirable plants selectively over crop seeds, reducing the spread of aggressive weeds (Honek et al., 2003).

Increases in land conversion and agricultural intensification accelerate the loss of soil biodiversity and, as a result, have contributed to the reduction of approximately 60% of soil ecosystem services (Díaz et al., 2006; Veresoglou et al., 2015;

Singh et al., 2019). Many insects that depend on soil for portions of their life cycle, like ground beetles and ground-nesting bees, as well as terrestrial insects and mites in North America, have declined greatly in recent decades (Forister et al., 2019; Sánchez-Bayo and Wyckhuys, 2019; van Klink et al., 2020; Sullivan and Ozman-Sullivan, 2021). Habitat loss due to agricultural intensification and pollution, primarily from synthetic agricultural pesticides and fertilizers, are thought to be the major driving factors in recent insect declines and are an increasing threat (Hallmann et al., 2017; Forister et al., 2019; Seibold et al., 2019; Sánchez-Bayo and Wyckhuys, 2019; Miličić et al., 2020). In a 2019 survey of member countries of the Food and Agriculture Organization of the United Nations (FAO), overuse of chemical control mechanisms (e.g., pesticides, antibiotics, etc.) was identified as the most impactful practice that has been driving the loss of soil biodiversity in the last 10 years (FAO, 2020).

From 1992 to 2014, DiBartolomeis et al. found that increased use of neonicotinoid insecticides and the environmental persistence of those insecticides drove a 48- and 4-fold increase in oral and contact toxicity load, respectively, for insects in agricultural environments using the European honeybee *Apis mellifera* L. as a proxy species (DiBartolomeis et al., 2019). The study focused on honeybees because they are the most extensively studied non-target insect within United States agroecosystems; in fact, they are the only terrestrial invertebrate for which the United States Environmental Protection Agency (EPA) requires testing during pesticide registration (Legal Information Institute, 2020). However, the results of this study also signify an increasing threat to soil invertebrates. Neonicotinoid insecticides accounted for 92% of the increase in invertebrate toxicity loading (DiBartolomeis et al., 2019), and 60% of neonicotinoid use is through seed treatments and soil application as of 2011 (Jeschke et al., 2011). Neonicotinoid seed treatments are estimated to be used in over half of soybean acres and nearly all non-organic corn grown in the United States (Douglas and Tooker, 2015; Mourtzinis et al., 2019). Because 80% or more of the active ingredients from neonicotinoid seed treatments remain in the soil (Sur and Stork, 2003; Alford and Krupke, 2017) soil organisms in these systems are likely to be exposed to high doses of these insecticides.

Large-scale use of seed-applied fungicides presents another risk, as almost all United States corn is also treated with seed-applied fungicides (Lamichhane et al., 2019). In addition to the pesticides that are currently on the market, several new seed- and soil-applied pesticide active ingredients are currently going through the registration process in the United States, such as the pyrazolecarboxamide fungicide ipyrrfluxam (U.S. EPA, 2020a), the diamide insecticide tetraniliprole (U.S. EPA, 2020d), and the novel insecticide broflanilide (U.S. EPA, 2020c).

The trend toward wider use of soil-applied pesticides — both individually and in combination with other active ingredients — will likely continue to grow. While soil organisms are at particular risk from exposure via direct soil applications, they can also be exposed to pesticides through other routes, such as drift from foliar sprays (Sánchez-Bayo, 2011), inclusion of pesticides in irrigation water (Sánchez-Bayo, 2011), or absorption of pesticides

into plant tissue that eventually returns to the soil through senescence of crop residues (Doublet et al., 2009). As this threat to soil organisms increases, it is important to strive for a more complete understanding of the impacts of all pesticides on soil invertebrates.

The word “pesticide” is an umbrella term used to describe an agent that targets a pest — in plant agriculture a pest is defined as an organism that causes harm to crops through direct damage or competition for nutrients and water — and includes insecticides, herbicides, fungicides, and bactericides, among others. Most review papers assessing pesticide impacts to soil organisms have focused on the environmental risks of specific classes of pesticides (Biondi et al., 2012; Pisa et al., 2015; Douglas and Tooker, 2016; Wood and Goulson, 2017), specific taxa affected (da Silva Souza et al., 2014; Pelosi et al., 2014; Römbke et al., 2017), or have analyzed only lab data (Frampton et al., 2006) or field data (Jänsch et al., 2006), making it difficult to identify big-picture trends and hazards that are necessary to inform general pesticide policy. To the best of our knowledge, there has only been one comprehensive overview on the effects of pesticides on a wide variety of soil organisms (Puglisi, 2012), which focused on soil microorganisms, particularly bacteria and fungi. Here we present a review focused on how broad types of pesticides affect the health of soil invertebrates. Given the scope of such a review, special attention is also paid to general effect trends among pesticide classes, taxa, and endpoints, as well as data gaps that should be addressed. Considering the key ecosystem services performed by soil invertebrates and the increase in the use of seed- and soil-applied pesticides, one of our objectives is to understand the overall hazard that pesticides pose to soil invertebrates to inform whether they should be included in regulatory ecotoxicological risk assessment to ensure that environmental harm is adequately estimated.

METHODS

Literature Search Methods

We conducted a preliminary literature search using Google Scholar to assess the impacts of pesticides on soil organisms. We used the following search terms: (pesticide OR herbicide OR insecticide OR fungicide) AND (soil OR terrestrial OR non-target OR invertebrate) to identify review papers that could be mined for further resources. The soil taxa included in the corresponding bibliographies informed more specific search terms for a second round of literature search combining (pesticide OR herbicide OR insecticide OR fungicide, and the three bactericides approved for agriculture in the United States, streptomycin, kasugamycin, and oxytetracycline, were also used as keywords) with taxa-specific search terms. To ensure an exhaustive search, multiple, diverse strings of keyword searches were used in the following databases and journals between the dates of October 2019 and January 2020; Agricola, Agris, Wiley-Blackwell, Directory of Open Access Journals, Karger, PubMed, Nature, SpringerLink, Environmental Toxicology and Chemistry, Chemosphere, Ecotoxicology and Environmental Safety, Ecotoxicology, Applied Soil Ecology, European Journal of Soil Biology, Environmental Pollution, Soil

and Tillage Research, Pesticide Biochemistry and Physiology, Science Direct/Elsevier, Jstor, PlosOne, Frontiers. The title and abstract of each identified paper were briefly scanned for relevance. From this we identified 1,028 studies. After further reviewing all abstracts, the number of studies was reduced to 394 (see **Supplemental References**). The majority of removed studies did not meet our inclusion criteria (outlined below) and a small number of studies could not be accessed, were not in English, or were not an original study. We separated studies conducted in a laboratory from those conducted in a field setting and present each as such. Some studies were conducted in both the laboratory and the field and, in those cases, the individual tested parameters in each study were designated as being done either in the laboratory or field.

Criteria and Relevance

For inclusion in this review, we established the following criteria: (1) The studied organism must be a soil-dwelling terrestrial invertebrate (see definition below) - aquatic organisms or terrestrial microbial organisms, such as bacteria and fungi, were not in the scope of our review; (2) The study must include soil-dwelling organisms that are not target invertebrates or common agricultural pests (for example, thrips, snails, or root feeding beetle larvae); (3) The pesticide studied must not be banned in the United States; and (4) The study must look at pesticide effects on non-target organism(s) with measurable endpoints. We did not include studies that focused on the behavior of pesticides, which includes the topics of sorption, transport, runoff, volatilization, degradation, dissipation, half-life, persistence, and leaching. While these interactions in the soil are necessary to understanding the environmental fate of pesticides, they are beyond the scope of this review. We also did not include studies that dealt with the efficacy of ecotoxicity testing methods, such as species-specific sensitivity, recommendations for test protocols, or new technologies for data collection.

Pesticides

Pesticides were limited to any agricultural insecticide, fungicide, herbicide, or bactericide that is not currently banned in the United States, using (Donley, 2019) to identify banned pesticides. This was done to exclude mainly legacy pesticides that are no longer subject to regulatory review in many parts of the world. We only included data on parent pesticide molecules and not their degradates or metabolites. We included studies done on formulations of pesticides as well as on individual active ingredients. Studies with two or more pesticide active ingredients applied together were categorized as mixtures and included in our analysis. Studies on fertilizers, genetically modified organisms, living pesticidal organisms, antibacterial agents not used in crop production, or any chemical not registered through the United States EPA as a pesticide were outside of the study scope. We only included studies on common veterinary medicines, such as abamectin, if the chemical was applied as a pesticide to plants and not directly to livestock. We included all soil studies on the antibiotics oxytetracycline, kasugamycin, and streptomycin,

because they are the only antibiotics currently approved for agricultural application to crops in the United States.

Soil Organisms

We defined soil organisms as any non-target invertebrate that has egg, larval, or immature development in the soil (Paoletti and Purrington, 1991). We excluded taxa from our data when there was any uncertainty of the location of the egg, larval, and/or immature development of the organism. Many soil-associated invertebrates such as various species of Arachnids, Diptera, or Hymenoptera were therefore not included. In addition, no aquatic organisms or terrestrial microbial organisms such as bacteria and fungi were included. The soil organisms that fit our criteria from the relevant studies are organized by taxa in the Results section and include Oligochaeta (earthworms), Enchytraeidae (potworms), Nematoda (roundworms), Tardigrada (water bears), Acari (mites), Myriapoda (centipedes and millipedes), Isopoda (woodlice), Collembola (springtails), Protura (coneheads), Isoptera (termites), Coleoptera (beetles), Formicidae (ants), *Bombus* spp. (bumble bees), other ground-nesting bees, and parasitic wasps, as well as various combinations of these taxa studied together.

Organization of Data, Definition of “Tested Parameter,” and Determination of Effects

We identified and extracted the relevant tested parameters in each study. A “tested parameter” was defined as a unique combination of the following variables: pesticide, organism, and endpoint. All tested parameters measured a specific endpoint following exposure of a specific organism to a specific pesticide. For instance, if a study tested how three different pesticides (chlorpyrifos, imidacloprid, and permethrin) affected two endpoints (mortality and DNA damage) on one species (*Caenorhabditis elegans*), then we would be able to extract six unique tested parameters, any of which could result in a negative effect, positive effect or no significant effect to the tested species. To manageably organize the data, we distilled variables other than “pesticide,” “endpoint,” or “species” into one tested parameter as often as possible. In studies that analyzed multiple substrates, we only entered data from soil tests but also included studies if they only utilized surrogate substrates, such as filter paper. Similarly, if there were multiple time periods utilized to determine LC50 or EC50, we reported data from only the standard 48 h or seven days, whichever was relevant. If there were multiple soil types, temperatures, pesticide concentrations, or moisture contents, we extracted the full data range rather than separating each variable as its own tested parameter. For instance, if three different concentrations of chlorpyrifos were tested on *C. elegans* mortality, only one tested parameter was recorded. If any of the three concentrations caused a positive or negative effect on *C. elegans* mortality, then the tested parameter was categorized as having a positive or negative effect. If all

three concentrations caused no significant effect, then it was categorized as having no effect.

We considered an effect negative or positive if the authors reported a statistically significant change from that of the control. If a study reported an effect on an endpoint over a time course, we considered the effect positive or negative if there was a significant effect in any of the tested times (e.g., earthworms significantly affected three out of ten days or one out of six years). An effect was considered “positive” if there was an unambiguous benefit to the organism from the associated effect, such as an increase in abundance or reproduction compared to control. All other significant changes were categorized as “negative.” Tested parameters that measured biochemical biomarker endpoints were always considered “negative” if there was any significant change compared to the control. A small minority of studies lacked statistical analyses. For instance, some studies used specific thresholds to determine significance (e.g., < 15% reduction in survival equaled no effect, or exposure exceeding 5% of LD50 was a negative effect). In these rare cases, we scored each tested parameter as “no effect,” “negative effect,” or “positive effect” based on the authors’ conclusions.

Organization of Endpoints

We classified tested endpoints into nine major categories that measured the following:

1. Mortality — survival and/or average lifespan.
2. Abundance — number of individuals and/or activity-density.
3. Biomass — weight of organisms.
4. Behavior — behavioral responses, including avoidance, mounding or burrowing activity, litter decomposition, food consumption and predation on or parasitism of target pests, cast or fecal production, locomotor functioning, defensiveness and aggression, and foraging and flight efficiency.
5. Reproduction — fecundity, reproductive anatomy and function, and offspring production, including egg laying rate, hatching rate, juvenile number, larval ejection, ovary development and sperm deformation, sex ratio, brood number and production, sterility, and viable brood cells.
6. Biochemical biomarkers — biochemical or molecular responses from toxic exposure, including oxidative stress (ROS), enzymatic, protein, and lipid activity or content, gene expression, cellular energy allocation or energy available, mitochondrial response, metabolism, neutral red retention time (NRRT), DNA damage, and synapsin levels.
7. Growth — weight and development of individuals, including adults and juveniles, molting rate, larval growth, and cocoon production.
8. Richness and diversity — community structure and composition, including richness, diversity, and evenness.
9. Structural changes — visible, physical histological and/or morphological changes, such as damage to the epidermis.

RESULTS

Non-target Effects of Pesticides on Soil Invertebrates Overall

A total of 394 laboratory and field studies fit our criteria, yielding 2,842 tested parameters representing 275 unique species, taxa, or combined taxa of soil organisms and 284 different pesticide active ingredients or unique mixtures of active ingredients. The majority of studies (257) tested the impact of insecticides on 1,592 tested parameters, followed by herbicides, fungicides, bactericides, and pesticide mixtures with 67, 55, 49, and 2 studies looking at 541, 465, 218, and 26 tested parameters, respectively. We present the negative and positive effect percentages below, and the percentage of parameters with no significant effect can be gleaned from these values, as the total percentage always equals 100%.

Overall, we found that pesticides negatively affected 70.5% and positively affected 1.4% of tested parameters (Table 1). By pesticide type, 74.9% of tested parameters were negatively affected by insecticides, 63.2% by herbicides, 71.4% by fungicides, 57.7% by bactericides, and 56.4% by pesticide mixtures (Table 1). The impact of pesticide mixtures depended on the type; of the 49 mixtures, those consisting of insecticides negatively affected tested parameters 83.7% of the time compared to 61.5, 38.6, and 49.5% caused by herbicide mixtures, fungicide mixtures, and cross pesticide mixtures, respectively.

Overall, insecticides, herbicides, fungicides, and mixtures each negatively affected a similar number of parameters in the laboratory setting – roughly 80% (Figure 1). Comparatively, studies conducted in the field generally resulted in fewer significant negative impacts, with insecticides negatively impacting about 60% of tested parameters, fungicides 40%, and herbicides 30% (Figure 1). Bactericides generally resulted in fewer negative results in lab studies; however, they were underrepresented compared to other pesticide types.

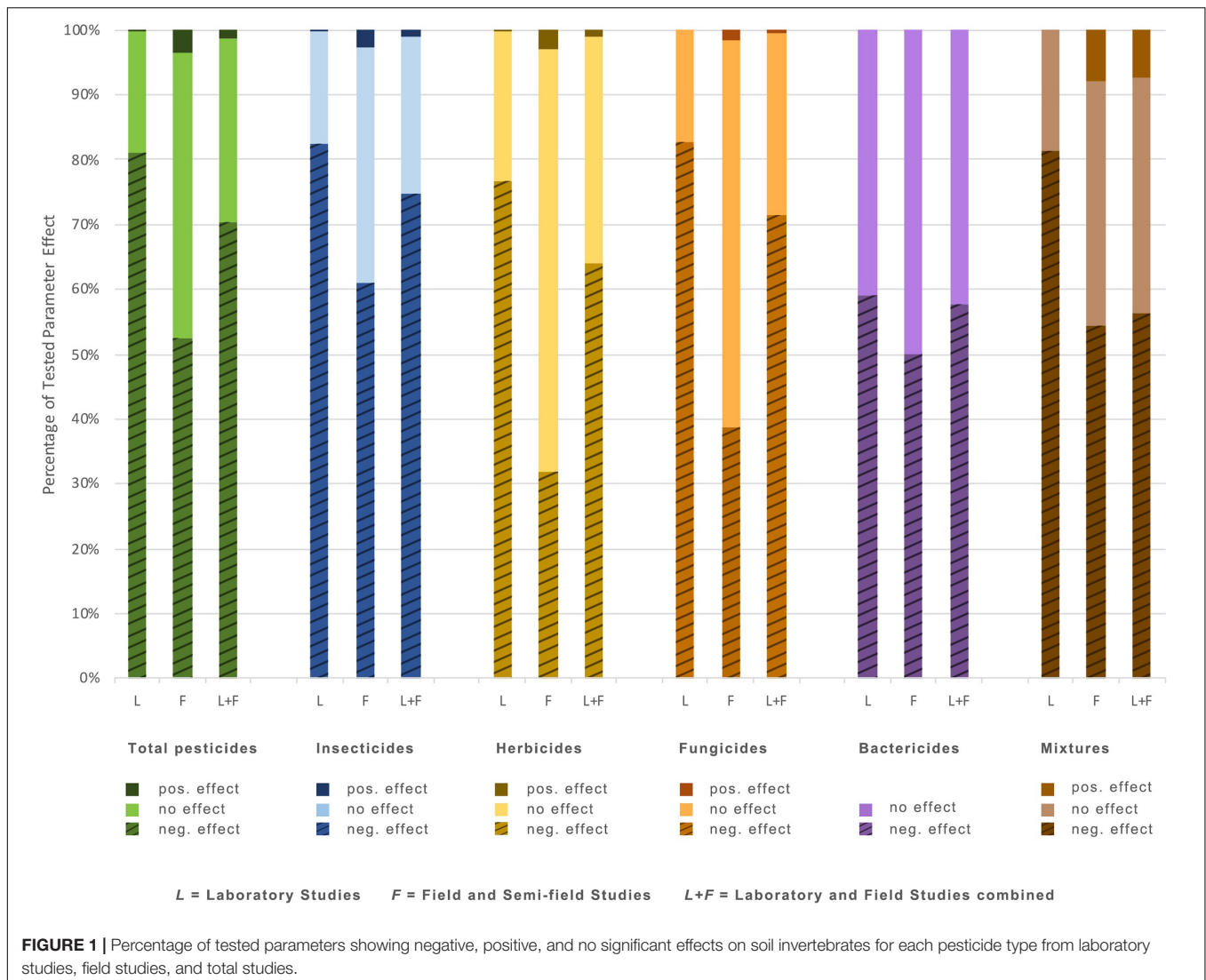
Organophosphates and neonicotinoids were the most studied classes of insecticides; of herbicides, phosphonoglycines (glyphosate) and triazines; and of fungicides, inorganic compounds such as copper and zinc, as well as conazoles (Supplementary Table 1). Among these, organophosphate, neonicotinoid, pyrethroid and carbamate insecticides, amide/anilide herbicides, and benzimidazole and inorganic fungicides negatively affected soil taxa more than 70% of the time (Figure 2).

Of endpoint categories, structural changes and biochemical biomarkers were the most impacted by pesticides followed by reproduction, mortality, behavior, growth, richness and diversity, abundance, and lastly, biomass (Table 2). Insecticides, fungicides, and pesticide mixtures had a greater than 70% negative effect on mortality endpoints; insecticides and fungicides on reproduction; fungicides on growth; and insecticides on behavior (Table 2). Supplementary Table 2 presents the effects of the most studied classes on the endpoint categories.

TABLE 1 | The number of tested parameters (# par.) and the percentage that resulted in negative effects (% neg.) to each soil taxa after exposure to different pesticide types.

Soil Dwelling Taxa	Total lab + field		Insecticides		Herbicides		Fungicides		Bactericides		Mixtures	
	# par.	% neg.	# par.	% neg.	# par.	% neg.	# par.	% neg.	# par.	% neg.	# par.	% neg.
Oligochaeta (earthworms)	1321	78.6%	697	84.2%	315	71.7%	260	77.7%	11	54.5%	38	63.2%
Enchytraeidae (potworms)	175	80.6%	38	71.1%	49	87.8%	83	83.1%	0	N/A	3	33.3%
Nematoda	39	71.8%	6	66.7%	27	66.7%	5	100.0%	0	N/A	1	100.0%
Tardigrade	1	100.0%	1	100.0%	0	N/A	0	N/A	0	N/A	0	N/A
Acari (mites)	136	56.6%	81	65.4%	18	38.9%	18	50.0%	0	N/A	19	42.1%
Myriapoda (millipedes, centipedes, Pauropoda)	15	53.3%	6	83.3%	2	50.0%	2	100.0%	0	N/A	5	0.0%
Isopoda (woodlice)	83	73.5%	57	80.7%	9	22.2%	16	81.3%	0	N/A	1	0.0%
Collembola (springtails)	314	72.0%	204	74.5%	46	65.2%	34	82.4%	8	55.6%	22	50.0%
Protura (coneheads)	1	0.0%	1	0.0%	0	N/A	0	N/A	0	N/A	0	N/A
Isoptera (termites)	13	61.5%	12	58.3%	0	N/A	0	N/A	1	100.0%	0	N/A
Coleoptera (beetles)	264	55.3%	121	79.3%	59	23.7%	22	4.6%	0	N/A	62	56.5%
Formicidae (ants)	42	59.5%	32	71.9%	4	0.0%	3	0.0%	2	50.0%	3	100.0%
<i>Bombus</i> spp. (bumble bees)	318	54.1%	271	54.2%	0	N/A	17	23.5%	0	N/A	30	70.0%
Ground-nesting bee (non- <i>Bombus</i>)	45	77.8%	33	81.8%	0	N/A	0	N/A	0	N/A	12	66.7%
Parasitic wasp	19	78.9%	14	78.6%	2	100.0%	2	50.0%	0	N/A	1	100.0%
Mixed taxa	56	39.3%	22	40.9%	10	20.0%	3	33.3%	0	N/A	21	47.6%
Total parameters and negative effect%	2842	70.5%	1596	74.7%	541	63.2%	465	71.4%	22	59.1%	218	56.4%
No effect%		28.1%		24.2%		34.6%		28.2%		40.9%		36.2%
Positive effect%		1.4%		1.0%		1.1%		0.4%		0.0%		7.3%

The bottom three rows identify the total number of tested parameters analyzed and percentage of tested parameters that found negative, positive and no effect findings for each pesticide type.



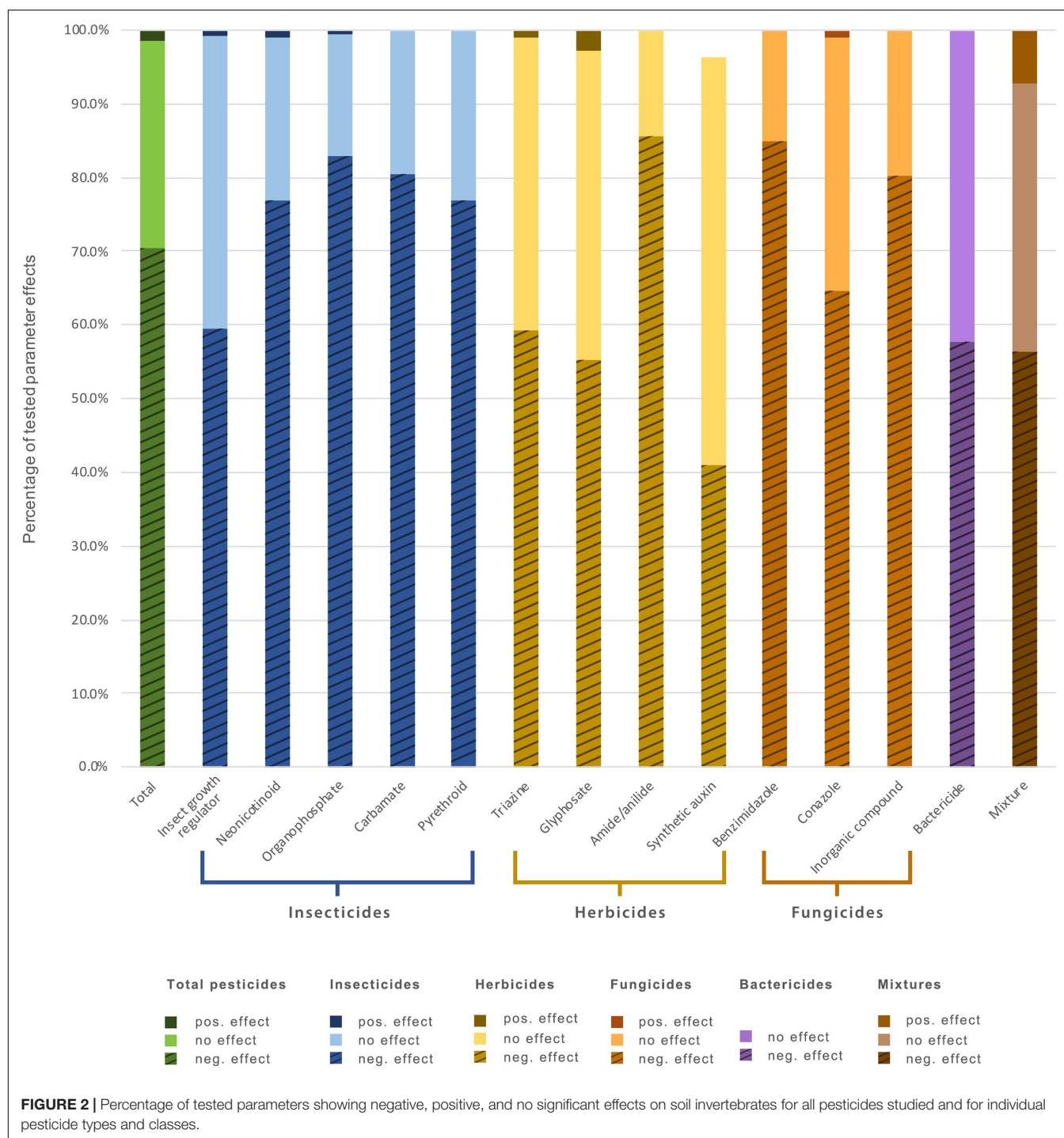
Overall trends by taxa revealed that Coleoptera (beetles) were more negatively affected by insecticides (79.3% of tested parameters) than herbicides (23.7%) or fungicides (4.6%) (Table 1). Of insecticides, neonicotinoids were the most detrimental to Coleoptera, with 91.5% of tested parameters being negatively affected, while pyrethroids were lowest at 50% (Supplementary Table 1). Eighty-four to 90% of tested parameters in Oligochaetes (earthworms) were negatively affected by the most-studied classes of insecticides, yet insecticides of less-studied classes (“other” category) resulted in a lower proportion of negative effects (55.4%) (Supplementary Table 1). Amide/anilide herbicides and benzimidazole fungicides were especially harmful to earthworms (Supplementary Table 1). Enchytraeids (potworms) were one of the only taxa that were more negatively impacted following exposure to herbicides (87.8% of tested parameters) and fungicides (83.1%) than insecticides (71.1%) (Table 1). Collembola were more negatively affected when exposed to fungicides (82.4%) than insecticides (74.5%) or herbicides (65.2%), at least in lab tests (Table 1).

Laboratory Studies

A total of 281 studies with 1,789 tested parameters conducted in the lab fit our criteria. The endpoints most studied in the lab were biochemical biomarkers (541), mortality (510), reproduction (343), behavior (195), growth (164), and structural changes (36) (Supplementary Table 3). Of taxa, earthworms (Oligochaeta) accounted for 60.4% (1,080) of the tested parameters in lab studies. Collembola, Isopoda, Acari, and *Bombus* spp. were also well-represented. There were fewer than 30 tested parameters for the remaining taxa – Coleoptera, Formicidae, parasitic wasps, non-*Bombus* ground-nesting bees, Isoptera, and Nematoda. Pesticides negatively affected 81.0%, positively affected 0.1%, and did not significantly affect 18.9% of tested parameters in laboratory studies (Supplementary Table 3).

Field and Semi-Field Studies

A total of 122 studies containing 1,053 tested parameters conducted in a field or semi-field setting fit our criteria. There were 51 studies conducted within Europe, 30 in the



United States, eight in Australia, seven in Canada, and five or less in Argentina, Brazil, Cameroon, Columbia, Egypt, India, Japan, Madagascar, Mexico, New Zealand, Sri Lanka, South Africa, and Yemen. The endpoints most studied in field settings were abundance (408), mortality (204), behavior (194), reproduction (66), growth (66), biomass (55), richness and diversity (34), and biochemical biomarkers (26) (**Supplementary Table 4**). Coleoptera was the most studied taxon in field

studies (245 tested parameters), followed closely by Oligochaeta (241 tested parameters). Acari, Collembola, *Bombus* spp., non-*Bombus* ground-nesting bees, and Formicidae were also relatively well-represented. There were fewer than 30 tested parameters for the remaining taxa – parasitic wasps, Isopoda, Isoptera, Nematoda, Tardigrada, and Myriapoda. Additionally, 56 tested parameters were associated with mixed organism groups. Pesticides negatively affected 52.6%, positively affected 3.6%,

TABLE 2 | The number of tested parameters (# par.) and the percentage that resulted in negative effects (% neg.) on each endpoint category tested in soil invertebrates after exposure to different pesticide types.

Endpoint Category	Total lab + field		Insecticides		Herbicides		Fungicides		Bactericides		Mixtures	
	# par.	% neg.	# par.	% neg.	# par.	% neg.	# par.	% neg.	# par.	% neg.	# par.	% neg.
Mortality	714	75.0%	468	79.1%	129	66.7%	95	70.5%	5	0.0%	18	72.2%
Abundance	408	45.3%	207	51.7%	56	26.8%	41	34.1%	0	N/A	103	46.6%
Biomass	55	40.0%	23	60.9%	15	13.3%	12	41.7%	0	N/A	5	20.0%
Reproduction	409	78.0%	238	79.8%	78	58.9%	73	86.3%	6	66.7%	14	64.3%
Behavior	389	70.2%	214	72.4%	81	65.4%	56	60.3%	1	100.0%	37	78.4%
Biochemical	567	85.4%	291	88.3%	114	86.0%	144	78.7%	7	85.7%	11	81.8%
Growth	230	58.3%	130	60.0%	52	50.0%	31	74.2%	3	66.7%	14	35.7%
Richness and diversity	34	47.1%	7	57.1%	10	30.0%	1	0.0%	0	N/A	16	56.3%
Structural changes	36	97.2%	18	100.0%	6	100.0%	12	90.0%	0	N/A	0	N/A
Total parameters and negative effect%	2842	70.5%	1596	74.7%	541	63.2%	465	71.4%	22	59.1%	218	56.4%
No effect%		28.1%		24.2%		34.6%		28.2%		40.9%		36.2%
Positive effect%		1.4%		1.0%		1.1%		0.4%		0.0%		7.3%

The bottom three rows identify the total number of tested parameters analyzed and percentage of tested parameters that found negative, positive and no effect findings for each pesticide type.

and did not significantly affect 43.8% of the field study tested parameters (**Supplementary Table 4**).

Annelida

Oligochaeta (earthworms) and *Enchytraeids* (potworms) lab studies

Enchytraeids (potworms) were analyzed in 38 lab studies with 159 tested parameters, of which pesticides negatively affected 84.3%. Specifically, insecticides negatively affected 75.0%, herbicides 89.6%, fungicides 86.3%, and bactericides 50.0% of tested parameters. Of endpoints, pesticides negatively affected biochemical biomarkers, reproduction, survival, and behavior 81.3%, 87.2%, 84.4%, and 90.9% of the time (**Supplementary Table 3**). Two tested parameters analyzed growth, with copper oxychloride negatively affecting worm body mass and a mixture of epoxiconazole and dimoxystrobin having no effect (Bart et al., 2017).

All other Oligochaetes, primarily Lumbricidae, but also Eudrilidae, Glossoscolecidae, Megascolecidae, and Moniligastridae were analyzed in 179 lab studies with 1,080 tested parameters. Of these, pesticides negatively affected 85% (**Supplementary Table 3**). Specifically, insecticides negatively affected 89.0%, herbicides 79.3%, fungicides 82.2%, bactericides 54.5%, and pesticide mixtures 83.3% of tested parameters (**Supplementary Table 5**). Biochemical biomarkers, which included subcellular events such as enzyme activity, membrane stability, gene expression, metabolism, DNA damage, and general oxidative stress, were the most studied parameters and were negatively affected in 85.5% of 408 tested parameters (**Supplementary Table 3**). Pesticides negatively impacted earthworm survival, reproduction, growth, and structural changes in 87.7, 82.7, 79.4, and 97.1% of corresponding tested parameters, respectively (**Supplementary Table 3**). Behavior, which included feeding rate, activity, burrowing, cast production, litter decomposition, avoidance,

and respiration, was negatively affected in 80.6% of 98 tested parameters.

Oligochaeta (earthworms) and *Enchytraeids* (potworms) field studies

Pesticides negatively affected potworms in 43.8% of 16 tested parameters in five field studies and earthworms in 49.8% of 241 tested parameters in 41 field studies. Specifically, fungicides negatively affected 60.0% and pesticide mixtures 33.3% of potworm tested parameters and insecticides negatively affected 54.5%, herbicides 37.5%, fungicides 53.8%, and pesticide mixtures 53.8% of earthworm tested parameters (**Supplementary Table 6**). Potworm reproduction was negatively affected in six of eight tested parameters and feeding behavior was reduced by imidacloprid and cyfluthrin seed coatings, but not by the fungicide, thiram (**Supplementary Table 4**) (Larink and Sommer, 2002). Earthworm mortality, abundance, and biomass were negatively affected in 77.8% of 18 tested parameters, 38.2% of 68 tested parameters, and 39.6% of 48 tested parameters, respectively (**Supplementary Table 4**). Earthworm richness and diversity and behaviors like avoidance of pesticides, surface and cumulative activity, burial of organic matter, cast production, feeding activity, and litter decomposition were also negatively impacted in about half to two-thirds of the tested parameters. Pesticides negatively impacted earthworm reproduction in 81.8% of 11 tested parameters (**Supplementary Table 4**). Imidacloprid (Kreutzweiser et al., 2008), oxychloride (Eijsackers et al., 2005), and chlorpyrifos (Reinecke and Reinecke, 2007) all reduced earthworm growth, while dimethoate, 2,4-DB, and glyphosate had no effect (Dalby et al., 1995).

Nematoda

Nematoda (roundworms) lab studies

Pesticides negatively affected nematodes in 84.6% of 26 tested parameters in eight lab studies. Specifically, insecticides and

fungicides negatively affected 100.0% and herbicides 80.0% of tested parameters (**Supplementary Table 5**). The survival of various nematode species was reduced by the fungicide fludioxonil (Haegerbaeumer et al., 2019), and the herbicides acetochlor (Zhang et al., 2011, 2013) and terbuthylazine (Salminen et al., 1996) (**Supplementary Table 3**). Nematode reproduction was negatively affected in six of seven tested parameters, by the fungicide fludioxonil (Haegerbaeumer et al., 2019), the insecticides imidacloprid (Gomez-Eyles et al., 2009) and chlorpyrifos (Martin et al., 2009), and the herbicide acetochlor (Zhang et al., 2011, 2013). Biochemical biomarkers were negatively affected in all of six tested parameters (**Supplementary Table 3**); specifically, oxidative stress response in *Caenorhabditis elegans* was induced by the herbicides, glyphosate and paraquat (Kronberg et al., 2018), and heat shock protein and B-galactosidase activity were negatively affected by the fungicide, mancozeb (Easton et al., 2001). Growth was negatively affected in all of five tested parameters; acetochlor reduced growth in *Acroboloides nanus*, *Pristionchus pacificus*, and *C. elegans* (Zhang et al., 2011, 2013), and larval and adult growth of *C. elegans* was negatively affected by mancozeb (Easton et al., 2001) and glyphosate (Kronberg et al., 2018), respectively.

Nematoda (roundworms) field studies

In seven field studies, pesticides negatively affected nematodes in 46.2% of the 13 tested parameters. Specifically, insecticides negatively affected 50.0%, herbicides 28.6%, and fungicides and pesticide mixtures 100% of the tested parameters (**Supplementary Table 6**), including nematode mortality, biomass, and abundance (**Supplementary Table 4**). Carbendazim reduced survival (Burrows and Edwards, 2004), glyphosate reduced biomass (Hagner et al., 2019), and chlormethoxynil (Ishibashi et al., 1983), chloropicrin (Carrascosa et al., 2014), and a mixture of thiobencarb and simetryne (Ishibashi et al., 1983) reduced population abundance, while oxadiazon, paraquat (Ishibashi et al., 1983), azadirachtin (neem), and chlorpyrifos (Stark, 1992) had no effect on nematodes in the field. Nematode diversity was also reduced by 1,3-Dichloropropene, which also was found to negatively affect Tardigrade (water bear) density (Carrascosa et al., 2014).

Arthropoda

Acari (mites) lab studies

Pesticides negatively affected mites in 79.6% of 49 tested parameters in 14 lab studies. Specifically, insecticides negatively impacted 78.9%, herbicides 83.3%, and fungicides 80.0% of the tested parameters (**Supplementary Table 5**). Mite survival and reproduction were negatively affected 79.2% and 73.7% of the time, respectively (**Supplementary Table 3**). Acari also avoided soils treated with chlorpyrifos, dimethoate, deltamethrin, copper, (Owojori et al., 2014), and spinosad (Rahman et al., 2011), and dimethoate significantly reduced the growth of the mite *Hypoaspis aculeifer* (Canestrini) (Folker-Hansen et al., 1996).

Acari (mites) field studies

Pesticides negatively affected mites in 43.7% of 87 tested parameters in 19 field studies; specifically, insecticides negatively affected 53.5%, herbicides 16.7%, fungicides 38.5%, and pesticide

mixtures 42.1% of tested parameters (**Supplementary Table 6**). Pesticide impacts to mite abundance varied between species, but was significantly reduced 44.3% of the time (**Supplementary Table 4**). Oribatid mite reproduction was negatively impacted in two of five tested parameters and Oribatid mite diversity was negatively impacted by exposure to a combination of mancozeb, copper oxychloride, and metalaxyl (Al-Assiuty et al., 2014). In one tested parameter, glyphosate positively affected Acari by increasing activity by nearly 50% (Al-Daikh et al., 2016).

Myriapoda: Diplopoda (millipedes) lab studies

The insecticide deltamethrin negatively affected Diplopoda in three tested parameters (**Supplementary Table 5**), causing a significant reduction in survival and neurological functioning, as well as altering millipede behavior by causing agitation, release of defensive secretion, gonopod externalization, and hemolymph leakage (Francisco et al., 2016).

Myriapoda: Diplopoda (millipedes), Chilopoda (centipedes), Pauropoda field studies

Pesticides had a negative effect in 41.7% and positive effect in 25.0% of 12 tested parameters in seven field studies measuring the combined abundance of Diplopoda, Chilopoda, and Pauropoda (**Supplementary Tables 4, 6**). Diflubenzuron and mancozeb decreased Myriapod abundance by 73.0% and 43.0%, respectively (Adamski et al., 2009). Carbendazim and lambda-cyhalothrin reduced abundance of the millipede *Trigoniulus corallinus* (Gervais) (Förster et al., 2006). Monuron reduced overall Diplopod abundance, while atrazine had no significant effect (Fox, 1964). Imidacloprid (Peck, 2009) and a mixed pesticide regimen (Lundgren et al., 2013) had no significant effect on Chilopoda abundance. In pesticide-treated vineyards, the relative abundance of Diplopoda, including *Julidae* sp., and Pauropoda increased, while Chilopods were not affected (Vaj et al., 2014).

Isopoda (woodlice) lab studies

Pesticides negatively affected Isopods in 75.9% of 79 tested parameters in 15 lab studies; specifically, insecticides negatively impacted 80.4%, herbicides 28.6%, and fungicides 81.3% of the tested parameters (**Supplementary Table 5**). Pesticides negatively affected 80% of 20 behavioral parameters measuring Isopod feeding rate, feces production, avoidance, and locomotion and 85.7% of 35 biochemical biomarkers, including enzymatic activity and metabolism (**Supplementary Table 3**). Survival of *Porcellio scaber*, *P. dilatatus*, and *Porcellionides pruinosus* was reduced by diazinon (Drobne et al., 2008), dimethoate (Engenheiro et al., 2005; Ferreira et al., 2015), lambda-cyhalothrin (Jänsch et al., 2005), chlorpyrifos (Morgado et al., 2016), and pyrethrins (Zidar et al., 2012). Isopod growth was also negatively impacted in four out of seven tested parameters, reduced by imidacloprid (Drobne et al., 2008), dimethoate (Fischer et al., 1997), chlorpyrifos, and mancozeb (Morgado et al., 2016). Reproduction was negatively affected in two out of three tested parameters by dimethoate (Fischer et al., 1997) and lambda-cyhalothrin (Jänsch et al., 2005), and the epithelial thickness of *P. scaber* was significantly reduced by imidacloprid (Drobne et al., 2008).

Isopoda (woodlice) field studies

Pesticides negatively affected Isopods in one of four tested parameters from three field studies (**Supplementary Table 6**). The insecticide lambda-cyhalothrin reduced *Circoniscus ornatus* abundance at field rates (Förster et al., 2006), while total Isopoda abundance was not significantly affected in pesticide treated vineyards (Nash et al., 2010). Glyphosate did not induce avoidance behavior or reduce reproduction in *P. scaber* (Niemeyer et al., 2018).

Collembola (springtails) lab studies

Pesticides negatively affected springtails in 79.1% of 225 tested parameters in 59 lab studies; specifically, insecticides negatively affected 78.1%, herbicides 72.7%, fungicides 96.4%, and bactericides 62.5% of tested parameters (**Supplementary Table 5**). Collembola mortality was negatively affected in 72.9% and reproduction in 89.2% of corresponding tested parameters. Pesticides negatively impacted 75.0% of 20 parameters measuring avoidance and locomotion behavior, 41.2% of 17 parameters measuring growth, and 88.0% of 25 parameters measuring DNA damage, gene expression, metabolism, gut microbial diversity, and enzymatic activities (**Supplementary Table 3**).

Collembola (springtails) field studies

Pesticides negatively affected springtails in 53.9% of 89 tested parameters in 30 field studies; specifically, insecticides negatively affected 63.3%, herbicides 46.2%, fungicides 16.7%, and pesticide mixtures 47.6% of tested parameters (**Supplementary Table 6**). Collembola survival was negatively impacted by dimethoate (Joy and Chakravorty, 1991) and chlorpyrifos (Wiles and Frampton, 1996), but not cypermethrin (Wiles and Frampton, 1996) and collembola abundance was reduced 50.7% of the time (**Supplementary Table 4**). The insecticide ethoprophos caused a total loss in reproduction of *Folsomia candida* (Willem) (Leitão et al., 2014), while glyphosate had no effect (Niemeyer et al., 2018). Atrazine (Al-Assiuty and Khalil, 1996), and the glyphosate formula, Zapp Qi 620 (Niemeyer et al., 2018) both caused avoidance behavior in Collembola, and fungicide and insecticide seed treatments both significantly increased *Sinella curviseta* (Brook) surface activity (Zaller et al., 2016). Chlorpyrifos significantly reduced springtail species richness in one study (Fountain et al., 2007), which was not significantly affected by chlorpyrifos or dimethoate in another (Endlweber et al., 2006). Ethoprophos reduced biomass of *F. candida* by 60% (Leitão et al., 2014), growth was impaired by dimethoate in another (Al-Haifi et al., 2006), and chlorothalonil altered *F. candida* gene expression in a third tested parameter (Simões et al., 2019). In the only study of another Order of Entognatha — Protura — abundance was unaffected by imidacloprid (Peck, 2009).

Isoptera (termites) lab studies

One lab study with termites found that streptomycin induced aggressive fighting behavior between individual termites in the same nest (Gao et al., 2018).

Isoptera (termites) field studies

Insecticides negatively impacted termites in 58.3% of 12 tested parameters in three field studies (**Supplementary Table 6**).

In one tested parameter, chlorpyrifos significantly reduced termite abundance (De Silva et al., 2010). Fipronil significantly reduced survival and activity in colonies of the termite *Coarctotermes clepsydra* (Sjöstedt), as 0–30% of termites were active in treated colonies, compared to 100% in untreated colonies (Peveling et al., 2003). Fipronil also reduced wood and cardboard bait consumption in *Microcerotermes*, *Armitermes*, and *Drepanotermes* termites in multiple soil types (Steinbauer and Peveling, 2011). Additionally, the termite *Drepanotermes rubriceps* (Froggatt) only repaired 19% of damaged mounds when treated with fipronil compared to 53% in unsprayed plots (Steinbauer and Peveling, 2011).

Coleoptera (beetles) lab studies

Pesticides negatively affected ground beetles (Carabidae) in 21.1% of 19 tested parameters in four lab studies (**Supplementary Table 5**). Thiamethoxam seed treatment significantly reduced Coleoptera survival (Douglas et al., 2015) and beetle behavior, including feeding rate was significantly impacted in three of seven tested parameters, while beetle growth was unaffected (**Supplementary Table 3**).

Coleoptera (beetles) field studies

Beetles, primarily in the family Carabidae, but also Staphylinidae, Latridiidae, Cryptophagidae, Tenebrionidae, Cleridae, Nitidulidae, and Elateridae, were the most represented taxa in field studies and pesticides negatively affected 58.0% of 245 tested parameters across 29 field studies. Specifically, insecticides negatively affected 79.2%, herbicides 28.3%, fungicides 5.0%, and pesticide mixtures 55.9% of tested parameters (**Supplementary Table 6**). Beetle abundance was reduced in 56.0% and reproduction in 2/3rds of corresponding tested parameters. Beetle behavior, such as feeding rate, predation, avoidance, and locomotory response were negatively affected in 77.4% of 31 tested parameters. Beetle richness and diversity, as well as growth parameters measuring larval development, body size, muscle mass, and lipid mass were negatively affected in two of seven and 16.0% of 25 parameters, respectively. However, beetle enzymatic activity, hemocyte count, and plasmatic basal and phenoloxidase activities, were negatively affected in five out of six tested parameters (**Supplementary Table 4**).

Formicidae (ants) lab studies

Of two lab studies with eight tested parameters on ant behavior, insecticides negatively affected seven, or 87.5% (**Supplementary Table 5**). Environmentally relevant concentrations of imidacloprid impaired *Pogonomyrmex occidentalis* (Cresson) navigation and foraging success (Sappington, 2018) and the activity and foraging behavior of *Lasius flavus* F. and *L. niger* L. (Thiel and Köhler, 2016). Specifically, a significant increase in aggressive behavior in *L. flavus* toward other nest-mates following exposure to imidacloprid reduced survival probability by 60%, but there was no significant effect on aggression or nest-mate recruitment in *L. niger* (Thiel and Köhler, 2016).

Formicidae (ants) field studies

Pesticides negatively affected ants in 52.9% of 34 tested parameters across eight field/semi-field studies; specifically, insecticides negatively affected 62.5% of 24 tested parameters, pesticide mixtures negatively affected all three tested parameters, and herbicides and fungicides had no significant effects on four and three tested parameters, respectively (**Supplementary Table 6**). Ant abundance was significantly reduced 51.6% of the time by chlorpyrifos (Armenta et al., 2003), diazinon (Potter et al., 1990; Armenta et al., 2003), deltamethrin (Rodriguez et al., 2003), fipronil (Steinbauer and Peveling, 2011), a mixture of fipronil and fenitrothion (Walker et al., 2016), and a pesticide mixture containing glyphosate, S-metolachlor, lambda-cyhalothrin and esfenvalerate (Lundgren et al., 2013). Behavior was studied in three tested parameters with two negative effects (**Supplementary Table 4**); clothianidin and bifenthrin applied together significantly reduced ant predation of black cutworm eggs, and bifenthrin significantly reduced the mound-building activity of *L. neoniger* (Emery) and *Solenopsis molesta* (Say) by 90% and 65%, respectively (Larson et al., 2012).

Bombus spp. (bumble bees) lab studies

Pesticides negatively affected bumble bees in 56.3% of 128 tested parameters in 18 lab studies. Insecticides negatively impacted 57.6% of 118 tested parameters and four of the remaining 10 tested parameters were negatively impacted by fungicides (**Supplementary Table 5**). Bumble bee survival was significantly reduced by pesticides in 57.4% of 68 tested parameters and reproduction negatively impacted in 48.0% of 25 tested parameters. Bumble bee behavior and growth were negatively affected by pesticides about 55–60% of the time (**Supplementary Table 3**). Of the two biochemical biomarker parameters, clothianidin and thiamethoxam altered *B. terrestris* gene expression (Colgan et al., 2019).

Bombus spp. (bumble bees) field studies

Pesticides negatively affected bumble bees in 52.6% of 190 tested parameters across 23 field studies; specifically, insecticides negatively affected 51.6%, and pesticide mixtures 70.0% of the tested parameters. Herbicides were not studied and fungicides had 85.7% non-significant effects on seven tested parameters (**Supplementary Table 6**). Neonicotinoids were present in 64.2% of all pesticide treatments tested, 58.1% of which negatively affected bumble bees. Abundance, survival, and reproduction were significantly reduced in 38.5, 53.3, and 48.6% of corresponding tested parameters, respectively (**Supplementary Table 4**). Nest condition was severely impacted by imidacloprid, while bumble bee biomass was not significantly affected by imidacloprid or chlorpyrifos (Moffat et al., 2015). Pesticides negatively impacted bumble bee behavior in 60.9% of 64 tested parameters, which included the specific measures of pesticide avoidance, foraging efficiency, feeding rate, worker defensive response, flight activity, flower visitation, and flower handling. Pesticides also negatively affected 41.7% of 24 bumble bee growth measurements, including colony development, strength, and mass (**Supplementary Table 4**). Bumble bee neural function was analyzed in two studies with five tested

parameters, of which four (80%) were negatively impacted by pesticide exposure.

Other (or non-Bombus) ground-nesting bees lab studies

Insecticides negatively affected non-*Bombus* ground-nesting bees in seven (87.5%) of eight tested parameters in two lab studies (**Supplementary Table 5**). Specifically, survival was significantly reduced in all tests from exposure to permethrin, mexacarbamate, aminocarb, fenitrothion, carbaryl and spinosad (Helson et al., 1994; Mayer et al., 2001). Spinosad had no significant effect on the feeding rate of *Nomia melanderi* (Cockerell) (Halictidae) (Mayer et al., 2001).

Other (or non-Bombus) ground-nesting bees field studies

Pesticides negatively affected non-*Bombus* ground-nesting bees in 75.7% of 37 tested parameters in six field studies; specifically, insecticides and pesticide mixtures negatively affected 80.0% and 66.7% tested parameters, respectively, while other pesticide types were not tested (**Supplementary Table 6**). Bee survival was negatively affected in 81.8% of 22 tested parameters and abundance in six out of 11 tested parameters (**Supplementary Table 4**). Both neonicotinoids (Main et al., 2020), and mixed pesticide regimens (Tuell and Isaacs, 2010; Mallinger et al., 2015) negatively impacted richness and diversity of ground nesting bees.

Parasitic wasp (Figitidae) lab studies

Many parasitic wasps develop in the soil, including *Trybliographa rapae* (Westwood) (Figitidae), which was analyzed in one lab study with four tested parameters. Although *T. rapae* was seven times less sensitive to chlorfenvinphos than its host, the agricultural pest cabbage root fly (*Delia radicum* L.), the wasp's mortality, longevity, fecundity, and sexual development were all significantly negatively impacted by pesticide exposure (Alix et al., 2001).

Parasitic wasp (Tiphidae) field studies

Parasitic wasps were analyzed in two field/semi-field studies with 15 tested parameters, of which 73.3% were negatively affected by pesticides. Specifically, insecticides negatively affected 70.0%, fungicides 50%, and herbicides and pesticide mixtures 100% of tested parameters (**Supplementary Table 6**). The survival of *Tiphia vernalis* (Rohwer) (Tiphidae) was reduced by bifenthrin, carbaryl, chlorpyrifos, imidacloprid, oryzalin, pendimethalin, chlorothalonil, thiophanate-methyl, and a mixture of 2,4-D and dicamba, but not halofenozide (Oliver et al., 2006). Imidacloprid reduced *T. vernalis* egg laying and pest control efficiency, as measured by the ability to locate, recognize, and successfully parasitize its host, the Japanese beetle [*Popillia japonica* (Newman)] (Rogers and Potter, 2003).

Mixed taxa field studies

Mixed groups of soil organisms were analyzed in 19 studies with 56 tested parameters, of which pesticides negatively affected 39.3%. Specifically, insecticides negatively affected 40.9%, herbicides 20.0%, fungicides 33.3%, and pesticide mixtures 42.9% of tested parameters (**Supplementary Table 6**). Soil taxa abundance and richness were negatively affected in 25.8% and 41.7% and positively affected in 19.4% and 8.3% of the 30 and 12

corresponding tested parameters, respectively (**Supplementary Table 4**). Litter decomposition and feeding rate behaviors were significantly reduced in 69.2% of 13 parameters testing the impact of pesticides on those ecosystem functions.

DISCUSSION

In reviewing 394 studies on pesticide impacts to soil invertebrates, we found that negative effects dominated, with 70.5% of 2,842 total tested parameters between lab and field studies identifying a negative impact to soil organisms from pesticide exposure. All of the 12 highly studied pesticide classes (**Figure 2**), other than synthetic auxin herbicides, negatively affected greater than 50% of the tested parameters that were analyzed. These findings indicate that a wide variety of soil-dwelling invertebrates display sensitivity to pesticides of all types and support the need for pesticide regulatory agencies to account for the risks that pesticides pose to soil invertebrates and soil ecosystems.

Impacts by Pesticide Type

Insecticides were by far the most-studied pesticide type and, unsurprisingly, because they are designed to target invertebrates, had the largest negative impact on soil invertebrates of any pesticide type analyzed. We found that insecticides consistently negatively affected approximately 60% to 85% of all tested parameters among the taxa studied (**Table 1**). Bumble bees (*Bombus* spp.) and mixed soil taxa were the notable exceptions but were still negatively impacted by insecticides 54.2% and 40.9% of the time, respectively. Herbicides and fungicides, however, varied greatly depending on the chemical, taxa, and endpoint studied, resulting in negative impacts on 5% to 100% of tested parameters in the reviewed studies. Some of this variability likely stems from fewer studies on herbicide and fungicide impacts compared to insecticides and, as a result, very few tested parameters for some taxa (**Table 1**). These results indicate that, in general, soil invertebrates are more variable in their sensitivity to fungicides and herbicides than insecticides. There were too few studies that met our search criteria to identify clear trends regarding the impact of bactericides on soil invertebrates, likely because we did not include research on microorganisms like bacteria or fungi. Negative effect percentages of pesticide mixtures varied between 33.3% and 100%, with most negatively impacting 40–60% of the tested parameters across taxa.

We found that positive effects were rare (1.4% overall) but occurred most often when abundance was measured in field studies with the application of insecticides versus other pesticide types. A positive effect indicates a benefit to one soil organism that may come at the detriment to other soil taxa or soil ecosystem functioning. For example, abundance of certain soil taxa could increase if a pesticide reduces competitors or predators, either through mortality or emigration from the area. Therefore, while certain effects were designated as “positive” to one species or taxa in this analysis, it does not indicate, nor is it likely, that pesticides had a positive effect on the ecosystem as a whole.

Our search criteria only identified three studies that tested the sensitivity of soil invertebrates to commonly used soil fumigants, specifically 1,3-D, dazomet, chloropicrin and metam sodium. Most studies we found on fumigants studied the sensitivity of microbial/fungal organisms or target pests like plant parasitic nematodes and were beyond the scope of this review. Of the nine tested parameters involving fumigants, seven resulted in negative effects to non-target soil invertebrates. The lack of available studies on harm from soil-applied fumigants to non-target invertebrates indicates that this is an area in need of more research.

Mixtures vs Individual Active Ingredients

Few studies measure the effects of pesticide mixtures as opposed to individual active ingredients, though research shows that mixtures of pesticide residues in the soil are the rule rather than the exception (Silva et al., 2019). Nearly all corn grown in the United States is treated with multiple pesticides (Douglas and Tooker, 2015; Lamichhane et al., 2019) and pesticide mixtures have the potential to increase toxicity due to chemical interaction (Sgolastra et al., 2016). We included pesticide mixture studies in our analysis, which surprisingly had fewer overall negative effects than single pesticides. This is likely because mixture studies were overwhelmingly done in a field or semi-field setting rather than a laboratory. However, among only field/semi-field studies, pesticide mixtures had a higher negative effect percentage than all pesticide classes except insecticides (**Figure 1**). Other variables could also contribute to the overall lower negative effect percentage of mixtures. For instance, mixture studies are often done with concentrations of individual components that are known to not produce an effect individually in order to maximize the ability to identify interactive effects (Kortenkamp, 2007). Furthermore, there was considerable variability in the outcome of mixture experiments based on pesticide type analyzed (see Results Section). Therefore, we caution against comparing the negative effect percentage for mixture studies with those of individual pesticide types in this analysis. Ultimately, considering that environmental exposure to pesticide mixtures is the rule and not the exception, research on pesticide mixtures is a major gap in the literature that we hope receives future focus.

Laboratory vs Field Studies

Overall, we found fewer negative impacts of pesticides in field studies compared to laboratory studies. One likely reason for this finding is that the pesticide concentrations used in lab studies were generally higher, while field studies often applied concentrations at or below the recommended use rate. Higher concentrations of pesticides are more often associated with negative effects on soil organisms (Puglisi, 2012). For instance, a study on the effects of pyrimethanil on Enchytraeids conducted across two labs in Portugal and Germany found contrasting results based on the tested pesticide concentration (Bandow et al., 2016). Due to the scope of this review, we did not identify the concentrations expected to be encountered in the environment for every pesticide used in each study. Since pesticide use rates and approved application methods can differ between countries and different areas within a country, a “field-relevant” pesticide

concentration in one region may be over- or under-representative of that expected in another region. Therefore, this review is inclusive of the wide variety of exposure concentrations found in the literature and is focused on identifying hazards, not necessarily risks.

In addition, uncontrolled, confounding environmental variables could provide some buffering capacity for pesticide effects in field and semi-field studies. Climatic conditions and various seasonal or yearly variations to the agricultural setting outside of pesticide application, such as cropping system changes or irrigation, make it difficult to fully assess pesticide effects under short-term trials (Ewald et al., 2015; Pelosi et al., 2015). For example, imidacloprid applied to turfgrass without irrigation significantly impacted the pesticide's effect on foraging bumble bees, while imidacloprid applied with irrigation did not (Gels et al., 2002). Significant differences in the soil microbial community structure were observed when pyrimethanil was applied during heavy rainfalls versus drought (Ng et al., 2014), and moisture increased Collembola sensitivity to lambda-cyhalothrin (Bandow et al., 2014). Different substrates can also determine variation in organism response to pesticide treatment (Velki and Ežimović, 2015), illustrated by the effects of phenmedipham on Enchytraeid reproduction which varied greatly between 18 different tested soils (Amorim et al., 2005a). While laboratory studies often use artificial soil or a standardized natural soil, agricultural soil environments vary widely in factors such as organic matter, water holding capacity, and pH (Amorim et al., 2005b). Additionally, ecotoxicology tests in the lab may use contact filter paper instead of soils, to which soil organisms typically show much higher sensitivity to pesticides. For example, the LC50 for earthworms treated with cypermethrin in artificial soils was 9.83 mg/kg while it was 0.30 mg/kg on contact filter paper, which is only used for testing in laboratory settings (Saxena et al., 2014).

Despite the major advantage of field studies being conducted under more realistic conditions than laboratory studies, there are some downsides to relying on them exclusively. Since the complex logistics and expensive nature of field experiments can lead to lower sample sizes and fewer replicates, they can often lack a high statistical power. This can lead to statistical results that are highly variable between studies even when the overall effects are more-or-less consistent, leading researchers to advise conservative interpretations of non-significant results in low-powered field studies (Douglas and Tooker, 2016). Some endpoints — such as reproduction or individual growth — or organisms that are smaller or less abundant can be difficult to study in the field. Additionally, since pesticides are generally approved for use in different regions with highly variable agricultural practices, geography, precipitation, temperature, air quality, background soil contamination, soil mineral content, pH and organic matter, field studies done in one region may not be representative of effects in another region. The majority of field studies we found took place in Europe and the United States, while very few field studies were conducted in countries from other continents. The disproportionate data from these temperate regions could over- or underestimate the risk of pesticides to soil organisms in other regions of the world, or

even subregions in the studied countries. Controlling many of the fluctuating variables found in field tests in a laboratory setting can be useful, and both types of studies should be considered helpful in identifying potential harms that could come from pesticide use in or near soil.

Endpoints

The most sensitive endpoint category was structural changes, followed closely by biochemical biomarkers, then reproduction, mortality, behavior, growth, richness and diversity, abundance, and lastly, biomass (Table 2). All observable effects in a whole organism are preceded by subcellular events which can be measured in biochemical biomarker tests, yet all subcellular events will not necessarily lead to these larger, gross changes. Therefore, having the negative effect percentage of tested parameters gradually decrease from biochemical effects to sublethal effects to lethal effects to more macro-changes (like richness and diversity) was expected. While mortality is largely studied, it is often the least sensitive parameter; for example, acute mortality tests did not provide the most sensitive risk estimates for earthworms in 95% of cases (Frampton et al., 2006). Instead, when an organism is engaged in the detoxification process of pollutants in order to ensure survival, normal functions such as reproduction, growth, and feeding or burrowing behaviors are likely to suffer (Pelosi et al., 2014). For example, earthworms exposed to copper fungicides entered quiescence — a period in which development is suspended — in order to resist contamination, which resulted in a significant reduction of biomass (Bart et al., 2017).

Most studied endpoints for soil organisms provided a clear indication of harm, while others, such as avoidance behavior, inform us of an organism's response that could indicate other negative effects either to the organism or the ecosystem (Niemeyer et al., 2018). As a test metric, avoidance behavior has high variability and lower sensitivity than other endpoints and has been suggested for better use as a screening evaluation of soil contamination (Loureiro et al., 2005; Natal-da-Luz et al., 2008; Novais et al., 2010). Avoidance represented 35% of behavior endpoints in our analysis, and pesticide exposure led to an increase in avoidance in 77% of 135 tested parameters. Observation of avoidance may explain a reduction in abundance or species richness. However, for taxa that do not avoid pesticide treated soil, reductions in abundance may result from higher mortality. There can also be false negatives in avoidance tests; for example, dimethoate did not cause avoidance behavior in *Folsomia candida* but did cause stress or paralysis that prevented movement (Pereira et al., 2013). Additionally, certain taxa like Annelids and Isopods possess chemical receptors that allow them to detect and therefore respond to pesticides more readily than other taxa (Loureiro et al., 2005; Amorim et al., 2008; Marques et al., 2009).

Indirect Effects

Soil toxicology research tends to focus on the impacts of direct exposure and largely ignores the indirect effects on ecosystems when soil organisms are harmed. As such, our review was only able to account for the direct, measured harm to soil organisms

and does not account for any additional harm to ecosystems through indirect effects. For instance, herbicides had a greater negative effect on small arthropod population dynamics through changes to surface litter structural complexity, composition, and nutrition than from direct toxicity (House et al., 1987). Additionally, the direct effects of pesticides on soil organisms can have indirect consequences to ecosystem functioning on a larger scale, including contaminating or reducing food sources for terrestrial vertebrates such as birds (Hallmann et al., 2014; Gibbons et al., 2015), and decreasing crop yield by disrupting pollination services (Reilly et al., 2020) and biological control of target pests (Douglas et al., 2015). As an example, slug predation by the beetle *Chlaenius tricolor* (Dejean) was reduced by 33% following exposure to thiamethoxam, causing a 67% increase in slug activity and density, which resulted in a 19% and 5% decrease in soybean crop density and yield, respectively (Douglas et al., 2015). The importance of these indirect effects of pesticides are underappreciated and, when unaccounted for, can result in an underestimation of risk posed by pesticide use.

Persistence, Recurring Use, and Recovery

Persistence of pesticides in the soil varies greatly across different environmental conditions, like soil type or temperature, and among different pesticides, with particular classes like neonicotinoids (Gibbons et al., 2015) and triazines (Jablonowski et al., 2011) having consistently long soil half-lives. Both burrowing soil taxa and those that develop in the soil are likely to be more vulnerable to the effects of soil-persistent pesticides and conditions that contribute to their persistence.

Some studies in our analysis found that soil invertebrates recovered from negative effects after removal from contaminated soil or following a single pesticide application. We chose not to account for recovery in our data because it would have greatly increased the complexity of our analysis, and its relevance under typical agricultural practices is questionable considering the widespread practice of recurring treatments. For instance, agricultural fields in Great Britain received an average of 17.4 pesticide applications per year in 2015 (Goulson et al., 2018). The United States Department of Agriculture estimates that Washington apples are treated with an average of 51 different pesticides in a total of 6–17 applications per year (USDA, 2016). East coast apples are also treated 15–25 times with pesticides throughout a given year (USDA, 2016). With some pesticides persisting in soil for months or years and the real prospect of recurrent pesticidal applications during the growing season in many fields, soil organisms may not fully recover as they might in the lab or following a single application to a field.

Trends in pesticide application methods are also leading to an increase in the potential for soil contamination. Due to widespread harms associated with pesticide drift, mitigation measures are increasingly being adopted in the United States to cut down on atmospheric presence of pesticides. This includes measures that can increase soil deposition to an area, such as increasing spray droplet size, adding anti-drift adjuvants to formulations and lowering boom height (U.S. EPA, 2016a). In

conjunction with other pesticide application methods that have increased considerably, such as pesticide seed treatment (Hitaj et al., 2020), agricultural soils are increasingly being exposed to pesticides at higher levels. The trend away from foliar pesticide application to soil/seed pesticide application will also increase soil exposure throughout the growing season.

It has been suggested that recovery of the soil invertebrate community is slow and can take more than 15 years (Menta, 2012). Therefore, while recovery from some of these sublethal negative effects is possible, it necessarily depends on quick elimination of the soil pesticide followed by a sufficient period for recovery to take place before another application is made. This will likely vary considerably from field to field.

Representation of Soil Organisms

In observing the effects of pesticides on soil organisms, scientists and regulators have tended to focus on a handful of surrogate species that are conducive to studying in a lab or field environment (Frampton et al., 2006; Banks et al., 2014). The selection of soil organisms for ecotoxicology studies usually relies upon an organism's amenability to the lab and effective use as a bioindicator (Cortet et al., 1999). A review by Jänsch et al. (2006) found that pesticide studies on soil invertebrates are strongly biased toward laboratory testing, and that soil organisms are chosen based upon their ease in testing environments rather than their ecological relevance (Jänsch et al., 2006). Earthworms are the most studied soil organism in ecotoxicology, partially because of their ubiquity in the soil, their pivotal role as ecosystem engineers, and the ease with which they are studied (Luo et al., 1999; Jänsch et al., 2006; Bart et al., 2018). *Eisenia* spp. were particularly common in studies in this review; however, they are not naturally found in agroecosystems and are often less sensitive to pesticides than other earthworms, like *Apporectodea* spp. (Pelosi et al., 2013; Bart et al., 2018). Earthworms are less sensitive to pesticides than other soil invertebrates in general (Frampton et al., 2006; Jänsch et al., 2006; Daam et al., 2011), so standardized test methods have been developed for different taxa also amenable to laboratory studies: *Folsomia candida* (springtail: Collembola), *Enchytraeus albidus* (potworm: Enchytraeidae), and *Hypoaspis aculeifer* (mite: Acari) (Kula and Larink, 1997; Frampton et al., 2006; Jänsch et al., 2006).

Field studies typically look at diverse soil communities, often with a focus on beneficial predators like ground beetles (Carabidae) and rove beetles (Staphylinidae); larger taxa that are easier to capture and identify. Still, there is high variability in sensitivity between beetles, often depending on size and seasonal variations of life cycle; as an example, dimethoate applied at lower rates resulted in harm to smaller species like the Carabids, *Agonum dorsale* (Pontoppidan) and *Bembidion* sp., and the Staphylinid, *Tachyporus hypnorum* F., while not affecting larger Carabids like *Pterostichus melanarius* (Illiger) and *Calathus erratus* (Sahlberg), and generally having more harmful effects on the beetles in the autumn than in the summer (Gyldenkrone et al., 2000).

In general, the smaller and more cryptic Protura, Diplura, Pseudoscorpionida, Symphyla, and Pauropoda are the least commonly investigated soil taxa (Menta and Remelli, 2020).

In our analysis, Protura, Pauropoda, and Tardigrada were each analyzed in only one study (Peck, 2009; Carrascosa et al., 2014; Vaj et al., 2014), and Symphyla and Diplura were only analyzed in the context of mixed organism groups (Al-Haifi et al., 2006; Atwood et al., 2018). In addition, there were fewer than 20 tested parameters for parasitic wasps, termites, and Myriapods, including millipedes and centipedes.

Bumble bees were commonly represented in the studies in our analysis, yet comparatively less negatively impacted by insecticides than other soil taxa. One potential reason is that there was a higher number of field studies for bumblebees compared to other soil taxa, and, as discussed above, field studies tended to reveal fewer negative effects. Pesticides, dominated by insecticides and, in particular, neonicotinoids, negatively affected about 50–60% of bumble bee tested parameters, including survival, reproduction, neural function, and behavior. Bumble bees are one of the few taxa in our analysis that spend much of their life above ground, sometimes nest above ground, and are eusocial. Thus the exposure potential in bumble bee studies may differ from that of other soil organisms (Gradish et al., 2019). Non-*Bombus* ground-nesting bees, though less-studied, were negatively impacted by neonicotinoids and other insecticides at a rate more typical to other taxa in our analysis (i.e., 75–90%), suggesting bumble bees are not good surrogates for ground-nesting bees and soil organisms in general.

Surrogacy in Pesticide Regulation

The European honey bee (*Apis mellifera*) is the only terrestrial invertebrate for which the U.S. EPA (2018) requires testing for pesticide toxicity, and only on an acute-contact exposure basis (Legal Information Institute, 2020). This is the case even for pesticides that are applied directly to the soil. When harm to *pollinators* is expected, the agency will often require additional studies on chronic toxicity to honey bees and/or studies on both adult and larval honey bees (U.S. EPA, 2016b). Testing on other bee species (often *Bombus terrestris* L.) or field or semi-field studies are sometimes, but rarely, requested (U.S. EPA, 2019b). Honey bees have unique life histories and behaviors that result in very different pesticide exposure risk to most invertebrates, even when compared to bumble bees, members of the same taxonomic family that share the very rare trait of being eusocial. While bumble bee colonies often consist of < 500 individuals nesting underground, honey bee colonies of > 10,000 individuals are generally kept in artificial boxes as a domesticated agricultural animal (Gradish et al., 2019). Therefore, pesticide risk to all soil invertebrates in the United States is essentially estimated by harm to a species that generally does not come into contact with the soil and does not share any of the same exposure pathways. For example, most neonicotinoid seed coatings end up in the soil, and concentrations of neonicotinoids can be drastically higher in soil than in pollen (Goulson, 2015; Willis Chan et al., 2019; Dubey et al., 2020; Main et al., 2020).

Not only is *A. mellifera* a highly specialized species whose sensitivity to chemical stressors is not typically representative of other terrestrial invertebrates (Hardstone and Scott, 2010), other arthropods differ from honey bees in their seasonal timing of emergence, life span, degree of sociality, nesting behavior, and

foraging, and are thus subjected to different pesticide exposure routes and levels than honey bees - such as via cuticle contact or soil-covered prey consumption. For example, bumble bees in underground nests experience contact exposure via residues on soils, including as developing larvae and hibernating queens (Gradish et al., 2019). Over 80% of bees, the vast majority of which are solitary, are ground-nesting (Anderson and Harmon-Threatt, 2019) and are at great risk of pesticide exposure, as adult females spend the majority of their life cycle constructing nests in the soil (Willis Chan et al., 2019). Thus, even for soil invertebrates in the same superfamily as honey bees, exposure to, and harm from, pesticide residues in soil and soil cell water are not adequately estimated in current EPA pesticide ecological risk assessments.

Implications for Regulation

From these data it is apparent that, as a set of chemical poisons, pesticides pose a clear hazard to soil invertebrates. A previous review has identified similar hazards to soil microorganisms (Puglisi, 2012). Each individual pesticide will pose a unique risk profile to each soil-dwelling species that is exposed based on exposure potential and sensitivity. This review supports the need for soil health endpoints in regulatory risk assessment of pesticides to assess the probability that pesticide use will negatively impact these ecosystems.

Due to the sheer number of species and endpoints that can be impacted by pesticide use and researchers' limited capacity to test every permutation, pesticide risk assessment is heavily reliant on surrogate species that are used to generalize toxicity across taxa, and in the case of soil, across entire ecosystems. The level of detachment of soil surrogate species from the taxa they are supposed to represent varies widely across regulatory agencies around the globe. Of the pesticide regulatory agencies whose regulatory process on soil organisms could be easily obtained, none differ as much in their treatment of soil organisms as the European Food Safety Authority (EFSA) and the United States EPA. While the EFSA has testing requirements in place to quantify risk to multiple soil dwelling organisms, the EPA has none.

For pesticides that are likely to contaminate soil, the EFSA currently requires chronic toxicity tests on one earthworm species (*Eisenia fetida* or *Eisenia andrei*), one springtail species (*Folsomia candida*) and one mite species (*Hypoaspis aculeifer*) (Ockleford et al., 2017). The EFSA also requires a study on nitrogen transformation as a readout of soil microbial activity (Ockleford et al., 2017). If the ratio of exposure to toxicity exceeds a predetermined threshold, higher tier tests, such as field testing, may be required. Additional protection goals have recently been identified, and the EFSA is currently considering strengthening its requirements to include additional exposure pathways and to require tests on Isopods and mycorrhizal fungi (Ockleford et al., 2017).

The EPA's practice of using only the honey bee as a surrogate for other terrestrial invertebrates underestimates harm to many taxa and can be even more consequential when it comes to identifying mitigation measures for a pesticide. For instance, mitigation measures the EPA has put in place for pesticides that

pose a significant risk to the honey bee (which should indicate risk to all terrestrial invertebrates due to its use as a surrogate) are generally designed to reduce risk only to pollinators (U.S. EPA, 2019a, 2020b). Mitigations, like spray restrictions during flower bloom, increasing droplet size to reduce drift, or label language identifying a pollinator hazard, will likely have little impact on soil organism exposure to a pesticide. Of the 219 individual pesticides represented in our review, 74% are currently approved in the United States and 26% are not in current use but could be registered at any time, indicating that our results are highly relevant to pesticide regulation in the United States.

CONCLUSION

This paper constitutes a comprehensive review of the impacts of agricultural pesticides on soil invertebrates. We found that pesticide exposure negatively impacted soil invertebrates in 70.5% of 2,842 tested parameters from 394 reviewed studies. We also identified several broad trends and directions for future research. Insecticides were the most studied type of pesticide and generally had greater negative impacts on soil invertebrates than herbicides, fungicides and other pesticide types. Herbicides and fungicides still had a high proportion of negative effect findings, however negative effects varied much more widely between different pesticide classes and studied taxa than with insecticides. Fewer studies evaluated the impacts of pesticide mixtures. Given that pesticide mixtures are more commonly found in agricultural soils than individual active ingredients, this is a gap in the literature that should be addressed.

Studies evaluating pesticide impacts often use a narrow range of surrogate species that are easy to rear, identify, or study, while smaller and more cryptic organisms are rarely analyzed. In some cases, the organisms that are the most extensively studied are known to be less sensitive to pesticides than other organisms, suggesting that we have limited knowledge of the extent of harm caused by pesticides.

The prevalence of negative effects in our results underscores the need for soil organisms to be represented in any risk analysis of a pesticide that has the potential to contaminate

soil, and for any significant risk to be mitigated in a way that will specifically reduce harm to the soil organisms that sustain important ecosystem services. The United States Environmental Protection Agency does not have sufficient testing requirements or tools in place to quantify risk to soil dwelling organisms. The European honey bee is the only terrestrial invertebrate included in mandatory ecotoxicological testing of pesticides. The practice of using the honey bee as a surrogate underestimates harm to many taxa and often results in narrow efforts to mitigate pesticide impacts solely to honey bees and other pollinators, not soil organisms.

This review presents extensive evidence that pesticides pose a serious threat to soil invertebrates and the essential ecosystem services that they provide. Given the widespread and increasing adoption of seed and soil applied pesticides that pose a particular threat to soil organisms, we strongly support the inclusion of a soil health analysis in the United States pesticide risk assessment process.

AUTHOR CONTRIBUTIONS

TG, TC, and ND conceptualized the project and, with KK, developed the structure for the review. TG researched, compiled data, and prepared an initial manuscript. All authors discussed the results and contributed specific knowledge of the relevant literature. All authors contributed to the editing and finalization of the manuscript and figures.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2021.643847/full#supplementary-material>

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Conflict of Interest: ND, on behalf of his employer, has submitted public comments to the Environmental Protection Agency that have been critical of the agency's pesticide risk assessments of terrestrial invertebrates and use of honey bees as surrogate species. ND and TC, on behalf of their employer, have expressed public positions on the scientific evidence of harm to terrestrial invertebrates from some pesticides.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Green Experiential Marketing, Experiential Value, Relationship Quality, and Customer Loyalty in Environmental Leisure Farm

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Environmental/green marketing has emerged as one of the dominant paradigms of marketing in recent years. However, aspects, such as internationalization, the development of artificial intelligence, and stress from growing global competitive forces, have brought about changes in the way leisure farms approach experiential marketing with a significant environmental focus. In this context, the concept of relationship quality offers an opportunity for environmental leisure farms to understand how green experiential marketing impacts consumers' perceived value and the ongoing interaction relationship. This study adopts a comprehensive perspective that includes green experiential marketing and relationship marketing that leisure farms use in order to enhance customer loyalty, and analyzes the effect of a series of elements inherent to customer psychic or personal needs. Seven hundred fifty-four valid copies of questionnaire were adopted in total. To verify the proposed model empirically, a survey of customers of environmental leisure farms in Taiwan was conducted. Structural equation modeling is conducted to examine the research hypotheses. The findings show that, overall, green experiential marketing has positive direct effects on experiential value and experiential value has positive direct effects on trust, commitment, and satisfaction. At the same time, trust and satisfaction have positive effects on attitudinal and behavioral loyalty. In addition, attitudinal loyalty has a positive influence on behavioral loyalty.

Keywords: green experiential marketing, green marketing, experiential value, relationship quality, customer loyalty

JEL Classifications: C83, M31, Q13, Z33.

INTRODUCTION

In the context of economic booms, people in urbanized environments are exposed to nonenvironmental, non-green, and unhealthy things and are trapped in an oppressive and tense situation. With increasing awareness of the need for environmental protection, consumers are paying more attention to the green experience and pursuing their physical health (Wu H. C. et al., 2018). This has urged leisure farms to enhance their environmental image, so as to give a positive impression to tourists and promote consumer loyalty to the farms. According to Owens (2000),

the advent of new technologies, increasing numbers of competitors, and increasing wealth of consumers will change economic industries that focus on services, and lead a new consumption trend that focuses on experience (Chang and Horng, 2010; Maklan and Klaus, 2011; Lemon and Verhoef, 2016). In recent years, the experience economy has become a hot topic of discussion for economists and management scholars (Pine and Gilmore, 1998; Mathwick et al., 2001; Hosany and Witham, 2010; Brun et al., 2017), and the concept of experiential marketing has also emerged (Chang and Horng, 2010; Maklan and Klaus, 2011). According to Brun et al. (2017), current generic conceptualizations of experience may be too broad to be actionable and relevant in any one context. Indeed, measurement of variables could require differentiation based on the context (Hosany and Witham, 2010; Lemke et al., 2011; Maklan and Klaus, 2011), such as green experiential marketing. In this regard, green experiential marketing can be defined as that something environmentally friendly is added into a brand, product, or service, which brings consumers unforgettable experiential memories and enhances their sense of identity with the concept of environmental demands. Under the circumstances of sustainable development of environment and leisure farms, it is necessary to figure out how to make tourists perceive the significance of environmental protection and enhance their consuming intention. Thus, this study explores the role that green experiential marketing plays in the sustainable development of environment and leisure farms from the perspective of green marketing.

In the field of service marketing, better pre- and post-sales services are also required, after establishing the relationship, so as to maintain and enhance it (Maklan and Klaus, 2011; Monferrer-Tirado et al., 2016). Moliner et al. (2007) claimed that customers' overall assessment of service providers is reflected in the perceived relationship quality (RQ), which is also a key mechanism for guiding customer behaviors (Arcand et al., 2017). The perceived RQ is defined as the degree of appropriateness in meeting the demands for customer relations (Hennig-Thurau and Klee, 1997; Olavarria-Jaraba et al., 2018). However, no previous studies have analyzed the possible RQ antecedents from the perspective of green marketing. Or, put another way, studies have failed to include the set of green experiential marketing and experiential value aspects needed by environmental leisure farms so that customer value and relations can be maintained (Olavarria-Jaraba et al., 2018). Based on the above arguments, this study aims to explore the mediating role of RQ in the relationship between green experiential marketing and consumers' intentions to visit leisure farms.

Based on consumer decision-making theory, Kotler (1997) proposed that the consumer decision-making process comprises a "black box." Because consumers' decisions are deeply influenced by their cultural background, social culture, and personal psychological factors, they only make purchase decisions—which also comprise a subsequent behavior—following a complicated psychological process after being stimulated by external factors (Chang and Horng, 2010; Srivastava and Kaul, 2016). Consumer decision-making theory describes the antecedent variables as external stimuli

to consumers, whereas the RQ is the psychological process of dealing with stimuli (Monferrer-Tirado et al., 2016); an attitude toward the stimuli is formed, and this is subsequently reflected as loyalty (Brakus et al., 2009; Srivastava and Kaul, 2016; Brun et al., 2017). However, in terms of RQ, there is still a question of whether loyalty behaviors are affected by attitude, which is derived from the antecedent variables (stimuli), or from the interaction effect of different attitudes originating from the psychological process (Maklan and Klaus, 2011; Olavarria-Jaraba et al., 2018). In fact, consumer motivation and decision-making for green product or service purchase differ from those for common product purchase. For consumers, green consumption is often not based on immediate self-interest, but on environmental altruism. Thereby, compared with the purchasing behavior pattern of common products, the purchase decision of green consumption is often more complicated. Despite green experiential marketing is an important source of stimulation, it still needs to judge the feelings brought by the stimulation *via* information processing and further transform it into positive emotions and attitudes. Experiential value may be an important intermediary variable between green experiential marketing and RQ thereinto. Mathwick et al. (2001) defined experiential value as consumers' perception of and relative preference for product attributes or service performance. Thus, this study regards experiential value as an important antecedent variable that affects RQ.

According to our research purposes, this study attempts to provide several contributions. Firstly, this study approaches the consumer experience marketing in terms of green or environmental awareness. Secondly, we adopted the theory of consumer decision-making to verify the framework generated from green and relationship marketing. Thirdly, this study employs consumer decision-making theory to examine the relationships among green experiential marketing, experiential value, and RQ, and consumer loyalty is deepened in the view of management, through which it may develop during the process of stimuli, cognition, emotion, and action. Hence, recommendations for managers relevant to environmental sustainability will be put forward.

LITERATURE REVIEW AND HYPOTHESES DEVELOPMENT

Theoretical Foundation

Referring to the study by Oliver (1997), this study proposes that the loyalty should be formed through the path of cognition–affect–conation–action. In the cognition phase, consumers will make evaluation based on stimulus or previous experience and knowledge (experiential value) (Brun et al., 2017). If satisfaction arises, it will enter the phase of affect. The affect phase focuses on the psychological level of consumers and is based on the cumulative experience of satisfaction (RQ). The conation phase means that consumers have repeated positive emotions and the commitment to repurchase. Finally, the action phase is the action control, indicating that the consumer's purchasing pattern has been formed and any obstacle that prevents the repurchasing

will be overcome (customer loyalty) (Srivastava and Kaul, 2016). Notwithstanding a lot of research support for the relationship among the variables of this study in a previous literature (as shown in **Table 1**), such as experiential value and loyalty, RQ and loyalty, experiential value and RQ, etc., but in allusion to the issues of environmental sustainable development and leisure farms, there are few research studies combining the concept of green marketing and experiential marketing for discussion and then developing the potential gaps in the literatures to be discussed in this study.

Customer loyalty has always been a hot topic in the field of marketing, and it has also driven a lot of research on loyalty marketing (Srivastava and Kaul, 2016; Brun et al., 2017). Oliver (1997) defined customer loyalty as “despite environmental changes and competitors’ efforts in marketing have a potential impact on the conversion of consumer behaviors after purchase, consumers are still willing to promise to buy or consume the same goods and services in the future, thereby resulting in repetitive purchases of the same brand or brands.” According to Oliver’s definition, customer loyalty can be divided into two parts: attitudinal loyalty and behavioral loyalty (Srivastava and Kaul, 2016). Attitudinal loyalty reflects the consumer’s psychological level, whereas behavioral loyalty mainly reflects the actual purchase behavior of consumers. Singh and Sirdeshmukh (2000) stated that customer loyalty is a behavioral tendency for consumers to continue to maintain relationships with service providers. Chao et al. (2007) argued that competitors in the retail industry will provide heterogeneous services and competitive prices to attract customers, but the customer loyalty may not follow (Monferrer-Tirado et al., 2016). The background of this research is similar to the retail industry. Managers of leisure farms offer differentiated and customized services to attract customers, expecting the generation of loyalty. However, Chao et al. (2007) measured customer loyalty only by the behavioral loyalty, holding that although the attitudinal loyalty is important to the expected cash flow in the future, it cannot be immediately reflected on the company’s financial performance. This study proposes that according to the view of relationship marketing, the operators of this industry should develop long-term stable relationships with customers for the purpose of establishing and maintaining customer relationships, although the behavioral loyalty can produce substantial benefits in the short term (Monferrer-Tirado et al., 2016). Therefore, subsequent studies are recommended to consider both attitudinal loyalty and behavioral loyalty when measuring brand loyalty (Zeithaml et al., 1996; Oliver, 1997; Palmatier et al., 2006). Particularly, the behavioral loyalty, also known as purchase loyalty, refers to the customer’s repeated purchase.

Green Experiential Marketing

Schmitt (1999) claimed that experience comprises an event in which an individual responds to certain stimuli, including the essence of the whole life, where the experience derives from engagement in or direct observation of the event, no matter whether the event is real, fantasy, or virtual (Pine and Gilmore, 1998; Lemke et al., 2011; Lemon and Verhoef, 2016). In this regard, the experience is translated into interaction between

TABLE 1 | Studies on experiential marketing and loyalty.

Research	Dimensions	Research context	Methodology
Tsaur et al., 2007	Experiential marketing, emotion, satisfaction, behavioral intention	Tourism 484 visitors	Questionnaires SEM
Yuan and Wu, 2008	Customer satisfaction, experiential marketing, experiential value	Restaurant 420 customers	Questionnaires SEM
Slåtten et al., 2009	Atmospheric experiences, customer emotion, feelings of joy, customer loyalty	Tourism 162 visitors	Questionnaires SEM
Luo et al., 2011	Virtual experiential marketing, site browsing intentions, purchasing and loyalty	Web 976 buyers	Questionnaires SEM
Jin et al., 2013	Experiential value, relationship quality, loyalty, gender	Restaurant 368 customers	Questionnaires SEM
Wu and Ai, 2016	Experiential quality, excitement, equity, experiential satisfaction, experiential loyalty	Golf 542 customers	Questionnaires SEM
Srivastava and Kaul, 2016	Customer experience, attitudinal loyalty, behavioral loyalty, share of wallet	Retail 840 customers	Questionnaires SEM
Brun et al., 2017	Customer experience, channel, loyalty	Tourism 484 customers	Questionnaires SEM
Wu H. C. et al., 2018	Experiential quality, green relationship quality, green support, green desire, green experiential loyalty	Hotel 517 customers	Questionnaires SEM
Ahn et al., 2019	Experiential value, co-creation attitude, co-creation behavior	Resort 500 customers	Questionnaires SEM

the body, the cognition, and the affect in the context of the environment, and any effort or skill of experience introduction will affect that interaction (Arnould et al., 2004; Chang and Horng, 2010; Maklan and Klaus, 2011; Brun et al., 2017). Affect and cognition are inseparable. Affect refers to the evaluation process in the mind, which derives the physical state and the additional mind, making experience the core of consumer behavior (Maklan and Klaus, 2011). Pine and Gilmore (1998) believed that experience crosses two dimensions, of which the core is customer participation and passive participation and active participation are contained. Hence, customers are critical to performance or event creation which yields experience. The greatest difference between experiential and traditional marketing is that traditional marketing puts emphasis on product features and benefits, whereas experiential marketing shifts the focus to the overall consumption situation and brand experience (Hosany and Witham, 2010; Lemke et al., 2011). However, environmental issues are now a core competitive factor in product markets (McDonagh and Prothero, 2014). Compared with the experiential and/or traditional marketing era, where the emphasis was on front-line polluters, environmentally friendly behaviors are being more widely adopted across all industries (Papadas et al., 2017). Some scholars have concluded that the green marketing has not performed to its full potential, and that existing research about environmental/green marketing is still in the stage of studying its applied value in practice (Fuentes, 2015; Papadas et al., 2017). Despite there is a lack of definitive results from previous research, this study is based on the current literature and aims to capture a more integrative perspective of green experiential marketing. To identify the purposes of environmental/green marketing, this study conceptualizes the green experiential marketing construct as a set of dimensions and defines it as a series of physical and psychological stimuli of environmental/green awareness while offering consumers products and services that have environmental value (Wu H. et al., 2018).

The green experiential marketing concept is based on the strategic experiential field. Schmitt (1999) proposed to cooperate with experiential providers to attract customers *via* five aspects of experience—sense, feel, think, act, and relation—which allow us to understand customer experience from a wide, integral and comprehensive angle. In this study, a multi-dimensional model is adopted for comprehension of perceived green experiential marketing. According to a large number of studies (Wu and Li, 2017), a multi-dimensional model is available for measuring green experiential marketing in an environmental leisure farm. These five aspects have been deemed as the basis for research in this field (e.g., Gentile et al., 2007; Brakus et al., 2009; Verhoef et al., 2009) and have become widely accepted (Lemon and Verhoef, 2016). The “sense” experience creates sensory impacts through perceptual stimuli, providing excitement, pleasure, and satisfaction, and creating a fresh and unique emotional or perceptual experience (Lemke et al., 2011). In the process of consumption at environmental leisure farms, consumers can experience psychological comfort and relaxation in the countryside. The “feel” experience aims to tap into consumers’ inner emotions and affects and enables consumers to generate

positive emotional responses to related products and brands by providing that experience. The emotional experience generated *via* environmental leisure farms enables consumers to relieve stress, relax, increase their emotional communication with the environment, and experience enhanced emotional value. The “think” experience encourages consumers to think differently and creatively compared with in their day-to-day lives. Consumers gain think experiences in environmental leisure farms, learn about farm life, and observe people’s affairs in rural contexts. The “act” experience comprises the connection between body experience and lifestyle. By increasing the body experience, alternative lifestyles and ways of doing things are found, which enrich the lives of consumers (Lemke et al., 2011). The act experience in environmental leisure farms serves to transform the attitudes of consumers and create a common drive to protect the environment through participation in activities related to environmental protection, thereby promoting a positive experience. Finally, the “relate” experience links individuals to the broad cultural and social contexts reflected in a brand, allowing individuals to link with others, specific communities or cultures, and abstract entities (Srivastava and Kaul, 2016).

Based on the above arguments, the previous studies suggested that when it comes to the green marketing strategies of an environmental leisure farm, the green experiential marketing is critical to consumer experience determination (Wu H. C. et al., 2018). Meanwhile experiential value based on the customer experience is also of great significance (Marković and Raspor Janković, 2013). Wu and Li (2017) argued in their study that good experiential quality contributes to the positive perception of the experienced products for consumers, which is better than the expected perceptual experiential value. Similarly, Tsaor et al. (2007) stated that the five variables of experiential marketing can strongly influence consumers’ positive emotions and enhance their dedication and enjoyment in the process of experience. At environmental leisure farms, consumers can look for the relevance with green environmental protection, so as to deepen their recognition of farms. On the basis of the above reasoning, this study suggests that green experience marketing will enhance consumers’ experiential value. Thus, the following hypotheses are proposed:

H1a: Sense experience has a positive and significant impact on experiential value.

H1b: Feel experience has a positive and significant impact on experiential value.

H1c: Think experience has a positive and significant impact on experiential value.

H1d: Act experience has a positive and significant impact on experiential value.

H1e: Relation experience has a positive and significant impact on experiential value.

Experiential Value

Lam et al. (2004) argued that customer value refers to the overall value that customers obtain from products or services, including product value, service value, personnel value, and

image value. Customer cost is the financial amount required to obtain value from products and services, including monetary cost, time cost, energy cost, and psychic cost. The true value of the customer comes from the gap between the total customer value and the customer cost. If the total customer value is greater than the customer cost, the value is positive; otherwise, it is negative (Huber et al., 2001; Flint et al., 2002, 2005). Mathwick et al. (2001) referred to customer value from the perspective of experience, calling it experiential value. They identified the relevant aspects of experiential value and defined it as a perception and relative preference for product attributes or service performance, stating that such value can be improved through interactions, but these interactions may help or hinder the achievement of consumer goals. Referring to customer value from the perspective of experience, as proposed by Holbrook (1999); Mathwick et al. (2001) classified experiential value into four types: customer return on investment, service excellence, aesthetics, and playfulness.

Zeithaml et al. (1996) advocated that improving experiential value leads to greater consumer satisfaction. Before buying, consumers already have a standard of service (i.e., an expectation), which is used to measure the service performance, resulting in a positive or negative evaluation (Oliver, 1997). If consumers experience cognitive value brought by their experiential activities, they will believe in the goods and services provided by the operators and respond in a kind and friendly manner (Duncan and Moriarty, 1997; Baker et al., 2002). Zeithaml (1988) argued that the greatest difficulty in value research lies in the multiple meanings value holds for consumers and the implicit symbolic meaning at the highest level. These aspects may involve an emotional payoff. The relationship between experiential value and RQ needs to be found for an overall comprehension of the various functions of experiential value in leisure farm settings. According to Wu and Li (2017), Wu H. C. et al. (2018) experiential value perceptions are closely related to interactions between direct experience and distant evaluations of feelings, and such interactions lay the basis for experiential value perception and determine the level of consumer RQ. Wu H. C. et al. (2018) found in the research results that the perceived experiential value, through the value-added concept of experiential marketing, can promote consumers' satisfaction with the friendly green environment and enhance their trust in the green environment after the experience, thus leading to green experiential loyalty. Jin et al. (2013) examined the effects of experiential value on RQ in a full-service restaurant, finding experiential functions and value to be important antecedents to RQ in US restaurants. In our context, when consumers obtain emotional value from a better experience value at a leisure farm, they will have a high-level emotional reaction to the leisure farm, thus generating enhanced expectations and support for the operator, as well as a willingness to maintain the mutual relationship in return for the emotional payoff involved (Maklan and Klaus, 2011). Therefore, hypotheses are developed as follows:

H2a: Experiential value has a positive and significant impact on satisfaction with leisure farm.

H2b: Experiential value has a positive and significant impact on trust with leisure farm.

H2c: Experiential value has a positive and significant impact on commitment with leisure farm.

Relationship Quality

Crosby et al. (1990) stated that RQ is an overall assessment of the relationship intensity between buyer and seller. This assessment is in line with the needs and expectations of both parties, and these needs and expectations are based on past successful or failed experiences or events of both parties. Smith and Bolton (1998) also indicated that RQ is a high-level contract built on the results of various positive relationships (Olavarria-Jaraba et al., 2018), reflecting the overall relationship intensity and the degree of satisfaction of the parties in terms of needs and expectations, in the manner of a reciprocal output (Wulf et al., 2001). However, to date, there have been no explicit discussions on the mediators of RQ in green marketing, and few previous studies have discussed RQ from the perspective of environmental protection, in that experiential value can impact loyalty through mediators such as trust, commitment, and satisfaction (Monferrer-Tirado et al., 2016). The present study aims to address this deficiency. Therefore, referring to the concepts put forward by Wulf et al. (2001); Beloucif et al. (2004), Rauyruen and Miller (2007); Olavarria-Jaraba et al. (2018), this study uses satisfaction, trust, and commitment as the main aspects of RQ. This study not only verifies the causal relationship among the measurement variables, such as antecedent variable, dependent variable, and RQ, but also discusses the relationship among the measurement variables within the RQ (Ferro et al., 2016; Arcand et al., 2017).

Morgan and Hunt (1994) defined trust as the degree to which a relationship member has confidence in the reliability and honesty of a trading partner. Trust not only is about having confidence in a relationship partner but also centers on the individual's willingness to take risky actions to support the partner (Garbarino and Johnson, 1999; Moliner et al., 2007; Olavarria-Jaraba et al., 2018). Trust can also be defined as the willingness to rely on a trusted and well-meaning partner. If customers receive high-quality services and are aware that service personnel are reliable and genuine, positive word of mouth will form (Prasad and Aryasri, 2008; Monferrer-Tirado et al., 2016). Caceres and Paparoidamis (2007) argued that long-term loyalty entails commitment, where if a customer has brand commitment, three important behavioral outputs will result: repeated purchase of the brand's products, offsetting of changes caused by competition, and offsetting of negative feelings caused by dissatisfaction. It has been indicated in the studies from Wu and Ai (2016), Wu H. C. et al. (2018) that high trust and satisfaction arising from the process of experience are conducive to generating high customer loyalty. In long-term relationships with customers, if customers' commitment can be continuously maintained, their relationship with the firm will be enhanced, which will increase purchase frequency (Prasad and Aryasri, 2008; Arcand et al., 2017). Furthermore, Caceres and Paparoidamis (2007) argued that the development

of trust and commitment is strategically important, because it creates a relationship atmosphere between the two parties and directly affects the customer's behavioral intentions (Monferrer-Tirado et al., 2016). Past research has illustrated that customer satisfaction is a key antecedent factor for customer loyalty and repeated purchases (Seiders et al., 2005). In the environmental setting, a leisure farm perceived to be environmentally friendly can make consumers more satisfactory with their product or service when they visit the farm (Wu H. C. et al., 2018). Prasad and Aryasri (2008) claimed that the quality of preferred service attributes will increase the satisfaction level and satisfaction strength (Hosany and Witham, 2010; Lemon and Verhoef, 2016). Additionally, Jin et al. (2013) investigated the mediating role of RQ on the connection between experiential value and relationship outcomes (attitudinal and behavioral loyalty) in full-service restaurants and found that the effective use of experiential value increased customers' RQ to the restaurant, thereby increasing loyalty. Therefore, the following hypotheses are derived:

H3a: Trust has a positive and significant impact on consumers' loyalty (behavioral and attitudinal) in leisure farms.

H3b: Satisfaction has a positive and significant impact on consumers' loyalty (behavioral and attitudinal) in leisure farms.

H3c: Commitment has a positive and significant impact on consumers' loyalty (behavioral and attitudinal) in leisure farms.

In the service industry, high contact between consumers and service providers is necessary. Customers with high satisfaction of service experience will have a high degree of trust in the service provided by the organization itself and the service provider (Arcand et al., 2017). Garbarino and Johnson (1999) demonstrated that satisfaction is an antecedent factor for trust, and that the higher the satisfaction, the higher the trust degree of consumers, no matter for product purchase or the business performance, between which there is a positive relationship (Moliner et al., 2007; Ferro et al., 2016). Lin et al. (2014) also believed that trust is a major element in the development of higher-order relationships, especially at the initial stage of relationship development, consumers' imagination degree of demand and expectation for relationship strength and satisfaction should be enhanced so as to establish the cornerstone of stable relationships (Arcand et al., 2017). In other words, in environmental leisure farms, satisfied consumers will be more likely to increase short-term and long-term consumption levels (Hsieh and Hsiang, 2004) than dissatisfied consumers by establishing trust with leisure farms (Ferro et al., 2016). Thus, this study explores that consumers show preference when they perceive services and environmental qualities provided by environmental leisure farms, then they will have higher trust in leisure farms. To sum up, the following hypothesis is proposed in this study:

H3d: Satisfaction has a positive and significant impact on trust in leisure farms.

Trust is the willingness to rely on trustworthy and good-faith exchange partners (Ferro et al., 2016). When consumers

receive good quality services, they perceive the service providers as reliable and benevolent, thus generating positive reputation (Prasad and Aryasri, 2008). Arcand et al. (2017) mentioned that the key element of trust lies in the fact that consumers believe in the intentions and motivations of sellers that are beneficial to consumers and have the creation of positive consumer output in consideration. If consumers perceive that the business operator does not have any speculative behavior, but consider their own welfare, they will have higher trust in the operator (Ferro et al., 2016). Besides, Caceres and Paparoidamis (2007) considered that the development of trust and commitment is of strategic importance, as it forms the atmosphere of relationship between the two sides and directly influences consumers' behavioral intention. In conclusion, the following hypothesis is proposed in this study:

H3e: Trust has a positive and significant impact on commitment in leisure farms.

Most previous studies have considered attitude as the antecedent factor of behavior (Zaltman and Wallendorf, 1983; Srivastava and Kaul, 2016). Indeed, Oliver (1997) proposed a loyalty model that divides customers' loyalty regarding products or services into four stages (Srivastava and Kaul, 2016): cognitive loyalty, emotional loyalty, attitudinal loyalty, and behavioral loyalty. These four stages have a sequential relationship. In addition, when the customer generates an intention to recommend and repurchase, the behavioral loyalty stage will start, resulting in substantial purchase behavior. McMullan and Gilmore (2003) echoed Oliver's (1997) view that loyalty can be divided into four stages, and that these stages come one after another. They also indicated that behavioral loyalty always comes after attitudinal loyalty (Srivastava and Kaul, 2016). Based on the results of past research on customer loyalty, the following final hypothesis is proposed.

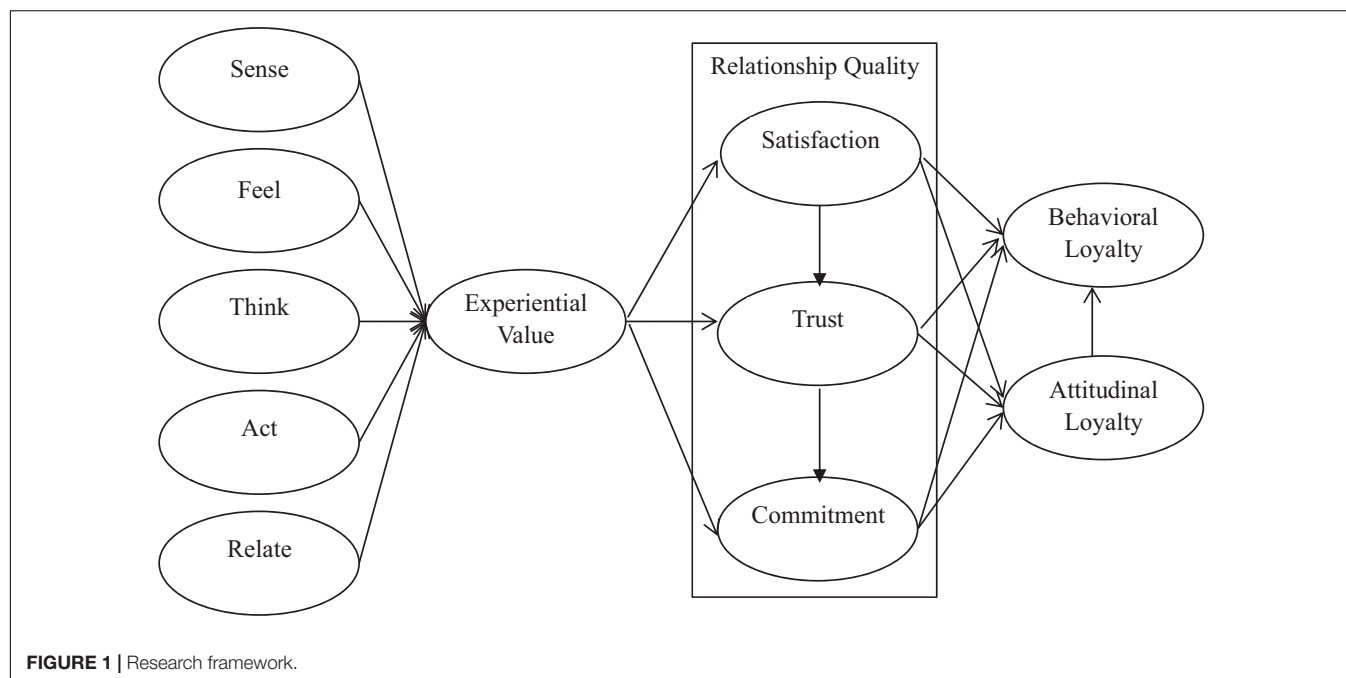
H4: Consumers' attitudinal loyalty has a positive and significant impact on behavioral loyalty.

Based on the above arguments, this study proposes the following research framework in **Figure 1**.

METHODOLOGY

Sampling

The questionnaire used in this study was designed based on extant literature about experiential marketing (e. g., Kumar et al., 1995; Garbarino and Johnson, 1999; Schmitt, 1999; Moliner et al., 2007). Two pre-tests and several revisions were undertaken. The final questionnaire comprised three sections focusing on the following areas: (1) the consumer's perceived experiential marketing and experiential value provided by an environmental leisure farm whose services they had used, (2) the consumer's overall satisfaction with, trust in, and commitment and loyalty to the environmental leisure farm, and (3) demographic information on the consumer. Sampling of all leisure farms is difficult on account of numerous leisure farms in Taiwan; thus, purposive sampling



was adopted. Moreover, some principles for sampling were set so as to accurately measure consumers' perceptions of the variables in the study and improve external validity. First, it intends for investigation of consumers' actual cognition to environmental leisure farms. Respondents are those who have been to environmental leisure farms, for those who have just been to general leisure farms may not have explicit expression of their feelings toward the importance of environmental protection; thereby, the effect of each variable on customer loyalty is impossible to measure. Second, since consumers with a clear environmental awareness are constituted by the sample, the question was contained to eliminate those who were less inclined to seek environmental leisure farms in the near future with the purpose of representativeness enhancement of samples, which expressed "Do you have environmental awareness?" One thousand copies of questionnaire were distributed among environmental leisure farm consumers, and 761 were returned, giving a 76.1% return rate. After removing the copies that were considered invalid, 754 remained for use in our empirical analysis.

Of these 754 samples, 53% of the participants were male, and 47% of the participants were female; 51.7% were aged less than 30 years; 82.1% had completed university courses; and the group was distributed into equal thirds regarding personal average monthly income (less than 600 USD, 600–1,300 USD, and more than 1,300 USD). In addition, 486 respondents reported having a relationship with a leisure farm for fewer than 2 years, 115 reported a relationship of 2–3 years, and 153 reported a relationship of longer than 3 years. To confirm whether there are differences among the samples in different background variables, this study conducted independent sample *t* test in terms of gender, income, age, and other factors. The results show that there is no significant influence when the sample is placed in

different background variables. Thus, sample segments are not a critical issue.

Measurements

Green Experiential Marketing

We developed the experiential marketing scale based on Schmitt's (1999) model. To enrich the items with the concept of green marketing, the study has modified the content of the items and conducted a pre-test before the formal test to check the availability of the items. In the pre-test, 53 samples were tested, and the factor analysis results show that the measurement variables can be extracted effectively and achieved good reliability (Cronbach's α values of all variables are higher than 0.7). Thus, this scale was adopted for a formal test. The objective was to measure the five main dimensions and the subdimensions thereof, using a short, user-friendly scale that would ensure a high response rate. Items were included to measure perceptions of sense (4 items), feel (3 items), think (2 items), act (4 items), and relation (3 items).

Experiential Value

We used items adapted from the dimensions of Mathwick et al.'s (2001) scale of experiential value. Items were included to measure perceptions of playfulness (4 items), aesthetics (3 items), customer return on investment (2 items), and service excellence (2 items).

Relationship Quality

Relationship quality refers to the overall nature of the relationship between the consumer and the firm and views fulfilling consumers' needs as central to relationship success. Beloucif et al. (2004); Rauyruen and Miller (2007) proposed that RQ is a three-dimensional construct consisting of satisfaction, commitment,

and trust. Specifically, satisfaction was measured using a five-point semantic differential scale, as recommended by Ganesan (1994). Commitment was measured using three items adapted from scales by Garbarino and Johnson (1999); Morgan and Hunt (1994). Trust was measured using a five-point semantic differential scale, as recommended by Morgan and Hunt (1994); Kumar et al. (1995), Garbarino and Johnson (1999).

Customer Loyalty

Previously, the literature has divided customer loyalty into two categories: behavioral loyalty, which means repurchase behavior, and attitudinal loyalty, meaning recognition and attitude (Oliver, 1997; Chaudhuri and Holbrook, 2002). This study applied a five-point Likert scale to measure customer loyalty with respect to the following items: behavioral loyalty (4 items) and attitudinal loyalty (3 items). All items are using five-point Likert scales (1 = totally disagree; 5 = totally agree) and shown in **Table 2**.

DATA ANALYSIS STRATEGY

This study tested the hypotheses of research framework and included paths *via* structural equation modeling (SEM). For construct with a higher-order factor structure (green experiential marketing, experiential value RQ, and customer loyalty), if the traditional regression model is used, the effect between parameters is difficult to be tested. Thus, SEM is adopted in this study for model test and hypothesis verification. Structural validity analysis was performed using the IBM-AMOS statistical program, v. 23.0 (New York, NY, USA) for Windows; this program was also used to construct the structural prediction model, specifically, verification of the structural linear prediction hypothesis (path analysis) (de la Fuente et al., 2020).

MODEL ESTIMATE

The latent variable SEM is conveniently divided into two parts: the latent variable model and the measurement model. The latent variable model (sometimes called the structural model) is (Eq. 1).

$$\eta = \alpha_{\eta} + \mathbf{B}_{\eta i} + \Gamma \zeta_i + \zeta_i \quad (1)$$

where η_i is a vector of latent endogenous variables for unit i , α_{η} is a vector of intercept terms for the equations, $\mathbf{B}$ is the matrix of coefficients giving the expected effects of the latent endogenous variables (η) on each other, ζ_i is the vector of latent exogenous variables, Γ is the coefficient matrix giving the expected effects of the latent exogenous variables (ζ) on the latent endogenous variables (η), and ζ_i is the vector of disturbances. The i subscript indexes the i th case in the sample (Bollen and Noble, 2011). In this study, sense (ζ_1), feel (ζ_2), think (ζ_3), act (ζ_4), and relation (ζ_5) are latent exogenous variables; experiential value (η_1), satisfaction (η_2), commitment (η_3), trust (η_4), attitudinal loyalty (η_5), and behavioral loyalty (η_6) are latent endogenous variables. According to 17 paths in our research framework, this study forms six equations. To illustrate this model in equation form, consider the equation for the latent green experiential

marketing variables [sense (ζ_1), feel (ζ_2), think (ζ_3), act (ζ_4), and relation (ζ_5)] for experiential value (η_1) (Eq. 2),

$$\eta_1 = \zeta_1\gamma_{11} + \zeta_2\gamma_{12} + \zeta_3\gamma_{13} + \zeta_4\gamma_{14} + \zeta_5\gamma_{15} + \zeta_1 \quad (2)$$

and the equation for satisfaction variable (η_2) (Eq. 3),

$$\eta_2 = \beta_{21}\eta_1 + \zeta_2 \quad (3)$$

and the equation for commitment variable (η_3) (Eq. 4),

$$\eta_3 = \beta_{31}\eta_1 + \beta_{34}\eta_4 + \zeta_3 \quad (4)$$

and the equation for trust variable (η_4) (Eq. 5),

$$\eta_4 = \beta_{41}\eta_1 + \beta_{42}\eta_2 + \zeta_4 \quad (5)$$

and the equation for attitudinal loyalty variable (η_5) (Eq. 6),

$$\eta_5 = \beta_{52}\eta_2 + \beta_{53}\eta_3 + \beta_{54}\eta_4 + \zeta_5 \quad (6)$$

and the equation for behavioral loyalty variable (η_6) (Eq. 7),

$$\eta_6 = \beta_{62}\eta_2 + \beta_{63}\eta_3 + \beta_{64}\eta_4 + \beta_{65}\eta_5 + \zeta_6. \quad (7)$$

DATA ANALYSIS

Assessing Measurement Model

As **Table 3** shows, all scales are reliable, with Cronbach's α ranging from 0.725 to 0.854. In order to gauge validity, this study adopted the confirmatory factor analysis (CFA) using AMOS 23.0 to verify the validity of the construct, which includes convergent and discriminant validity. Hair et al. (2010) recommended convergent validity criteria as follows: (1) standardized factor loading of items higher than 0.5, (2) average variance extracted (AVE) above 0.5, and (3) composite reliability (CR) above 0.7. As **Table 3** indicates, all three criteria for convergent validity are met.

The criterion for discriminant validity is the square root of the AVE for one dimension greater than the correlation coefficient with any other dimension(s). As **Table 3** shows, correlation coefficients are all less than the square root of the AVE within one dimension, suggesting that each dimension in this study has good discriminant validity.

An Examination of the Structural Model

In this study, the measurement patterns of the above-mentioned variables are established according to the research framework, and the model matching degree of the SEM is adopted. For mode matching tests, Bagozzi and Yi (1988) considered that the size of the sample should be considered, and suggested that when the mode fit is measured by the ratio of χ^2 to the degree of freedom (df), it generally does not exceed 3 (Hair et al., 2010). In the study, green experiential marketing, experiential value, RQ, and customer loyalty are often higher-order constructs in nature, with items measuring them as indirect reflective measures of both second- and first-order factors associated with them, where the green experiential marketing, experiential value, RQ, and customer loyalty are umbrella terms for multiple sub-constructs. Cadogan and Lee (2013) suggested that research

TABLE 2 | Scale of measurement variables.

Constructs	Variables	Items
Green experiential marketing	Sense	The green visiting experience is refreshing.
		The green components in the environmental leisure farm are comfortable.
		The environmental leisure farm is well lighted to for selection of merchandise.
		A green atmosphere is in the environmental leisure farm.
	Feel	A good smell in the environmental leisure farm.
		I feel relaxed and comfortable with the green visiting experience.
	Think	The surroundings in the environmental leisure farm are very pleasant.
		I do not feel deceived by the environmental leisure farm.
	Act	A commitment to satisfy my needs from the environmental leisure farm.
		The environmental leisure farm is a reflection of my green lifestyle while I am visiting it.
Experiential value	Playfulness	The green elements of the environmental leisure farm attract my eyes.
		Every indication in the environmental leisure farm provides me with an easy way to understand the green features.
		Coming here will contribute to my social life improvement with friends.
		I can be associated with other consumers here.
	Aesthetics	A desire of self-improvement arises here.
		Visiting the environmental leisure farm makes me cheerful.
	Customer return on investment	I feel happy when I have a visit to the environmental leisure farm.
		Visiting the environmental leisure farm makes me forget the problem I encountered.
	Service excellence	I enjoy visiting the environmental leisure farm for its own sake.
		Environmental leisure farm is aesthetically appealing.
Relationship quality	Satisfaction	I like the entire design of the environmental leisure farm.
		The enthusiasm of the environmental leisure farm is catching and picks me up.
		A visit to the environmental leisure farm makes me feel healthy.
		The green elements embedded in the environmental leisure farm fit my needs.
	Commitment	When I think of environmental leisure farm, I think of excellence.
		I consider the environmental leisure farm as a green expert in the environmental awareness it offers.
	Trust	I am satisfied with this environmental leisure farm.
		I am happy with the green goods offered by this environmental leisure farm.
	Behavioral loyalty	This environmental leisure farm meets my needs and satisfies my expectations.
		I feel this environmental leisure farm close to me.
Customer loyalty	Attitudinal loyalty	I like feeling a link to this environmental leisure farm.
		I feel a sense of belonging to the environmental leisure farm.
		I have trust in providing green goods by the environmental leisure farm.
		This environmental leisure farm is frank when having a deal with us.
	Behavioral loyalty	I can trust this environmental leisure farm due to its honesty.
		I am willing to revisit the environmental leisure farm.
	Attitudinal loyalty	I am willing to dedicate all my future traveling to this environmental leisure farm.
		Even in the case of mark-up, I would like to subsequently consume green products from the environmental leisure farm.
	Behavioral loyalty	I tend to compare the green goods from the environmental leisure farm with those from the general leisure farm.
		I consider this environmental leisure farm as my only choice for visiting in the tourism site.

should avoid developing and evaluating a model containing a direct link from the antecedent variable to the aggregate endogenous variable. However, this study aims to explore the role of green experiential marketing in the consumer decision-making process. Furthermore, according to research purposes and hypotheses, the experiential value is measured from the first-order construct in order to optimize the overall fitness index of

the model and lower the complexity of the model. In this study, 754 valid copies of questionnaire were analyzed. The results are shown in **Table 4**. The ratio of χ^2 to its DOF is less than 3; PNFI is greater than 0.5; goodness of fit index (GFI), adjusted goodness of fit index (AGFI), normed fit index (NFI), comparative fit index (CFI), and incremental fit index (IFI) are all greater than 0.9; root mean square error of approximation (RMSEA) is less than 0.08

TABLE 3 | Measurement.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(1) Sense	0.751													
(2) Feel	0.565**	0.732												
(3) Think	0.560**	0.573**	0.744											
(4) Act	0.634**	0.598**	0.601**	0.788										
(5) Relate	0.514**	0.585**	0.623**	0.718**	0.782									
(6) Play	0.544**	0.513**	0.537**	0.611**	0.574**	0.757								
(7) Aesthe.	0.572**	0.640**	0.651**	0.646**	0.706**	0.683**	0.754							
(8) CRI	0.513**	0.548**	0.605**	0.630**	0.631**	0.610**	0.710**	0.736						
(9) SC	0.591**	0.530**	0.624**	0.662**	0.618**	0.700**	0.713**	0.600**	0.730					
(10) Satis.	0.608**	0.558**	0.577**	0.693**	0.629**	0.541**	0.634**	0.538**	0.645**	0.762				
(11) Com.	0.514**	0.565**	0.629**	0.716**	0.741**	0.564**	0.666**	0.630**	0.692**	0.686**	0.759			
(12) Trust	0.327**	0.397**	0.473**	0.422**	0.484**	0.328**	0.487**	0.395**	0.394**	0.509**	0.624**	0.729		
(13) AL	0.615**	0.544**	0.568**	0.654**	0.663**	0.584**	0.651**	0.599**	0.715**	0.683**	0.757**	0.500**	0.747	
(14) BL	0.456**	0.466**	0.598**	0.585**	0.602**	0.475**	0.632**	0.579**	0.601**	0.612**	0.655**	0.488**	0.716**	0.756
Means	4.070	4.064	3.990	4.005	3.998	3.884	3.957	3.962	4.055	3.921	4.019	3.614	3.854	3.993
SD	0.656	0.719	0.759	0.700	0.764	0.675	0.738	0.705	0.668	0.717	0.736	0.791	0.769	0.719
Cronbach's α	0.792	0.787	0.725	0.784	0.827	0.637	0.758	0.735	0.785	0.800	0.854	0.824	0.767	0.753
AVE	0.564	0.536	0.554	0.621	0.611	0.573	0.569	0.542	0.533	0.581	0.576	0.532	0.558	0.572
CR	0.855	0.834	0.837	0.882	0.851	0.828	0.874	0.863	0.846	0.875	0.869	0.821	0.845	0.853

** $p < 0.01$. The diagonal bold and italic values are the square root values of AVE.

TABLE 4 | Fit indices of structural model.

Fit indices	Criteria	Findings	Results
χ^2/df	< 3.00	2.647	Confirmed
GFI	> 0.9	0.94	Confirmed
AGFI	> 0.9	0.92	Confirmed
RMSEA	< 0.08	0.05	Confirmed
NFI	> 0.9	0.97	Confirmed
CFI	> 0.9	0.97	Confirmed
IFI	> 0.9	0.97	Confirmed
PNFI	> 0.5	0.70	Confirmed

(Jöreskog and Sörbom, 1993). The moderation of this research model is acceptable.

Verification of Structural Model

Correlations among variables were detected using SEM. There are many items in the consideration of certain facet scales. The results are shown in **Figure 2**. The path coefficients of sense ($\beta = 0.146$, $p < 0.01$), feel ($\beta = 0.143$, $p < 0.01$), think ($\beta = 0.242$, $p < 0.01$), act ($\beta = 0.251$, $p < 0.01$), and relation ($\beta = 0.253$, $p < 0.01$) to self-efficacy are statistically positive and significant; thus, H1a–e is supported. Similarly, the path coefficients of experiential value for trust ($\beta = 0.716$, $p < 0.001$), commitment ($\beta = 0.427$, $p < 0.001$), and satisfaction ($\beta = 0.649$, $p < 0.001$) are statistically positive and significant, meaning that H2a–c are also supported. In addition, the path coefficients of trust ($\beta = 0.162$, $p < 0.01$; $\beta = 0.483$, $p < 0.001$) and satisfaction ($\beta = 0.179$, $p < 0.01$; $\beta = 0.287$, $p < 0.001$) for behavioral and attitudinal loyalty are statistically positive and significant. Thus, the higher a customer's degree of trust and satisfaction in an environmental leisure farm, the higher their behavioral and attitudinal loyalty. Thus, there is support for H3a and H3b. However, the path coefficients of commitment for behavioral and attitudinal loyalty are 0.006 and 0.007 ($p > 0.1$); then, H3c was not supported. The path coefficients of satisfaction $\rightarrow$ trust ($\beta = 0.323$, $p < 0.001$) and trust $\rightarrow$ commitment ($\beta = 0.475$, $p < 0.001$) were statistically positive and significant; thus, H3d and H3e were supported. The coefficient of attitudinal loyalty on behavioral loyalty is 0.463 ($p < 0.001$), so H4 is also supported. The R^2 and Stone–Geisser Q^2 values obtained through the blindfolding procedures for experiential value ($Q^2 = 0.368$; $R^2 = 0.385$), trust ($Q^2 = 0.322$; $R^2 = 0.573$), commitment ($Q^2 = 0.217$; $R^2 = 0.382$), satisfaction ($Q^2 = 0.482$; $R^2 = 0.534$), attitudinal loyalty ($Q^2 = 0.362$; $R^2 = 0.443$), and behavioral loyalty ($Q^2 = 0.324$; $R^2 = 0.485$) were larger than 0, supporting that the model has predictive relevance (Hair et al., 2017).

DISCUSSION

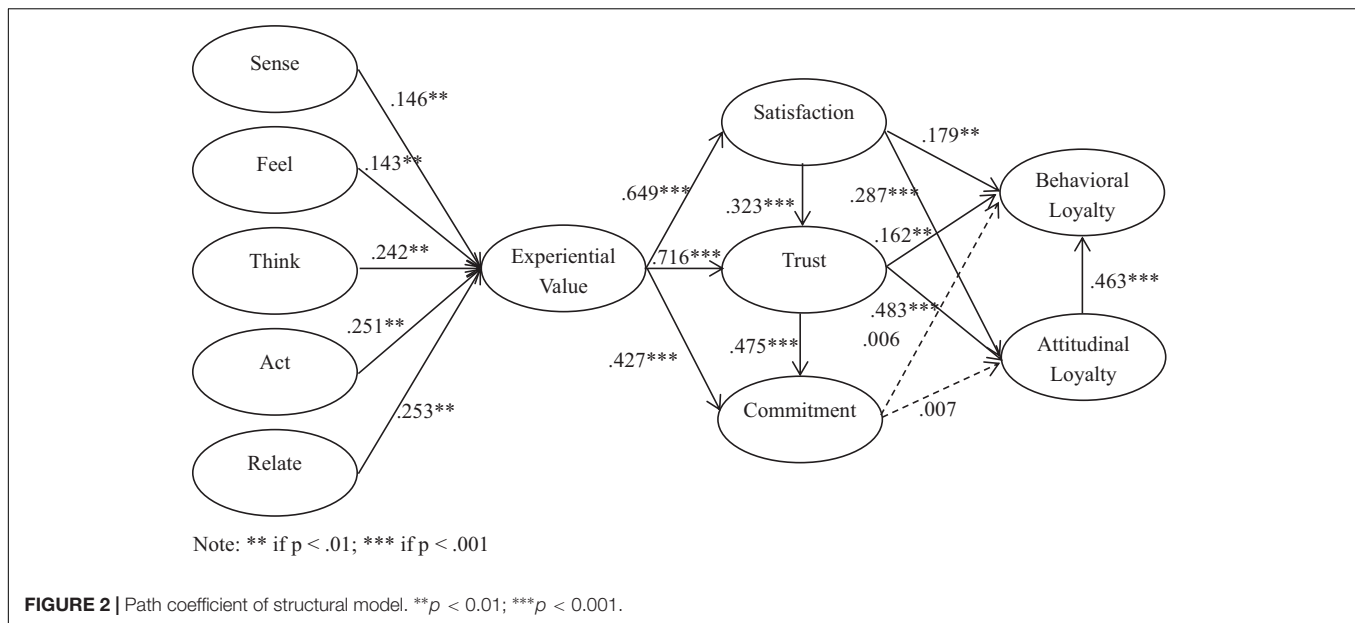
Previous studies have emphasized the significance of sustainability in intensely competitive markets. This study contributes to the literature from three perspectives: (1) we demonstrate, for the first time, an integral and comprehensive approach toward green experiential marketing for environmental

leisure farms; (2) by incorporating prior studies' findings on the role of green experiential marketing, experiential value, RQ, and customer loyalty, we examine the correlations among these constructs; and (3) by verifying the nomological network of the green experiential marketing scale, we provide support for the findings of previous studies in terms of the positive effect of green experiential marketing on experiential value. Based on the above research findings, it makes substantial contributions to theories and practice. In theoretical aspect, green experiential marketing proposed in this study is combined with the concepts of green marketing and experiential marketing; thus, specific measurement items are proposed, and good reliability and validity are achieved. Furthermore, the research framework is constructed through the formed sequence of consumer loyalty proposed by Oliver (1997), and the results obtained after verification enrich the degree of generalization of consumer decision theory in various fields. In regard to the practical part, the findings of this study provide administrators and marketers with emphasis on environmental awareness and green experience, which not only enhance consumer intention toward leisure farms but also demonstrate their social responsibility for environmental conservation, and further enhance business reputation and positive feelings of consumers. Specific theories and management implications are described as follows.

Theoretical Implications

To the best of our knowledge, this is the first study to (1) conceptualize and operationalize the broad meaning of green experiential marketing and (2) build an integrated and empirically tested framework of this concept. Our findings are significant to the further development of environmental/green marketing. Generally speaking, the results offer four main theoretical implications. First, the development of an economical green experiential marketing scale can be used in future studies of green marketing. From the methodological point of view, this study incorporates the green marketing concept into Schmitt's (1999) experiential marketing scale, which results in a holistic scale of green experiential marketing (Maklan and Klaus, 2011). The verification results of our measurement model confirm the reliability and validity of five dimensions of green experiential marketing and support its use in scholarly research in the future. Similar to Hosany and Witham (2010), our findings establish the importance of five dimensions in explaining the outcome variable of experiential value.

Second, this study provides an updated and comprehensive investigation into relationship marketing strategies, which extends the scope of earlier studies of green marketing (e.g., Menon and Menon, 1997) and relationship marketing (e.g., Morgan and Hunt, 1994; Garbarino and Johnson, 1999; Rauyruen and Miller, 2007; Olavarria-Jaraba et al., 2018). Most empirical studies on relationship marketing to date have emphasized the functional/emotional activities related to relationship marketing strategies (e.g., Maklan and Klaus, 2011; Lemon and Verhoef, 2016; Olavarria-Jaraba et al., 2018). Our results suggest that environmental leisure farms employ green initiatives at an individual consumer level. The confirmation of H1a–e agrees with Lemke et al.'s (2011),



Lemon and Verhoef's (2016), Brun et al.'s (2017), findings and seems to underline the need to focus on experiential value in the green context, and in particular as a key factor for environmental leisure farms to build relationships with consumers.

Third, in line with Brun et al.'s (2017) advocacy for additional research, this study explores the combination of green experiential marketing and relationship marketing and investigates the relationships among experiential value, RQ (satisfaction, commitment, and trust), and customer loyalty (behavioral and attitudinal loyalty). The findings with regard to these relationships are in line with those of Oliver (1997); Monferrer-Tirado et al. (2016), Srivastava and Kaul (2016). They suggest a four-stage framework that demonstrates a hierarchical approach to loyalty formation. Specifically, the results indicate that experiential value has direct effects on satisfaction, commitment, and trust (H2a–c); satisfaction and trust have direct effects on behavioral and attitudinal loyalty (H3a, H3c); and attitudinal loyalty has a direct effect on behavioral loyalty (H4). Similarly, Srivastava and Kaul (2016) reported that attitudinal loyalty leads to behavioral loyalty. In the green context, according to the barometer of Monferrer-Tirado et al. (2016) about RQ, the approach to RQ defined in recent works has been confirmed (Beloucif et al., 2004; Rauyruen and Miller, 2007; Olavarria-Jaraba et al., 2018) regarding three variables: trust, commitment, and satisfaction. Specifically, satisfaction and trust act as antecedents of behavioral and attitudinal loyalty.

Managerial Implications

This study analyzes the effect of experiential elements (green experiential marketing and experiential value) on RQ, where RQ is found to affect consumer loyalty. By integrating green experiential marketing into the relationship marketing framework, this study takes the lead in adopting managerial

issues. The findings help to explain the effect of RQ between customers and environmental leisure farms.

This study also provides insights for operators. First, our findings on the aspects of sense, feel, think, act, and relation in the green experiential marketing scale can help managers of environmental leisure farms to devise appropriate green marketing actions. For example, creating elaborate physical surroundings to induce customers' positive emotional perceptions of the green experience might be critical, whereas employing a green-specific experience design may facilitate customers to sense the importance of environmental protection and create significant differentiation from ordinary leisure farms.

Second, our findings offer interesting implications with regard to maintaining each RQ dimension. In an atmospheric context, the feel and sense stimuli at environmental leisure farms contribute to positively perceived value and enhance customer satisfaction, commitment, and trust. This also suggests that green experiential activities (e.g., organic vegetables, use of recycled materials) offer a differentiation strategy to managers for (1) improving the green brand image of environmental leisure farms in the long term and (2) adjusting their green experiential marketing strategy through increasing the drive for environmental protection. Moreover, RQ evolves over time, so environmental leisure farms need to pay constant attention to the evolution of customer demands and expectations so as to continually update their experiential value. As some customers psychologically tend to cultivate relationships than others to a certain extent, it may be critical to determine the level of experiential value for customers using services and natural facilities of the leisure farm, and for those who turn to competitors.

Third, the study provides new insights into loyalty by demonstrating the roles of customer RQ and green experiential marketing in a four-stage framework. Furthermore, the

findings can help operators to understand how outcomes from loyalty can be achieved and used. This study suggests that environmental leisure farms should enhance attitudinal loyalty first and then create specific consumption behavior. If customers perceive that the leisure farm has spent time addressing their needs and offering the best leisure environment, this constitutes a fundamental step in promoting emotional or psychological attachment and, therefore, engagement in desired (repurchase) behavior.

Limitations and Future Research

The above conclusions should be considered in light of the study's limitations, which also suggest future directions of research. First, the sampling region of the study was confined to Taiwan, which limits the generalization of the results. Taiwan is a popular tourist attraction in Asia, and the conditions of its environmental leisure farms are on a par with those of other Asian countries, such as China, Thailand, Japan, Malaysia, and Vietnam. Although environmental leisure farms have developed in Taiwan, they are still rare in other countries. Given this, new research could compare the proposed relationships in other countries to improve the generalization of results.

Furthermore, it would be interesting to study the possible influence of other factors related to customer demographics, such as age, gender, and education level, as well as potential

moderating effects related to loyalty, involvement, service quality, or personal interactions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Taipei. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

This study is a joint work of the four authors. T-CL and MP contributed to the ideas of educational research, collection of data, empirical analysis, data analysis, design of research methods, and tables. MP participated in developing a research design, writing, and interpreting the analysis. All authors contributed to the literature review and conclusions.

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Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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