GREENHOUSE GAS EMISSIONS AND EMISSIONS MITIGATION FROM AGRICULTURAL AND HORTICULTURAL PRODUCTION SYSTEMS

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GREENHOUSE GAS EMISSIONS AND EMISSIONS MITIGATION FROM AGRICULTURAL AND HORTICULTURAL PRODUCTION SYSTEMS

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Table of Contents

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EDITORIAL
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Editorial: Greenhouse Gas Emissions Mitigation From Agricultural and Horticultural Systems

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Editorial on the Research Topic

Greenhouse Gas Emissions Mitigation From Agricultural and Horticultural Systems

Global geopolitics were harmonized at COP26 when more than 150 countries pledged to the Glasgow Climate Pact, resulting in unified aspirations to constrain global average temperature rise to 1.5°C and well below 2°C by 2050 (UNFCCC, 2021). Achievement of this goal demands urgent, deep and sustained reductions in global greenhouse gas (GHG) emissions, with threshold targets of 45% by 2030 (relative to 2010) and net zero by mid-century (UNFCCC, 2021). With agriculture, forestry and other land use (AFOLU) contributing 24% of global GHG emissions each year, AFOLU represents the second largest contributor to global GHG emissions after the energy sector (IPCC, 2014).

Predominant GHG emissions from agri-food systems include methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) from livestock, savanna and crop residue burning, soil respiration and cultivation, fertilizer and lime application, burning of electricity and fuel (Harrison et al., 2016). Direct GHG emissions are generated from livestock enteric fermentation (48%) and excreta (22%), crop production systems with nitrogen (N) fertilizers (10%), and rice paddy cultivation (11.5%) (FAO, 2021). The magnitude of global AFOLU GHG emissions suggests that the development of skills, practices, and technologies for GHG emissions mitigation must be foremost priorities when proposing any systemic or transformational innovation for adaptation to the climate crisis (Ho et al., 2014; Alcock et al., 2015; Chang-Fung-Martel et al., 2017). The diversity of processes and GHGs *per se* from AFOLU does however provide significant latitude for GHG mitigation through manifold avenues, including carbon dioxide removal (CDR), enhanced reduction, avoidance, and/or displacement (Smith et al., 2008).

This Research Topic documents scientific advances in measurement protocols for field or greenhouse gas experimentation, together with improved modeling that allows upscaling and extrapolation of field measurements. Three papers focus on milk production in dairy systems (housed or grazing), five papers examine plant production systems, and one paper reviews the literature, synthesizing opportunities for strategic GHG emissions mitigation in grazing systems. For example, Häfner et al. fastidiously distinguish between organic-N and ammonium-N as potential N sources for denitrification in the field, while Prangbang et al. measure and model the regional applicability of alternate wetting and drying (AWD) of rice paddies as prospective pathways for methane mitigation. Sokolov et al. quantify the effects caused by acidifying manure inoculum on the CH_4, N_2O, and ammonia (NH_3) emissions from stored dairy manure by targeting

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Methyl Coenzyme M Reductase A genes, as well as bacterial abundance using real-time qPCR. Finally, March et al. compute the carbon footprints of milk production systems using Life Cycle Assessments (LCA). They demonstrate the importance of allocation method, livestock genetics and management in the attribution of GHG emissions. The same authors also examined the effects of nutritional quality on the carbon footprint of novel and conventional dairy systems. Differential allocation methods resulted in GHG emissions ranging from 0.95 to 3.79 kg CO_2e/kg fat and protein corrected milk, indicating the importance of quantifying footprints using multiple metrics, similar to work shown for cattle and sheep production systems elsewhere (Harrison et al., 2014; Alcock et al., 2015).

Durango Morales et al. demonstrate a clear need for development of site-specific N_2O emission factors (EF), as opposed to the more generic and granular Tier 1 EF used by the IPCC. They show that EFs can be reduced by decreasing urine deposits, by limiting N inputs to pastures. More strategic planning of nitrogenous fertilizer type (urea, green urea, slow release etc.), timing, rate and placement shown in other dairy studies (Christie et al., 2018, 2020) has similarly shown that improved use of N fertilizer reduces urea N in the milk.

Emissions of CH_4, N_2O, and NH_3 from liquid manure storages can be substantially reduced (>70%) by acidifying manure, however this usually comes with high financial costs (Sommer et al., 2017). Sokolov et al. propose acidification of only manure inoculum. To determine the feasibility of this idea, they elicit functional mechanisms by measuring methanogenic activity and abundance using Methyl Coenzyme M Reductase A (mcrA), a gene and transcript which encodes a subunit of the key enzyme that catalyzes the final step of methanogenesis. Sokolov et al. (2020) also used quantitative real-time PCR to quantify bacterial abundance using the 16S rRNA gene. They found that the 38–77% mitigation of CH_4 was caused by disruption of the mcrA gene and transcript abundance, while NH_3 and N_2O emissions were reduced by 33–73% by acidyfing inoculum. The authors concluded that future studies should test lower acid rates and less frequent acidification to further lower financial costs in commercial settings.

In a review of CH_4 and N_2O emissions from animal manure, Rivera and Chará converse that emissions depend on multiple factors and are highly variable, implying that "one size fits all" solutions are problematic at best, similar to observations by Durango Morales et al. Rivera and Chará found that promising options for reducing emissions from livestock manure include manipulation of livestock diet nutritional quality, [where practical] implementation of silvopastoral systems, use of nitrogen fixing plants, and management approaches for improving soil health, carbon storage and seasonal ground cover.

It is well-known that synthetic nitrogenous fertilizers in intensive agricultural and horticultural production systems are a key source of GHG emissions (Christie et al., 2018, 2020). Of the studies we are aware of, Karlowsky et al. is the first to measure how N fertilizers impact N_2O in hydroponic greenhouse production. They showed that N_2O emissions from tomato and cucumber account for 2.3 and 1.5 kg ha^{-1} yr^{-1}, respectively, lower than previously measured in laboratory experiments (Daum and Schenk, 1996). Kitamura et al. show that organic fertilizers (viz. manure and digestive fluid) had both positive effects on soil carbon stocks and caused greater reduction in N_2O relative to synthetic N fertilizer. By using organic fertilizers from legume-based crops grown for green N and incorporating the material into the soil, Singh et al. report that (i) post-cultivation N_2O emissions can be greater from non-legume green N crops compared with legume green N crops due to greater biomass productivity of the former, and (ii) emissions of N_2O could be mitigated by removing biomass of the green N crop for use as forage. Häfner et al. find that digestate application mainly resulted in N_2O emissions derived from existing soil N stocks, rather than N applied. Collectively, these findings suggest that comprehensive consideration of all plant genetic, environmental and management factors is necessary to help guide the development of best management practices regarding fertilizer use.

Water management is another tactical tool allowing reduction of GHG emissions from irrigated cropping systems. Alternate wetting and drying (AWD) was proposed by Prangbang et al. as a management approach that would enable both water savings and methane mitigation from rice paddy fields. However, future studies of this type should also examine the implications of trade-offs and co-benefits associated with GHG mitigation options (Harrison et al., 2011). Using AWD can result in greater rice biomass production and this requires greater N fertilization, ensuing increase in N_2O emissions (Christie et al., 2014). Such N_2O increases may well offset any mitigation caused by reduced CH_4 emissions, underscoring the need to holistically explore multiple GHG emissions in a closed systems, using multiple metrics (Harrison et al., 2012, 2021).

This Research Topic provides several promising avenues for sustained—and in some cases, substantial—reduction of GHG emissions, in line with aspirations posed in the Glasgow Climate Pact. However, to achieve deep cuts in emissions without adversely impacting productivity or agricultural economic prosperity, we call for more studies that transcend disciplinary boundaries. Such studies should focus on not just GHG emissions, but multiple sustainability metrics (environmental, social, economic, institutional) and across scales (plot, field, region, continent, global) allowing more comprehensively evaluation of the wider co-benefits and trade-offs associated with GHG emissions mitigation (Harrison et al., 2021).

AUTHOR CONTRIBUTIONS

DS and MH wrote the manuscript. All authors contributed to editing the manuscript. All authors contributed to the article and approved the submitted version.

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Acidification of Residual Manure in Liquid Dairy Manure Storages and Its Effect on Greenhouse Gas Emissions

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Liquid manure storages are an important source of greenhouse gases (GHG) on dairy farms. Methane (CH_4) and nitrous oxide (N_2O) are the predominant GHGs, while ammonia (NH_3) is an indirect source of N_2O. Addition of acid to manure has shown promising emission reductions, however, cost of acidification may be unfeasible for farmers. Fully cleaning storages has also shown to reduce CH_4, due to removal of inoculating effects of residual manure ("inoculum") on fresh manure (FM). However, complete removal of inoculum is practically impossible on large farms, thus acidifying only the inoculum may reduce GHGs without requiring acidification of all FM. This study aimed to quantify the effect of acidified inoculum on CH_4, N_2O, and NH_3 emissions from stored manure and quantify the changes in methanogen abundance and activity. Emissions were measured from six 10.6 m^3 storages filled with 20% inoculum (1-year-old manure) and 80% FM. Inoculum was treated in three ways: untreated (control); previously acidified (1-year prior); and newly acidified with 70% H_2SO_4 (1.1 L m^{-3} manure). The CH_4 and N_2O emissions were continuously measured from June—November using tunable diode trace gas analyzers coupled with venturi air flow systems. The NH_3 emissions were measured at 24-h intervals 3 × weekly using acid traps. The activity and abundance of methanogens were quantified by targeting the Methyl Coenzyme M Reductase A (*mcrA*) gene and transcript which encodes a subunit of the key enzyme that catalyzes the final step of methanogenesis. Bacterial abundance was quantified by targeting the bacterial 16S rRNA gene. Quantifications were performed using quantitative real-time PCR. CH_4 emissions were reduced by 77% using newly acidified inoculum and 38% using previously acidified inoculum, compared to the control with untreated inoculum (36.1 g CH_4 m^{-2}). Significant treatment reductions in *mcrA* gene and transcript abundance suggest that CH_4 reductions were caused by disruption of methanogen activity. NH_3 and N_2O emissions were reduced by 33 and 73% using acidified inoculum and 23 and 50% using previously acidified inoculum, respectively, compared to the control. Results suggest that lower acid rates and acidifying less frequently may still have good treatment effects while minimizing cost.

Keywords: manure management, methanogens, methane, inoculum, acidification

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INTRODUCTION

Liquid dairy manure is a substantial source of methane (CH_4) and moderate source of nitrous oxide (N_2O), and ammonia (Le Riche et al., 2016; Sokolov et al., 2019). Both CH_4 and N_2O are greenhouse gases (GHG) contributing to global warming and climate change, while ammonia (NH_3) is an indirect source of N_2O and is a toxic gas hazardous to human health (Jayasundara et al., 2016; Sokolov et al., 2019). Liquid manure is often stored on farms for >100 days prior to spreading onto fields. During this storage period considerable amounts of GHGs and NH_3 are emitted to the atmosphere (Jayasundara et al., 2016).

Dairy manure acidification (to pH 6–6.5) with sulfuric acid (H_2SO_4) was found to decrease CH_4 (>87%) and NH_3 (>40%) emissions (Sokolov et al., 2019). Sommer et al. (2017) reported 68% reductions of CH_4 and 62% of NH_3 with H_2SO_4 acidification (to pH 5.2–5.5). Kavanagh et al. (2019) reported 96% reductions of CH_4 and 85% of NH_3 with H_2SO_4 acidification (to pH of 5.5). The mechanism of CH_4 reduction is still unclear, as H_2SO_4 and pH reduction can disrupt microbial communities throughout all the processes of organic matter degradation as well as methanogens directly (Habtewold et al., 2018). Habtewold et al. (2018) reported a methanogen reduction of 6% in abundance and 20% in activity between untreated and acidified dairy manure but observed no difference in the microbial communities. This suggests that H_2SO_4 primarily disrupts methanogenesis rather than other microbial processes, however, more research is necessary to confirm these results. Petersen et al. (2012) reported substantial methanogen inhibitions (63–67%) from cattle slurry using potassium sulfate with no corresponding pH reduction. They suggest that sulfur transformations inhibit methanogenesis independent of any pH reduction. Therefore, lower rates of H_2SO_4 may reduce CH_4 production without necessarily aiming for a certain manure pH value. However, it is important to note that in the acid of acidification, decreasing NH_3 volatilization may still require lowering pH. Therefore, sulfate alone may not have the best overall treatment differences.

Due to the cost of acid, infrastructure and equipment, there is a need to make manure acidification more feasible. Treating only the inoculum (manure remaining in storage tanks after emptying) has been suggested to reduce the quantity and the frequency of acidification (Sokolov et al., 2020). As storages are difficult to completely empty, the residual manure becomes an inoculum for incoming fresh manure and increases subsequent CH_4 emissions by 34–52% (Ngwabie et al., 2016). If the inoculation process can be disrupted, then reductions can be expected (Sokolov et al., 2020). Sokolov et al. (2020) measured CH_4 production from manure incubated with 6-month-old, previously acidified inoculum and with 6-month-old, newly acidified manure inoculum. They reported 82 and 63% CH_4 reductions, respectively, compared to the manure with untreated inoculum. They suggest that long-term effects of acidification could lower inoculation effect and reduce the frequency of acidification to every other tank emptying. These laboratory results are promising, however there is need to evaluate inoculum acidification on a large scale in outdoor manure storage tanks.

The objectives of this research were to: (a) quantify the effect of acidified aged manure as inoculum on CH_4, NH_3, and N_2O emissions from dairy manure storages and (b) quantify changes in methanogen and bacterial abundance relative to CH_4 reductions.

METHODS

Meso-Scale Chambers

The study was conducted at the Bio-Environmental Engineering Center (BEEC) at Dalhousie University's Agricultural Campus in Truro, Nova Scotia, Canada (45°45' N, 62°50' W). The research site contained 6 in-ground, cement, meso-scale manure tanks (6.6 m^2 and 1.8 m deep). This site has been previously described by Wood et al. (2012) and Le Riche et al. (2016). Each tank was filled with 10.6 m^3 (160 cm depth) of liquid dairy manure, consisting of 20% inoculum (2.1 m^3) and 80% fresh manure (FM; 8.5 m^3). Manure was obtained from a local diary operation which housed 95 lactating cows in a free stall barn. The manure was gathered from an in-ground manure tank adjacent to the dairy barn and was a mixture of feces, urine, and sand bedding.

Two types of inoculum were used in this study: (i) 1-year-old untreated manure, and (ii) 1-year-old manure that was previously acidified (**Table 1**). This manure inoculum was obtained from the same farm in spring 2017 (12 months prior to the start of this trial). The previously acidified (PA) manure was acidified using sulphuric acid (70% H_2SO_4; 2.4 L m^{-3} manure) to pH 6. Both the PA manure and untreated manure inoculum remained in storage for 1-yr (Sokolov et al., 2019). Additional information about storage conditions of the inoculating manure prior to this study can be found in Sokolov et al. (2019).

The six manure tanks were assigned within two blocks, each containing two treatments and a control. Inoculum was prepared on May 15–16, 2018 by pumping out of old storages and distributing 2.1 m^3 to new storages using a pumping truck. The newly acidified (NA) inoculum treatment received 2.1 m^3 of 1-yr-old untreated inoculum and was acidified on May 17, 2018 with 1.1 L m^{-3} 70% H_2SO_4 (i.e., 12 L per 10.6 m^3 tank). The PA inoculum treatment received 2.1 m^3 of 1-yr-old inoculum which had been acidified the previous year (spring 2017) at 2.4 L m^{-3} (i.e., 5.04 L added to 2.1 m^3; **Table 1**). Lastly, the control received 2.1 m^3 of 1-year-old untreated manure inoculum. Fresh manure was added to each tank on May 28 and 29, 2018 using a pumping truck to transport manure from the farm to the research site. The NA inoculum treatment received 1.1 L m^{-3} H_2SO_4, which is half as much as a previous meso-scale study (Sokolov et al., 2019) but considerably more than in the laboratory study where rates were only 0.16 L H_2SO_4 m^{-3} of total manure (i.e., 0.03 mL of 98% H_2SO_4 in 180 mL of stored manure; Sokolov et al., 2020).

Sulfuric acid (industrial grade) was obtained from Bebbington Industries (Dartmouth, NS) and was pumped into the inoculum manure using acid resistant tubing and a peristaltic pump. The tubing was attached to an aluminum pole which was moved around the inoculum as the acid was being pumped.

TABLE 1 | Volume (L) of 70% sulfuric acid (H_2SO_4), inoculum, and manure added to the control, previously acidified inoculum treatment (PA), and newly acidified inoculum treatment (NA).

		Control	PA	NA
		Volume (L)		
Inoculum	12-month-old manure	2,120	2,120	2,120
	70% H_2SO_4	0	25	12
Fresh manure		8,480	8,480	8,480
Acid addition		NA	12-month-ago	Following tank emptying

Flux Monitoring

Emissions of CH_4 and N_2O were monitored continuously from Jun 8 to Nov 10, 2018 (155 days). Each manure storage tank was covered by a flow-through, steady-state chamber (∼13 m^3 headspace) consisting of an aluminum frame and 0.15 mm greenhouse plastic. Air was pulled through the chamber through intake slits at the front of the chamber and out through an exhaust fan and outflow exhaust duct and the opposite end. The rate of air flow within each chamber was approximately two full air exchanges per minute (∼0.5 m^3 s^{-1}). The airspeed was measured within each exhaust duct using cup anemometers recorded by a CR1000 data logger (Campbell Scientific, Edmonton, AB). The air temperature within each chamber was measured using copper-constantan thermocouples at 10 cm above the manure surface and along with manure temperature at 80 cm depth and 150 cm depth, recorded by the same CR1000 data logger (Campbell Scientific, Edmonton, AB). Ambient air temperature was obtained from the nearest Environment Canada climate station (Debert, NS, 45.42 N, 63.42 W; Climate ID: 8201380).

Methane and Nitrous Oxide

Air samples were continuously pumped (RC0021, Busch Vacuum Pumps and Systems, Boisbriand, QC) from the exhaust duct of each tank and two ambient inflow locations, and carried through polyethylene tubing (3.2 mm i.d.; Rubberline Products Ltd., Kitchener, ON) to a 8 × 2 manifold (Campbell Scientific In., Logan, UT) containing 12 V DC valves (The Lee Co., Essex, CT). The valves directed two samples every 30 s through high-flow air dryers (Perma Pure LLC.; Toms River, NJ) and into one of two tunable diode trace gas analyzers (TDLTGA, Campbell Scientific, Logan, UT). Sample CH_4 and N_2O concentrations were continuously recorded by a CR5000 data logger (Campbell Scientific Inc., Logan, UT) and an adjacent PC computer monitored the analyzer performance by running the TDLTGA software (Campbell Scientific, Logan, UT).

Concentrations were averaged hourly and used to calculate flux rates using to the following:

$$F = \frac{Q}{A}(C_o - C_i)$$

where F is the hourly flux (mg m^{-2} h^{-1}), Q is the flowrate of air out of the chamber [m^3 h^{-1}; calculated using average hourly windspeed × cross-sectional area of the exhaust duct (0.0645 m^2)], A is the surface area of the manure surface (6.63 m^2), and C is the concentration of gas (mg m^{-3}) in the ambient inflow air (C_i) and sample outlet air (C_o).

Due to technical issues, block one had missing flux data Aug 21-Sep 2, and block two had missing data Jul 18-Sep 2. This resulted in missing the peak fluxes in block two tanks. Linear interpolation was used to estimate the missing data, although the values were likely underestimated. All values are presented as treatment average.

Ammonia

Ammonia concentrations were determined using 125 mL 0.005 M H_3PO_4 acid traps. Three times per week, air was pumped (Model 2107CA20B; Thomas Pumps and Compressors, Sheboygan, WI) from the exhaust of each tank and two ambient inflow locations and bubbled through acid traps (dispersion tubes id = 35 mm) at 1.5 L min^{-1}. Air was continually pumped through the traps for 24 h at each deployment. Airflow for each sample was measured using inline flow meters (Gallus 2000; Actaris Metering Systems, Greenwood, SC). Following deployment, evaporated liquid was replaced to 125 mL and a sample frozen until analysis. Samples were shipped to Agriculture and Agri-Food Canada (Ottawa, ON) where they were analyzed for NH_3-N using the QuikChem® Method 12-107-06-2-A modified for 0.005 mol L^{-1} H_3PO_4 matrix using a Lachat QuikChem FIA+ Q8500 Series. Daily gas concentrations were calculated using the following:

$$C_{NH_3\ air} = \frac{C_{NH_3\ aq} \times V_{aq}}{V_{air}}$$

where $C_{NH_3\ air}$ is the daily NH_3-N concentration (mg m^{-3}), $C_{NH_3\ aq}$ is the NH_3-N concentration in sample liquid (mg L^{-1}), V_{aq} is the volume of liquid in the acid trap (L), and V_{air} is the volume of air pumped through the acid (m^3) (Hofer, 2003).

Ammonia emissions on days that were not sampled were estimated using linear interpolation and daily total NH_3-N losses were added together to find the entire monitoring period.

Manure Sampling and Analysis

Six FM composite samples were taken during tank filling (May 29). Manure in each tank was sampled monthly throughout the study with one sample per tank made from a composite of 12 subsamples. Subsamples were taken from each tank in a grid at two depths and six locations. All samples were kept frozen until analyzed at the Nova Scotia Department of Agriculture's Provincial Soils Lab (Bible Hill, NS). Samples were analyzed for total solids (TS) and volatile solids (VS) (American Public Health Association method 2540 B), total nitrogen (TN) (combustion method AOAC 990.03-2002), ammonium-N (TAN) (American Public Health Association method 4500-NH_3 B), and pH using an electrode (American Public Health Association method 4500-H^+) (Clesceri et al., 1998). To verify pH, a FieldScout pH 400 meter (Spectrum Technologies, Aurora, IL, USA) was used to

measure pH in the manure at 10, 50, 100, and 150 cm across 6 locations in each tank (24 pH points) on May 26, Jul 1, and Jul 31, 2018. These are not reported in the paper but verify the results of lab analysis.

For microbial analysis, duplicate composite samples were taken during storage tank filling (May 29, 2018) of FM, untreated inoculum, and previously acidified inoculum. Throughout the study, monthly composite manure samples were collected in duplicate and kept frozen until nucleic acid extraction. For each sampling, ∼2 g of manure sample was stored in 5 mL of LifeGuard soil preservation solution (MoBio Laboratories Inc., Carlsbad, CA).

Nucleic Acid Extraction and Quantitative Real-Time PCR

Based on the typical CH_4 emission curve, three sampling dates and starting FM and the two inoculums were chosen for analysis. The DNA or RNA PowerSoil total DNA/RNA isolation kit with RNA/DNA elution accessories (MoBio Laboratories, Inc., Carlsbad, CA) were used for DNA or RNA extraction. In triplicate, 8 μL of each extracted RNA sample was reverse transcribed to cDNA using Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA) following manufacturer's protocols.

Real-time qPCR was performed using an Applied Biosystems StepOnePlus real-time PCR system using clear 96-well PCR plates (Bio-Rad Laboratories, Inc., Hercules, CA). The total and active fraction of the methanogen populations were quantified by targeting methyl coenzyme A reductase (*mcrA*) genes and transcripts, respectively, using mlas-mod F and mcrA-rev-mod R primers (Habtewold et al., 2018). The total and active fraction of the methanogen populations were quantified by targeting methyl coenzyme A reductase (*mcrA*) genes and transcripts, respectively, using mlas-mod F and mcrA-rev-modR primers (Habtewold et al., 2018). Methyl Coenzyme M Reductase A gene is a fragment of DNA commonly found in methanogens that encodes the α-subunit of methyl-coenzyme M reductase enzyme which catalyzes the final step in methanogenic pathway (i.e., releases CH_4) (Evans et al., 2019). Although methanogens may involve one or more of the methanogenic pathways (i.e., hydrogenotrophic, acetoclastic, and methylotrophic), all pathways share the final step which requires methyl-coenzyme M reductase enzyme. Thus, the functional *mcrA* gene has been used extensively to effectively target all methanogens (Evans et al., 2019). Each reaction well-contained 10 μL of Ssofast EvaGreen supermix (Bio-Rad Laboratories, Inc.), 1 μL (10 pM) of each primer, 2 μL of DNA or cDNA, and 6 μL of PCR-grade water. Plasmid standard curves were prepared for *mcr*A from *Methanosarcina mazei* (ATCC 43340), and for 16S rRNA genes, plasmid with 16S rRNA gene insert from soil bacterium *Clostridium thermocellum* was used. Although primer sets used to target *mcrA* or 16S rRNA genes are specific to the respective gene fragments, target specificity of the primers were also confirmed by assessing the presence of a single district peak for melting curves (fluorescence *vs.* temperature) of each target gene. The *mcr*A gene standard curve had an efficiency of 101.6%, r^2 of 0.99, and slope of −3.29. The highest diluted standard had a cycle of quantification of 30.2 and no-template controls of 31.4. The 16S rRNA gene standard curve had an efficiency of 100.1%, r^2 of 0.998, and slope of −3.32. The highest diluted standard had a cycle of quantification of 27.0 and no-template controls of 28.9. StepOne software v2.3 (Bio-Rad Laboratories, Inc., Hercules, CA) was used to calculate sample copy numbers.

Data Analysis

To compare treatments based on their global warming potential, GHG emissions were converted to 100-yr CO_2-equivalent (CO_2-eq) values and summed. Conversion values for the global warming potentials of CH_4 and N_2O were 34 and 298, respectively (IPCC, 2014). The contribution of indirect N_2O emissions from NH_3 volatilization were calculated using the IPCC emission factor of 0.01 (Dong et al., 2006).

Given that PA inoculum could reduce the need for acidification following every other emptying event, to compare use of PA inoculum and NA inoculum it is necessary to compare estimated total emissions over two storage periods. The total PA inoculum over two storage periods was calculated using the following:

$$Total\ CH_4 = Acidified\ manure\ CH_4 + PA\ inoculum\ CH_4$$

where Total CH_4 is the total production over two storage periods, Acidified manure CH_4 is the production from one storage period where all manure was acidified (reported by Sokolov et al., 2019), and PA inoculum CH_4 is the total CH_4 production from the PA inoculum treatment.

The NA inoculum for two storage periods was calculated by doubling the total CH_4 production from the NA inoculum treatment. Lastly, the control for two storage periods was calculated by doubling the total CH_4 production from the controls. Note that this assumes both storage periods to have the same temperatures. Therefore, the two storage periods do not represent spring/summer and fall/winter, as emissions would be dramatically different during cold weather storage.

For each treatment the methane conversion factors (MCF) was calculated following IPCC methods (Dong et al., 2006). The calculation used the average VS of FM (disregarding VS of the inoculum) and maximum potential CH_4 production (B_o) of 0.24 m^3 CH_4 kg^{-1} VS. Cumulative N_2O and NH_3-N emissions for each tank were scaled by TN and TAN in FM and then averaged for each treatment.

Treatment effects were assessed using repeated measures, mixed linear model analysis using PROC Mixed in SAS software (SAS Institute Inc., Cary, NC) using the Kenward-Roger fixed effects method on total biweekly CH_4, N_2O, and NH_3 emissions. The CH_4 data was skewed and therefore log transformed to conform to normality. The spatial Gaussian covariance structure was chosen based on best fit statistics. Significance was considered when $p < 0.05$. Treatment effects on *mcrA* and 16S rRNA gene and transcript copy numbers over all dates were assessed using a general linear model using PROC GLM in SAS software, which uses ordinary least squares with Sidak adjustment to control familywise error. Effect size was calculated

using partial eta^2 (η_p^2). Significant results were followed up with a *post hoc* Sidak groupings comparison using a significance of $p < 0.05$.

RESULTS

Manure Characteristics

Average ambient air temperature during the study (Jun 1–Oct 31, 2018; 160 d) was 15.1°C (with an average relative humidity of 77%) as recorded by the closest Environment Canada climate station. The 30-years normal for this location, Jun–Oct, is 14.7 ± 0.2°C (Mean ± SD). The temperature inside the tank chambers (10 cm above the manure surface) was 17.6 ± 0.8°C, which was, on average, 2.6°C warmer than the ambient air (**Figure 1**). The average manure temperature in the tanks was 13.7 ± 0.1°C at 150 cm depth and 17.6 ± 0.1°C at 80 cm depth. The manure temperature peaked at week 12 (d 68, Aug 14) at 80 cm (20.9°C) and week 15 (d 91, Sep 6) at 150 cm (15.6°C). The CH_4 production followed a similar pattern, peaking a week earlier (d 61, Aug 7). Following the peak, the 80 cm temperature quickly fell. By the end of the study the temperature at 80 and 150 cm were both on average 14.3°C (∼122 days, Oct 7).

The manure pH had no clear treatment differences until days 85 into the study (**Figure 1**). The control was expected to have the highest pH, but was on average the lowest on Jun 15, 2 weeks after the start of the study. The pH dropped throughout storage until Sept when it increased. By Sept 8, the control tanks had the highest pH and the NA inoculum tanks had the lowest. This trend continued in Oct as well (**Figure 1**).

The TS, VS, and TN were all highest in the FM and fell markedly by Jun 15 (**Table 2**). This is most likely due to settling of solids which occurs rapidly following storage tank filling, and issues with unrepresentative sampling of the manure depth (Sokolov et al., 2019). The control had consistently the least VS, TS, and TN (**Table 2**). This may be due to faster degradation of organic matter and loss of TN to the atmosphere, although given the small differences, it may also be due to natural variability in manure (Sokolov et al., 2019).

Greenhouse Gas Emissions

Methane

Results of the mixed linear model show a significant CH_4 fixed effect due to treatment ($p < 0.0001$), time ($p < 0.0001$), and a combined effect of treatment and time ($p < 0.0001$). The average CH_4 emissions were 36.1, 22.3, and 8.2 g m^{-2} d^{-1} from the control (80% FM and 20% inoculum), PA inoculum (80% FM and 20% previously acidified inoculum), and NA (80% FM and 20% acidified inoculum) storages, respectively (**Table 3**). All treatments had similar lag phases of ∼40 d, although even during this time the control produced 31% more CH_4 than the NA inoculum tanks and 27% more than the PA inoculum tanks (**Figure 2**). The rate of growth following the lag was much higher in the control storage tanks. In fact, between day 40 (Jul 17) and day 110 (Sep 25) the largest treatment differences were recorded. At this time, NA inoculum tanks produced 82% less CH_4, while the PA inoculum tanks produced 47% less CH_4 compared to the control. After 110 d, fluxes were similar to the control and PA inoculum tanks (<25% difference). The NA inoculum tanks continued to produce less (56–80%) CH_4 than the control throughout the end of the study.

The total CH_4 production was 5.27, 3.26, and 1.20 kg m^{-2} from control, PA inoculum, and NA inoculum tanks, respectively (**Table 3**). The PA inoculum (38%; $p < 0.0001$) and NA inoculum (77%; $p < 0.0001$) treatments produced significantly less CH_4 compared to the control treatment. The NA inoculum treatment produced significantly less CH_4 (63%; $p < 0.0001$) than the PA inoculum. These treatment differences were the same on a VS basis.

The MCF values were 0.33, 0.20, and 0.08 for control, PA inoculum, and NA inoculum tanks, respectively (**Table 3**). Given that the average temperature inside the chambers was 17.6°C, the IPCC default MCF would be 0.32–0.35, which aligns with our control results (Dong et al., 2006). The PA inoculum and NA inoculum were both markedly different than the IPCC value.

Nitrous Oxide

Results of the mixed linear model show a significant N_2O fixed effect due to treatment ($p < 0.0001$), time ($p < 0.0001$), and a combined effect of treatment and time ($p = 0.0141$). The daily average N_2O emissions were 76.4, 38.4, and 22.3 mg m^{-2} d^{-1} from control, PA inoculum, and NA inoculum, respectively (**Table 3**). After interpolation, the total N_2O production was 11.2, 5.6, and 3.0 g m^{-2} from control, PA inoculum, and NA inoculum, respectively (**Figure 2**). This represented a significant ($p = 0.0015$) 50% reduction using PA inoculum and a significant ($p < 0.0001$) 73% reduction using NA inoculum, compared to the control. The NA inoculum produced 47% as much N_2O than the PA inoculum tanks ($p = 0.1091$). The treatment differences increased slightly (<10%) when scaled by TAN and TN in the manure.

Ammonia

Results of the mixed linear model show a significant NH_3 fixed effect due to treatment ($p < 0.0001$), time ($p < 0.0001$), and a combined effect of treatment and time ($p = 0.0351$). The average NH_3 emissions were 3.53, 3.28, and 2.76 g m^{-2} d^{-1} from control, PA inoculum, and NA inoculum, respectively (**Table 3**). The total NH_3 emissions over the entire study (160 days) were 540, 502, and 382 g m^{-2} from control, PA inoculum, and NA inoculum, respectively (**Figure 2**). This represented a significant ($p = 0.0001$) 7% reduction using PA inoculum and a significant ($p < 0.0001$) 29% reduction using NA inoculum, compared to the control. The difference in NH_3 volatilization between PA and NA inoculums was 25% ($p = 0.1326$), which is likely due to the similar manure pH.

CO_2-Equivalent Emissions

On a CO_2-eq basis, the total GHGs were 94–97% comprised of CH_4 emissions, due to the anaerobic conditions within the manure storages (**Table 4**). Clear treatment difference was observed, where PA inoculum reduced total GHGs by 38% and NA inoculum reduced total GHGs by 77%, compared to control. All sources of GHG were reduced due to PA and NA inoculums, although CH_4 was the most important in reducing total GHGs.

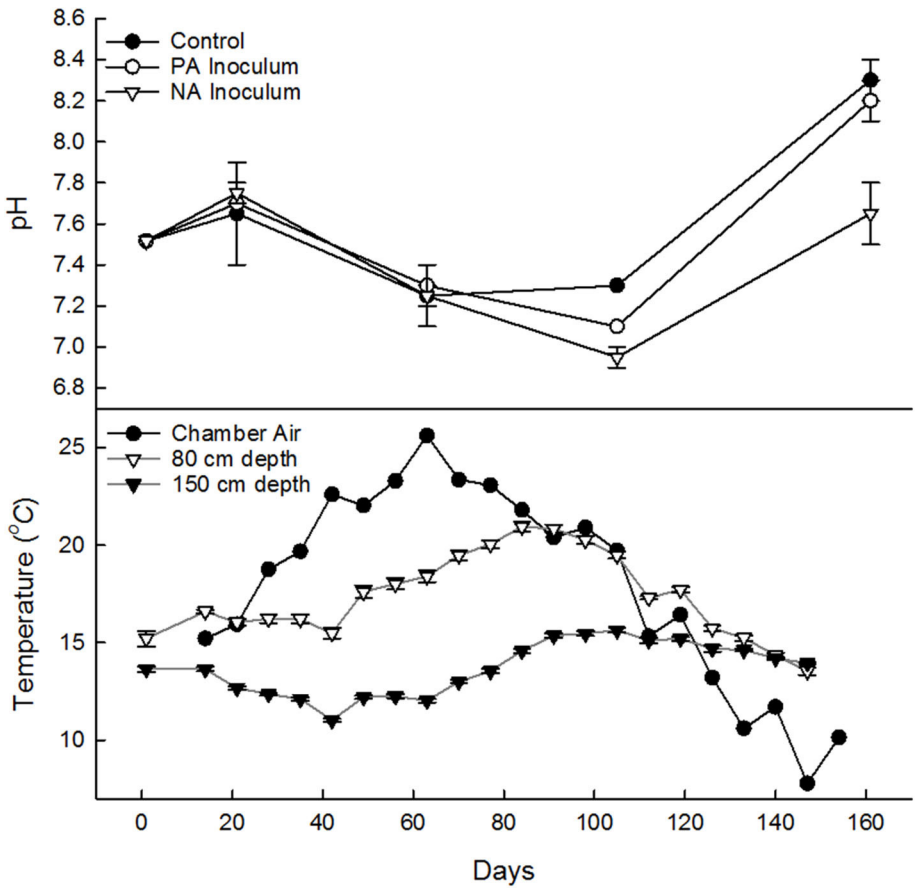

FIGURE 1 | Manure pH (top) in control (•), previously acidified (PA) inoculum (○), and newly acidified (NA) inoculum treatments (▽), samples from on May 29 from fresh manure and stored manure on June 15, July 27, Sept 8, and Oct 2018 (7, 49, 92, and 155 days). Weekly average temperature (bottom) averaged across all tanks, of chamber air 10 cm (•) above manure and of manure at 80 cm (▽) and 150 cm (▼) depth. Error bars denote standard deviation.

Methanogens and Bacteria

The results of the 2-way ANOVA on copies of *mcrA*, *mcrA* transcript, 16S rRNA, and 16S rRNA transcript are shown in **Supplementary Table 1**. There were significant treatment effects on *mcrA* ($p < 0.0001$) and 16S rRNA ($p < 0.0126$) but not in *mcrA* or 16S rRNA transcript.

The FM had higher copies per gram of dry manure of *mcrA* transcript and bacterial 16S rRNA genes and transcript than untreated inoculum (30–45%; percentages are calculated on values prior to log transformations) sampled prior to the start of the study (**Table 5**). An exception was *mcrA* gene in untreated, control inoculum which had 64% more copies per gram of dry manure of than FM. These results differed from Habtewold et al. (2018) who reported more (11–458%) copies of genes and transcripts of both *mcrA* and bacterial 16S in inoculum compared to FM. At the start of the trial, previously acidified inoculum had lower *mcrA* copies of genes and transcript (88%) and lower 16S rRNA genes and transcripts (90%) compared to the untreated inoculum. Given that both inoculums were stored for 1-year under the same conditions, the difference in abundance are likely due to acidification with H_2SO_4 1-year prior.

Averaged over the entire study period, the control had significantly more *mcrA* gene copies compared to PA inoculum (39%) and NA inoculum tanks (65%, $p < 0.05$; **Table 5**). The PA inoculum tanks have significantly more *mcrA* gene copies than NA inoculum tanks (43%, $p < 0.05$).

The *mcrA* transcript copies were variable over time, although the most marked difference between treatments was Jul 27 (d 42) when the NA inoculum and PA inoculum were 95 and 85% less than the control copies, respectively. This corresponds with the initial increase in CH_4 emissions. The average CH_4 emissions during the sampling week were 43.0, 7.70, and 4.12 g m^{-2} d^{-1} from control, NA inoculum, and PA inoculum treatments, respectively.

On Sept 8 (85 days) the NA inoculum treatment had the highest copies *mcrA* transcript, with the control and PA inoculum having 97 and 86% fewer copies, respectively (**Table 5**). This corresponds to CH_4 emissions during the sampling week of 43.4, 55.5, and 21.4 g m^{-2} d^{-1} from control, NA inoculum, and PA inoculum, respectively.

Lastly, on Oct 31 (108 days) the PA inoculum had the highest copies of *mcrA* transcript, with control and NA Inoculum

TABLE 2 | Manure total solids (%), volatile solids (%), total nitrogen (%), and ammonium-nitrogen (%) sampled from fresh manure (FM) during tank filling (May 29, 2018), and stored manure on 7 days (Jun 15, 2018), and 155 days (Nov 11, 2018).

		Control	PA-Inoc	NA-Inoc
Total solids (%)	FM	17.6	17.6	17.6
	15-Jun	12.5	12.7	11.9
	31-Oct	14.2	14.9	14.4
Volatile solids (%)	FM	8.06	8.06	8.06
	15-Jun	6.02	6.10	5.99
	31-Oct	5.59	5.99	6.40
Total nitrogen (%)	FM	0.39	0.39	0.39
	15-Jun	0.26	0.26	0.30
	31-Oct	0.27	0.30	0.29
Ammonium-N (%)	FM	0.16	0.16	0.16
	15-Jun	0.05	0.06	0.09
	31-Oct	0.09	0.10	0.10

having 81 and 86% fewer copies, respectively (**Table 5**). This corresponds to CH_4 emissions during the sampling week of 30.0, 34.2, and 11.0 g m^{-2} d^{-1} from control, NA inoculum, and PA inoculum, respectively.

The 16S rRNA gene copies varied less over time and between treatments (**Table 5**). The 16S rRNA transcript copies in the control treatment increased and decreased following the same pattern as the *mcrA* transcript copies. This pattern was not observed in the NA and PA inoculum, suggesting that the methanogen and bacterial communities had differing influences.

TABLE 3 | Total (g m^{-2}; g m^{-3}; kg; m^3) and daily mean (g m^{-2} d^{-1}) methane (CH_4), nitrous oxide (N_2O-N), and ammonia (NH_3-N) for manure with untreated inoculum (control), previously acidified inoculum (PA-Inoc), and newly acidified inoculum (NA-Inoc) in each block for the entire study period Jun 8–Nov 11, 2019 (155 days).

	Control	PA-Inoc	NA-Inoc
CH_4			
g m^{-2} d^{-1}	36.1 ± 27.7	22.3 ± 17.8	8.19 ± 5.64
g m^{-2}	5,266	3,258	1,196
g m^{-3}	3,291	2,036	748
Kg	34.9	21.6	7.9
m^{-3}	53.2	32.9	12.1
VS, kg	671	679	667
CH_4 potential B_0 × VS	161	163	160
MCF	0.33	0.20	0.08
N_2O-N			
mg m^{-2} d^{-1}	76.4 ± 65.1	38.4 ± 29.9	22.3 ± 23.7
g m^{-2}	11.2	5.61	3.00
g m^{-3}	6.97	3.51	1.88
g kg^{-1} TAN	13.3	5.93	2.23
g kg^{-1} TN	2.60	1.28	0.62
NH_3-N			
g m^{-2} d^{-1}	3.53 ± 2.28	3.28 ± 2.00	2.76 ± 1.52
g m^{-2}	540	502	382
g m^{-3}	338	314	239
g kg^{-1} TAN	643	514	270
g kg^{-1} TN	126	115	77.6

Cumulative CH_4 is also expressed as methane conversion factor (MCF), and maximum CH_4 production (B_0) × volatile solids (VS). Cumulative N_2O-N and NH_3-N are scaled by initial total nitrogen (TN) and initial total ammoniacal nitrogen (TAN).

DISCUSSION

Storages with NA inoculum reduced total GHGs by 77%, while PA inoculum reduced emissions by 38%, compared to the control. Sokolov et al. (2019) acidified manure with no inoculum at rates of 1.4 and 2.4 L 70% H_2SO_4 m^{-3} and reported 85 and 88% reductions in total GHGs, respectively. Our results were slightly lower, which is likely due to the lower rate of acid and the presence of an inoculum. In a lab study, Sokolov et al. (2020) stored FM with previously acidified (2.4 L 70% H_2SO_4 m^{-3}; 6-months old) inoculum and newly acidified inoculum at (0.17 L 98% H_2SO_4) 17, 20, and 23°C and reported average CH_4 reductions of 82 and 63%, respectively, across all temperatures. The PA inoculum in the lab study was more effective, with 82% reductions compared to the 38% reduction in this study. This difference may be due to the lab scale or age of the inoculum. The NA inoculum in the lab study had a much lower rate of H_2SO_4, (0.16 vs. 0.79 L pure H_2SO_4 m^{-3} total manure) which explains the lower (63%) reduction of CH_4.

All contributing GHGs were reduced using NA and PA inoculum. This is important to note, as often mitigating practices reduce one GHG in exchange for increasing another. Although there were clear GHG reduction treatment differences, the pH did not have corresponding differences. In fact, the pH was nearly identical among treatments until day 92 (Sept 7), thereafter the acidification treatments showed lower pH. Sokolov et al. (2019) also reported variable pH values in storage tanks following acidification, although the pH stabilized 35 days into the trial. Others have only observed increases in pH throughout storage due to natural processes re-establishing a neutral pH following acidification (Petersen et al., 2012; Shin et al., 2019). However, this might be due to better mixing of acid in initial short-term storage. The CH_4 reductions could be due to sulfide (derived from sulfuric acid) reactions inhibiting methanogenesis, rather than pH changes alone (Petersen et al., 2012). Previous research has suggested that sulfate reducing bacteria outcompete methanogens for substrate due to a higher affinity (lower K_s) for H_2 and acetate (Kristjansson and Schonheit, 1983). Future research should examine the mechanism of methanogenesis inhibition by sulfide reactions at different pH levels, corresponding hydrogen sulfide (H_2S) production, and resulting total GHG emission reduction from liquid dairy manure.

The NA inoculum treatments reduced total GHGs and total CH_4 by 77%. Both the control and the NA inoculum received the same untreated inoculum and FM, although NA inoculum received 1.2 L 70% H_2SO_4 m^{-3} (total manure in storage) into the inoculum prior to FM addition. This is similar to Sokolov

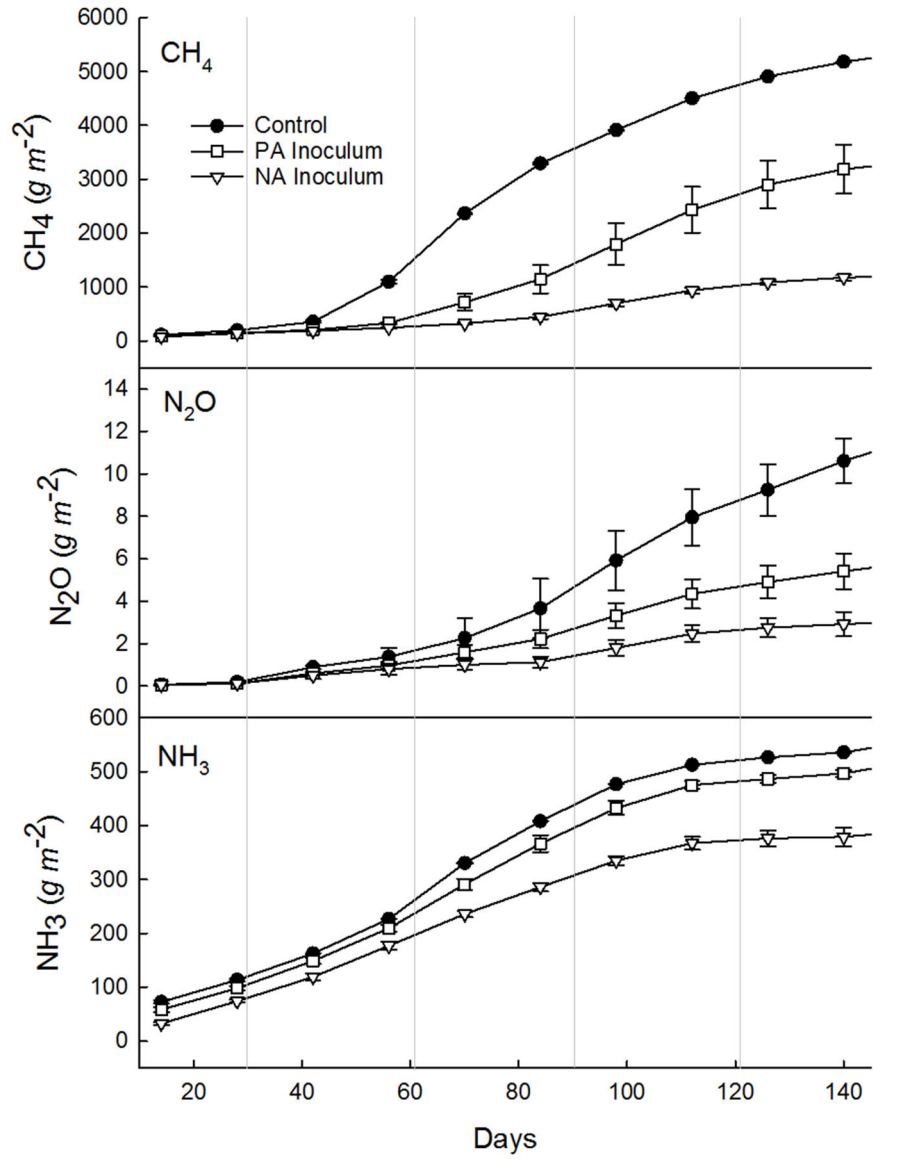

FIGURE 2 | Cumulative methane (CH_4), nitrous oxide (N_2O), and ammonia (NH_3) emissions summed every 14 days over the entire study period (Jun 8–Oct 31; 145 days), from manure with untreated inoculum (control, •), manure with previously acidified (PA, □) inoculum, and manure with newly acidified (NA, ▽) inoculum. Vertical gray lines denote the end of a month, starting with June and ending with Nov. Error bars show standard deviation (note some error bars are too small to see).

et al. (2019) who reported an average 88% reduction of CH_4 from acidifying FM using 1.4–2.4 mL 70% H_2SO_4 L^{-1} manure. Results of real-time qPCR suggest that the reduction is due to disruption in methanogen activity, which is expressed by lower *mcrA* transcript. On the sampling closest to peak emissions (Jul 27), the *mcrA* gene and transcript were lower in the NA inoculum tanks compared to the control. These reductions could be explained by the reduced activity of methanogens which was evidenced from the relatively lower abundance of *mcrA* genes and transcripts in the NA inoculum tanks compared to the control. A study by Habtewold et al. (2018) also found inhibition of methanogen abundance and activity following slurry acidification.

The PA inoculum treatments reduced total GHG and total CH_4 by 38% using no acid in this storage period and only inoculum that was acidified 1-year prior. Results of the real-time qPCR suggest that the reduction is due to reduced methanogen activity in the inoculum. The previously acidified inoculum had markedly lower *mcrA* gene and transcript compared to the untreated inoculum at the start of the trial. The same results are observed during the following sampling event on Jul 27, which was during the time of peak emissions (40–110 days). This suggests that the reduced methanogen activity, expressed as *mcrA* transcript, in the PA inoculum led to lower methanogen activity later in the storage. This was also suggested by Sokolov et al.

TABLE 4 | Total greenhouse gas emissions presented on a CO_2-equivalent basis (kg m^{-2}) for methane (CH_4), nitrous oxide (N_2O), and indirect N_2O from ammonia (NH_3) over the entire 145-d monitoring period from the control, previously acidified (PA) inoculum, and newly acidified (NA) inoculum treatments.

	Control	PA	NA
	CO_2-equivalent (kg m^{-2})		
CH_4	179	111	40.7
N_2O-direct	3.32	1.67	0.89
N_2O-indirect	2.26	1.73	1.52
Total	185	114	43

(2020), who reported that using PA inoculum had similar CH_4 production as FM with no inoculum in a laboratory incubation study. They reported similar CH_4 reductions of 49% using PA inoculum and 55% using no inoculum at 23°C. Ngwabie et al. (2016) similarly reported 36% reductions in CH_4 from manure with no inoculum compared to manure with 20% inoculum (163 days storage).

Given that PA inoculum can reduce the need for acidification to every other filling, it is important to compare estimated total GHG emissions from PA inoculum and NA inoculum over two storage periods. Acidifying all manure in the first storage period and using the PA inoculum in the second period reduced an estimated total GHG emissions by 62%, compared to the control. Using NA inoculum over two storage periods reduced total GHG emissions by 77%, compared to the control. The amount of acid using PA inoculum compared to NA inoculum was nearly identical in both treatments (1.1 vs. 1.2 L m^{-3} $year^{-1}$), although acidifying once accompanied a 38% decrease in GHG. Given that the cost of acid would be nearly the same, the best management practice would be to acidify each year. However, other factors are important to consider, such as the cost of the acidification process (acid delivery, equipment rental, labor, etc.) which is currently unclear and may be prohibitive to farmers. Additionally, removal of manure in the fall with PA inoculum accompanying winter storage may not reduce emissions further, as winter conditions cause very low GHGs regardless of inoculum and acid presence. However, spring emptying with PA inoculum accompanying summer storage could reduce the frequency of acidification and reduce GHG emissions by 62%. Additional research is necessary before we can conclusively state which is the most cost effective.

Manure pH appears to reach neutrality by the end of the storage period, therefore, it should not directly affect the soil pH following application. However, others have reported differences in nutrient composition in soil amended with acidified manure. Following soil application, delayed ammonium loss due to nitrification of ∼20 days has been observed, suggesting more N availability to plants (Fangueiro et al., 2010, 2013, 2015). Petersen et al. (2013) reported higher P availability in soil with acidified manure, while Roboredo et al. (2012) reported an increase in inorganic P in the labile fraction (Roboredo et al., 2012; Petersen et al., 2013; Fangueiro et al., 2015). Lastly, Eriksen et al. (2008) found that H_2SO_4 in pig slurry increased the S fertilizer value, reducing the need for additional mineral fertilizer for crops (Eriksen et al., 2008). Although the current research appears to be positive, more research is necessary to fully understand the effects of applying acidified manure onto crop lands. It is important to note that most research has utilized higher rates of acidification, therefore changes to dairy manure with our NA inoculum or PA inoculum acid rates may be much lower.

TABLE 5 | Copies (log_{10}) of *mcr*A and 16S rRNA gene and transcript from fresh manure (FM) and inoculum sampled on tank filling day (May 29, 2018), and from composite samples of stored manure on Jul 27, Sept 8, and Oct 31, 2018 from control (FM), newly acidified inoculum (NA-Inoc), and previously acidified inoculum (PA-Inoc) treatments.

	Control	PA-Inoc	NA-Inoc
***mcrA* gene copies log_{10}**			
FM	7.71 ± 0.003	7.71 ± 0.003	7.71 ± 0.003
Inoculum	8.16 ± 0.005	7.13 ± 0.011	8.16 ± 0.005
27-Jul	8.14 ± 0.005	7.81 ± 0.016	7.79 ± 0.013
8-Sept	8.46 ± 0.008	8.27 ± 0.009	8.02 ± 0.011
31-Oct	8.56 ± 0.003	8.36 ± 0.001	8.04 ± 0.12
***mcrA* transcript copies log_{10}**			
FM	6.73 ± 0.14	6.73 ± 0.14	6.73 ± 0.14
Inoculum	6.51 ± 0.07	5.68 ± 0.13	6.51 ± 0.07
27-Jul	8.16 ± 0.12	7.31 ± 0.05	6.83 ± 0.03
8-Sept	5.99 ± 0.16	6.71 ± 0.04	7.56 ± 0.04
31-Oct	6.73 ± 0.01	7.29 ± 0.05	6.78 ± 0.06
16S rRNA gene copies log_{10}			
FM	11.5 ± 0.02	11.5 ± 0.02	11.5 ± 0.02
Inoculum	11.2 ± 0.005	10.4 ± 0.02	11.2 ± 0.005
27-Jul	10.9 ± 0.02	10.8 ± 0.04	10.8 ± 0.02
8-Sept	10.9 ± 0.01	10.9 ± 0.01	10.9 ± 0.02
31-Oct	10.9 ± 0.02	10.9 ± 0.04	10.7 ± 0.08
16S rRNA transcript copies log_{10}			
FM	13.4 ± 0.14	13.4 ± 0.14	13.4 ± 0.14
Inoculum	13.2 ± 0.15	12.1 ± 6.98	13.2 ± 0.15
27-Jul	13.2 ± 0.08	13.1 ± 0.03	13.1 ± 0.06
8-Sep	10.5 ± 0.04	11.6 ± 3.82	13.1 ± 3.66
31-Oct	12.3 ± 0.06	10.9 ± 3.75	12.9 ± 0.09

CONCLUSION

Acidification of manure inoculum (1.1 L 70% H_2SO_4 m^{-3} total manure in storage) markedly reduced GHG emissions (77%) compared to the control (FM and untreated inoculum). This suggested that acidifying only the inoculum provided a positive treatment effect, while reducing the need to continuously acidify FM. CH_4 reduction (77%) using NA inoculum was attributed to disruption of methanogen activity due to significant treatment effects on *mcrA* gene and transcript. Using PA inoculum had a moderate reduction on GHG emissions (38%) and somewhat larger when considered over 2 years (62%). This means that cost of acid and the acidification process can be reduced by acidifying every other storage period while still reducing GHGs. The CH_4 reductions (38%) using PA inoculum was attributed to disruption of methanogenesis in the inoculum, hence removing

the inoculating ability of the residual manure. Over two storage periods, the amount of H_2SO_4 was nearly identical between PA and NA inoculum treatments, however PA inoculum allowed for fewer acidification events, while still retaining good GHG reductions. This may allow farmers to reduce expenses associated with acidification while still mitigating GHGs, although more research is needed to validate its applicability at the farm scale.

DATA AVAILABILITY STATEMENT

The datasets presented in the article may be requested by contacting bosa4400@mylaurier.ca or Robert.Gordon@uwindsor.ca.

AUTHOR CONTRIBUTIONS

VS was the graduate student who is the primary author and project lead. AV was a co-supervisor contributing intellectually and financially to overall project, particularly for GHG emission aspects. JH helped create microbial analysis methods and assist with methanogen and bacterial quantification. KD contributed intellectually and financially to particularly to methanogen and bacterial quantification. JT contributed intellectually, lab space and equipment for the methanogen and bacterial quantification. CW-R contributed intellectually and with manuscript editing, particularly with the GHG emissions aspects. JV contributed intellectually helping with statistical analysis and manuscript editing. Lastly, RG was the major funding holder for this project, graduate co-supervisor contributing intellectually and financially, particularly with the GHG emission aspects. All authors contributed to the article and approved the submitted version.

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Climate-Based Suitability Assessment for Methane Mitigation by Water Saving Technology in Paddy Fields of the Central Plain of Thailand

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The alternate wetting and drying (AWD) water management technique has been identified as one of the most promising options for mitigating methane (CH_4) emissions from rice cultivation. By its nature, however, this option is limited only to paddy fields where farmers have sustained access to irrigation water. In addition, large amounts of rainfall often make it difficult to drain water from paddy fields. Therefore, it is necessary to understand the specific conditions and suitability of an area in which AWD is foreseen to be applied before its CH_4 mitigation potential can be assessed in view of planning regional and national mitigation actions. In this study, we applied a methodology developed for assessing the climatic suitability of AWD to paddy fields in the central plain of Thailand in order to determine the potential spatial and temporal boundaries given by climatic and soil parameters that could impact on the applicability of AWD. Related to this, we also assessed the CH_4 mitigation potential in the target provinces. Results showed that the entire area of the six target provinces was climatically suitable for AWD in both the major (wet) and second (dry) rice seasons. A sensitivity analysis accounting for uncertainties in soil percolation and suitability classification indicated that these settings did not affect the results of the suitability assessment, although they changed to some extent the distribution of moderate and high climatic suitability areas in the major rice season. Following the methodologies of the Intergovernmental Panel on Climate Change Guidelines, we estimated that the AWD scenario could reduce annual CH_4 emissions by 32% compared with the emissions in the baseline (continuously flooded) scenario. The potential of AWD for annual CH_4 emission reduction was estimated to be 57,600 t CH_4 $year^{-1}$, equivalent to 1.61 Mt CO_2-eq $year^{-1}$, in the target provinces. However,

we recognize the possibility that other parameters not included in our current approach may significantly influence the suitability of AWD and thus propose areas for further improvement derived from these limitations. All in all, our results will be instrumental in guiding practitioners at all levels involved in water management for rice cultivation.

Keywords: rice, methane emissions, GIS, alternate wetting and drying, mitigation measures, low-emission farming

INTRODUCTION

Rice cultivation is one of the major sources of agricultural emissions of methane (CH_4) at a global scale. The contribution of CH_4 emissions from rice cultivation to the national budgets of greenhouse gas (GHG) emissions is significant in Southeast Asian countries, where rice cultivation is the dominant use of land. For example, in Thailand, rice cultivation occupies about 46% of the total agricultural land [OAE (Office of Agricultural Economics), 2018] and accounts for about 55% of the total GHG emissions in the agriculture sector, or 6.5% of the total national GHG emissions [ONREPP (Office of Natural Resources and Environmental Policy and Planning), 2017]. This highlights the fact that actions to reduce GHG emissions from rice cultivation in Southeast Asian countries could have a great potential in mitigating the impacts of climate change, both regionally and globally.

Considering agronomic management in practice, there exist a number of suggested technical options for mitigating CH_4 emissions from paddy fields. These include specific management techniques for the water regime, rice planting methods, selection of rice cultivar, and application of organic matter, fertilizer, and other amendments (Wassmann et al., 2000; Smith et al., 2014; Romasanta et al., 2017; Yagi et al., 2020). At present, however, only a few of these options have a proven track record as promising mitigation tools. In particular, water management options have been identified as an effective approach to consistently reducing CH_4 emissions (Itoh et al., 2011; Sander et al., 2015; Jiang et al., 2019). This was shown by a meta-analysis of experimental data from Southeast Asia (Yagi et al., 2020): Various water management options, including a single and multiple round(s) of draining of paddy fields (e.g., mid-season drainage and alternate wetting and drying [AWD]), significantly reduced CH_4 emissions by 35% on average (95% confidence interval: 41–29%).

AWD is a water management practice that was developed and is currently promoted by the International Rice Research Institute (IRRI) and its partners in many rice-producing countries, primarily in order to reduce the consumption of irrigation water (Lampayan et al., 2015). The principle of AWD is to switch from a continuously flooded rice field to a field that encompasses several dry phases during the growing season. Starting at about 2–3 weeks after transplanting (3–4 weeks after sowing) the field is left to dry out until the water table reaches a level of about 10–15 cm below the soil surface. Once the threshold is reached, irrigation water should be applied until 3–5 cm of standing water in the field is reached. A level of "−15 cm" has been identified as "safe" so that plants do not face drought stress and thus yields are not reduced (Bouman et al., 2007). Importantly, no significant effect of AWD on rice yield was reported by a meta-analysis of field data from Southeast Asia (Yagi et al., 2020).

Policy makers and rice value-chain operators all over the world are increasingly recognizing that alternative water management practices in rice cultivation could become a visible option for CH_4 reduction. Besides, the impact of climate change in the form of increased frequency of droughts already forces farmers to re-adjust their approach to water management. In view of this, all stakeholders involved may consider making low-emission rice farming as an important component of national and organizational commitments to tackle climate change and enhance the resilience and sustainability of the agriculture sector. In some of the major rice-producing countries, large-scale changes in water management are already part of the proposed actions for reducing GHG emissions in compliance with the national commitments to the Paris Agreement. For example, AWD is listed as one of the low carbon technologies with regards to the nationally determined contributions of Vietnam. The mitigation potential of using water drainage in rice fields was estimated to reduce GHG emissions by 4.1 Mt CO_2 from the South Central Coast and 21.9 Mt CO_2 from the Red River Delta. In addition, the Vietnamese government plans to promote the "System of Rice Intensification (SRI)" program, an innovative rice cultivation technique in which AWD is the central management option (Thakur et al., 2016), within a 500,000 ha of rice cultivation area (Ministry of Natural Resources and Environment, 2017). Similarly, the Indonesian government has included SRI in the list of national mitigation actions and reported that the program has already been applied to up to 435,999 ha of paddy fields by 2014 (Republic of Indonesia, 2017).

However, the application of alternative water drainage regimes may be limited to paddy fields in which the irrigation/drainage system has been well-developed. In particular, control of water is generally difficult or impossible in rainfed paddy fields. This poses a problem in the implementation of water management options for GHG mitigation. In addition, large amounts of rainfall often make it difficult to drain water from paddy fields. In the paddy fields of Southeast Asia, the extension of irrigated paddy fields is limited to <30% among the continental countries, while this value ranges between 60 and 70% in island countries (Dobermann and Fairhurst, 2000; Redfern et al., 2012). Even paddy fields classified as irrigated are not always capable of controlling water, in particular during the rainy wet season. Farmers need guidance regarding the appropriate timing of irrigating their fields within the rice-growing period. Hence,

it would be helpful to understand in which locations water management options are best applicable or suitable in order to achieve the best GHG mitigation results.

Responding to this need, Nelson et al. (2015) presented the first attempt of a spatial and temporal assessment of the climatic suitability for AWD at the province level in the Philippines. This study was based on a simple water balance model reflecting the possibility to drain or dry a rice field for a substantial duration during the rice-growing season. Later, Sander et al. (2017) extended this methodology to the entire rice area of the Philippines and developed country-scale climatic suitability maps for AWD for the wet and dry seasons. In addition, both studies illustrated how the assessment can be used to estimate the potential GHG emission mitigation. They estimated that a maximum of 60% of the rice area of the Philippines is climatically suited for AWD, reaching more than 90% in the dry and 34% in the wet season. The potential maximum annual reduction of CH_4 emissions from lowland rice in the Philippines was estimated to be about 265,000 t CH_4 $year^{-1}$ or around 15% of the country's annual emissions from the agriculture sector (Sander et al., 2017).

Here we apply the same methodology as described previously to the paddy fields in the central plain of Thailand. The six provinces selected for this study are located mostly in the irrigated area, which has a higher capacity of controlled irrigation compared to other areas in Thailand. Our work aimed at assessing the spatial and temporal boundaries of climatically suitable areas for application of AWD in the target provinces. At the same time, we estimated the potential for reducing CH_4 emissions from rice cultivation. We hope that our results will be helpful to agricultural practitioners and can contribute to the development of national policies for climate change actions.

MATERIALS AND METHODS

Study Area

This study focused on six provinces: Ang Thong, Ayuthaya, Chai Nat, Pathum Thani, Sing Buri, and Suphan Buri which are all located in the central plain of Thailand (**Figure 1**). The total area of all six provinces covers 13,736 km^2, between 13.92 and 15.42°N and between 99.28 and 100.95°E. All six provinces share boundaries, which provides for similar weather conditions in that area. The rainy season usually starts in May and lasts until the end of October. The period from November to January represents the cool and dry season, while the months from February to April represent the hot and dry period (Thai Meteorological Department, 2020). All six provinces are located in a plain, which includes major rivers, such as the Chao Phraya, Tha Chin, Pa Sak, and Lopburi. Most of the land use for agriculture in these six provinces is under irrigation, which is under the authority of the Regional Irrigation Offices. Therefore, water for rice cultivation is usually available in sufficient quantities, even during the dry season.

The average annual rainfall is around 1,150 mm, with most rain (960 mm) occurring during the rainy season from May to October. The average minimum and maximum temperature throughout the year is 23 and 34°C, respectively. In both major (wet) and second (dry) rice seasons, rice is cultivated under rainfed (5.9 and 1.6% of the rice area, respectively) and irrigated (94.1 and 98.4%, respectively) conditions according to data from the crop year 2018 published by the Office of Agricultural Economics (OAE) (OAE (Office of Agricultural Economics), 2018). In the event of a prolonged drought, however, even irrigated land may be in short supply of water.

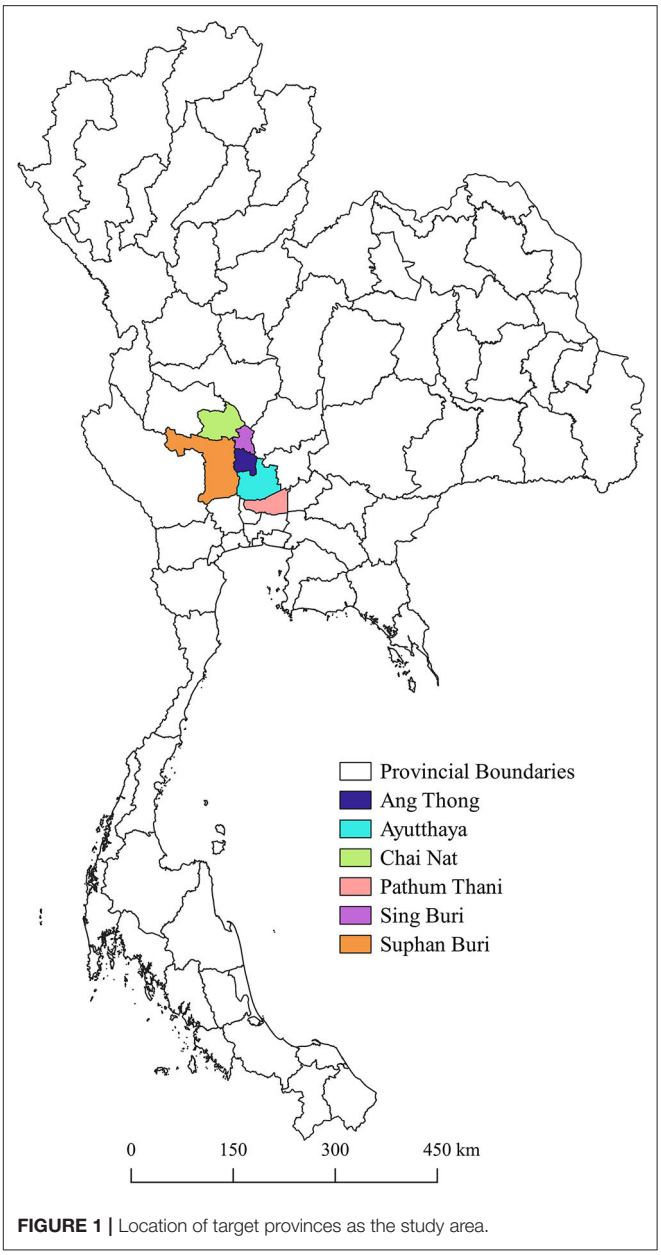

FIGURE 1 | Location of target provinces as the study area.

Outline of Suitability Assessment

We conducted the AWD suitability assessment by following the methodology described by Nelson et al. (2015). The outline of the methodology is summarized in a flow chart as shown in **Figure 2**. This approach for assessing climatic suitability of AWD was applied to paddy fields in the central plain of Thailand

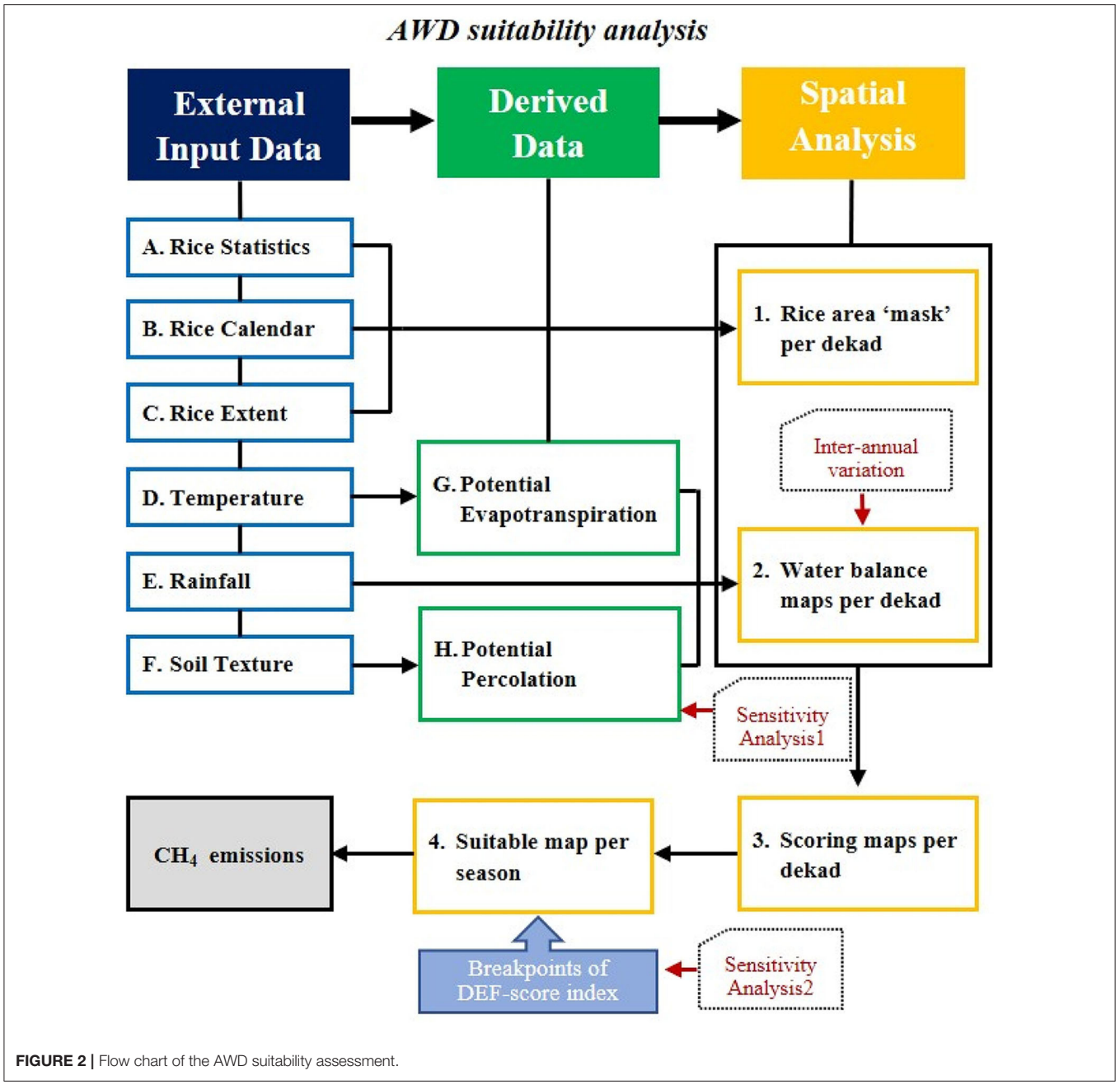

FIGURE 2 | Flow chart of the AWD suitability assessment.

for determining the potential spatial and temporal boundaries given by climatic and soil parameters that could influence the applicability of AWD. Related to that, the potential of CH_4 emission mitigation in the target provinces was estimated.

As shown in **Figure 2**, this study used the water balance model for climatic suitability assessment (Nelson et al., 2015). For running the model, six data sets were used as "input data," namely data set A: rice statistics, data set B: rice calendar, data set C: rice extent, data set D: temperature, data set E: rainfall, and data set F: soil texture. The water balance model is the central idea of the assessment: it takes into account the volumes of water entering and leaving the rice fields. The total amount of water flux is related to meteorological factors (temperature and rainfall) and characteristics of the soil. The products of derived input data are termed "derived data," including data set G: potential evapotranspiration and data set H: potential percolation. Data sets A–H were used to assess climatic suitability for AWD by following the four steps of "spatial analysis" described below.

The climatic suitability of the rice area for implementing AWD was calculated for a period of 1 year, which was divided into intervals of 10 days, called "dekad." A total of 37 maps were scored by counting the deficit (DEF) water dekads according to the rice calendar. We accounted for potential uncertainties related to the input data or criteria of

parameters by conducting sensitivity analyses for soil percolation rate data and breakpoint setting for ranking suitability were conducted. Finally, we estimated the potential of CH_4 mitigation by implementing AWD in the target provinces using the methodologies recommended in the Intergovernmental Panel on Climate Change (IPCC) Guidelines [IPCC (Intergovernmental Panel on Climate Change), 2019].

Input Data

Data Set A: Rice Statistics

Rice statistics included area, production, and yield of rice, in each province of Thailand as reported annually in the Agricultural Statistics of Thailand published by OAE. The reports for the years 2010–2017 (November 2010–October 2017) were available to us. According to the latest report for 2017, the average annual area of total rice harvested in the six target provinces was 1,047,000 ha, of which 584,000 ha are planted in the major rice season and 464,000 ha in the second rice season (OAE (Office of Agricultural Economics), 2018; **Table 1**). According to the monthly rice harvested area reports, the major rice season started in May and ended in October; the second rice season started in November and ended in April in all the target provinces.

Data Set B: Rice Calendar

The data used for feeding the "Rice calendar" were obtained from "RiceAtlas," a spatial database of global rice calendars and production (Laborte et al., 2017). The data in "RiceAtlas" are based on various published sources, such as global and regional datasets, international and national publications, online sources, and unpublished data sources like expert knowledge. The calendar summarizes the cultivation periods and provides the peak date of cultivation, which refers to the date when the majority of the crop is being planted or harvested.

According to "RiceAtlas," Thailand has five different rice calendars accounting for the double cropping of rice. Here, we have chosen the calendars of Supan Buri (type 1) and those of the five other provinces that share the same type (type 2). Rice calendars of both types and differentiated by season are listed in **Table 2**.

Data Set C: Rice Extent

Rice extent, or the spatial distribution of rice, was based on the product from MOD09A1 8-day composite remote sensing data in 2000–2012, which was derived from rice extent maps of a 13-years time series of "Moderate Resolution Imaging Spectrometer (MODIS)" at a 500 m resolution (Xiao et al., 2006; Nelson and Gumma, 2015). The maximum rice extent data during these years were used as input data for representing rice extent in the target provinces.

Data Set D: Temperature

Temperature data in degrees Celsius (°C) were obtained from daily minimum and maximum temperature records including the "Global Surface Summary of the Day (GSOD)" database [NOAA (National Oceanic and Atmospheric Administration), 2019]. These daily minimum and maximum temperatures in raster were averaged as mean daily temperature per dekad in 2007–2017. Consecutively, dekadal temperature data were again averaged as an 11-year mean temperature per dekad. Additionally, the dekadal temperature data of each year were used to analyze inter-annual variations of the suitability for AWD.

Data Set E: Rainfall

Rainfall data in mm day^{-1} were taken from the rainfall estimates from rain gauge and satellite observations included in the quasi-global rainfall database termed "Climate Hazards Group InfraRed Precipitation with Station (CHIRPS)" that provides daily rainfall data in raster grid format with a 0.05-degree (∼5.6 km) resolution (Funk et al., 2015). They were summed up to daily rainfall per dekad in 2007–2017 and calculated as the average of all 11 years per dekad. **Figure 3** shows the spatial distribution of the 11-years mean rainfall per dekad. In addition, the dekadal rainfall data of each year were used to analyze inter-annual variations of the suitability for AWD.

Data Set F: Soil Texture

We followed the definition of soil texture for each soil series in the soil database developed by the Land Development Department

TABLE 1 | Average size of the area of annually harvested rice in the target provinces for 7 years (2010–2017, except 2005) according to the Agricultural Statistics of Thailand [OAE (Office of Agricultural Economics), 2018].

Province	Province total area (km^2)	Annual rice harvested area (1,000 ha)				
		Major season		Second season		Total
		Average	Std.	Average	Std.	
Ang Thong	951	51	5	38	18	89
Ayutthaya	2,547	125	19	112	27	237
Chai Nat	2,466	119	8	81	34	200
Pathum Thani	1,515	49	5	47	13	96
Sing Buri	839	46	5	37	20	83
Suphan Buri	5,418	193	14	149	46	342
Total	13,736	584	56	464	158	1,047

TABLE 2 | Rice calendars in the target provinces according to RiceAtlas (Laborte et al., 2017).

Calendar	Province	Major season			Second season		
		Planting	Harvesting	Duration (day)	Planting	Harvesting	Duration (day)
Type 1	Ang Thong Ayutthaya Chai Nat Pathum Thani Sing Buri	DOY 135 (dekad 14)	DOY 306 (dekad 31)	172	DOY 15 (dekad 2)	DOY 135 (dekad 14)	121
Type 2	Suphan Buri	DOY 218 (dekad 22)	DOY 356 (dekad 36)	139	DOY 356 (dekad 36)	DOY 99 (dekad 10)	109

DOY, day of year.

(LDD), Thailand (LDD, 2019). This database is also available as a geographic information system (GIS) shapefile that presents spatial and attribute records. The information in the database compiled various soil characteristics and properties, including soil texture, for about 300 soil series over the entire area of Thailand. With regard to our target area, soil texture data for 67 relevant soil series were used as input data.

Derived Data

For the calculation of water balance, potential evapotranspiration (Pot_ET) and potential percolation (Pot_Pc) are required as inputs. Data sets G (Pot_ET) and H (Pot_Pc) were derived from data sets D (temperature) and F (soil texture), respectively, which are termed here "derived data."

Data Set G: Potential Evapotranspiration (Pot_ET)

One pathway of water loss from paddy fields is evapotranspiration. Here, Pot_ET was obtained by using the Hargreaves method (Hargreaves and Samani, 1985) which uses temperature and extra-terrestrial radiation for the calculation (Equation 1).

$$\text{Pot\_ET} = 0.0023\text{Ra}(\text{Tmean} + 17.8)\text{TD}^{0.5} \quad (1)$$

where 0.0023 is the Hargreaves coefficient, Ra is the extra-terrestrial radiation (MJ m^{-2} day^{-1}), Tmean is the average temperature (°C), and TD is the daily temperature range (Tmax–Tmin in °C). Ra is computed using the approach of Allen et al. (1998) for each day of the year and for different latitudes (Equation 2).

$$\text{Ra} = 0.408\frac{24(60)}{\pi}\text{G}_{sc}\text{d}_r[\omega_s \sin(\phi)\sin(\delta) + \cos(\phi)\cos(\delta)\sin(\omega_s) \quad (2)$$

where 0.408 is the inverse latent heat flux, G_{sc} is the solar constant, d_r is the inverse relative distance between the sun and the earth, ω_S is the sunset hour angle, ϕ is the latitude, and δ is the solar declination.

Pot_ET was measured for the period 2007–2017 and then displayed as data for spatial distribution for the 11-years mean Pot_ET per dekad as shown in **Figure 4**. In addition, the dekadal Pot_ET data of each year were used to analyze inter-annual variations of the suitability for AWD.

Data Set H: Potential Percolation (Pot_Pc)

Percolation rates are influenced by the range of characteristics pertaining to the physical and hydraulic properties of the soil. Nevertheless, the impact of texture is still observable by the higher percolation rate in sandy soils than that in clay soils. The basic setting of the value of Pot_Pc rate for the individual soil series was assigned from the soil texture data (data set F) by following the method of Sander et al. (2017). In addition, for evaluating uncertainties resulting from the setting of the Pot_Pc rates, two additional rates—lower (50% smaller than the basic setting) and upper (50% larger than the basic setting) settings—were applied across all soil textures in the database, as shown in **Table 3**.

The spatial data of the soil series were rasterized and interpreted with the Pot_Pc rate for each pixel in the target provinces. The Pot_Pc rate of each pixel is the weighted average of the Pot_Pc rates of its soil class composition. A total of 67 soil series were classified to 25 layers of Pot_Pc. **Figure 5** shows the distribution of Pot_Pc rates at the basic setting for the six target provinces.

Spatial Analysis

The four-step spatial analysis was carried out according to Nelson et al. (2015) to get the suitability maps for AWD. Since this was a spatial work, the following items were checked before evaluating procedures:

1. All layers should be in the same projection. This study used the World Geodetic System 1984 (WGS84).
2. All layers should be resampled to a uniform resolution. Resolution of the results was 0.002 degrees (224 meters).

Step 1. The provincial rice area per dekad (data sets A and B) was distributed to the physical rice extent pixels (data set C) on an equal area basis, resulting in 37 dekadal maps at 0.02 degrees (224 meters) resolution showing where rice is grown during which dekads.

Step 2. Data sets D–H were used to get the "water balance maps per dekad." As mentioned, data sets G and H were derived from temperature and soil texture values, respectively. Pot_ET, Pot_Pc, and rainfall were displayed for analyzing in the same resolution at 0.02 degrees (224 m). This step compared the aggregate of Pot_ET and Pot_Pc with rainfall.

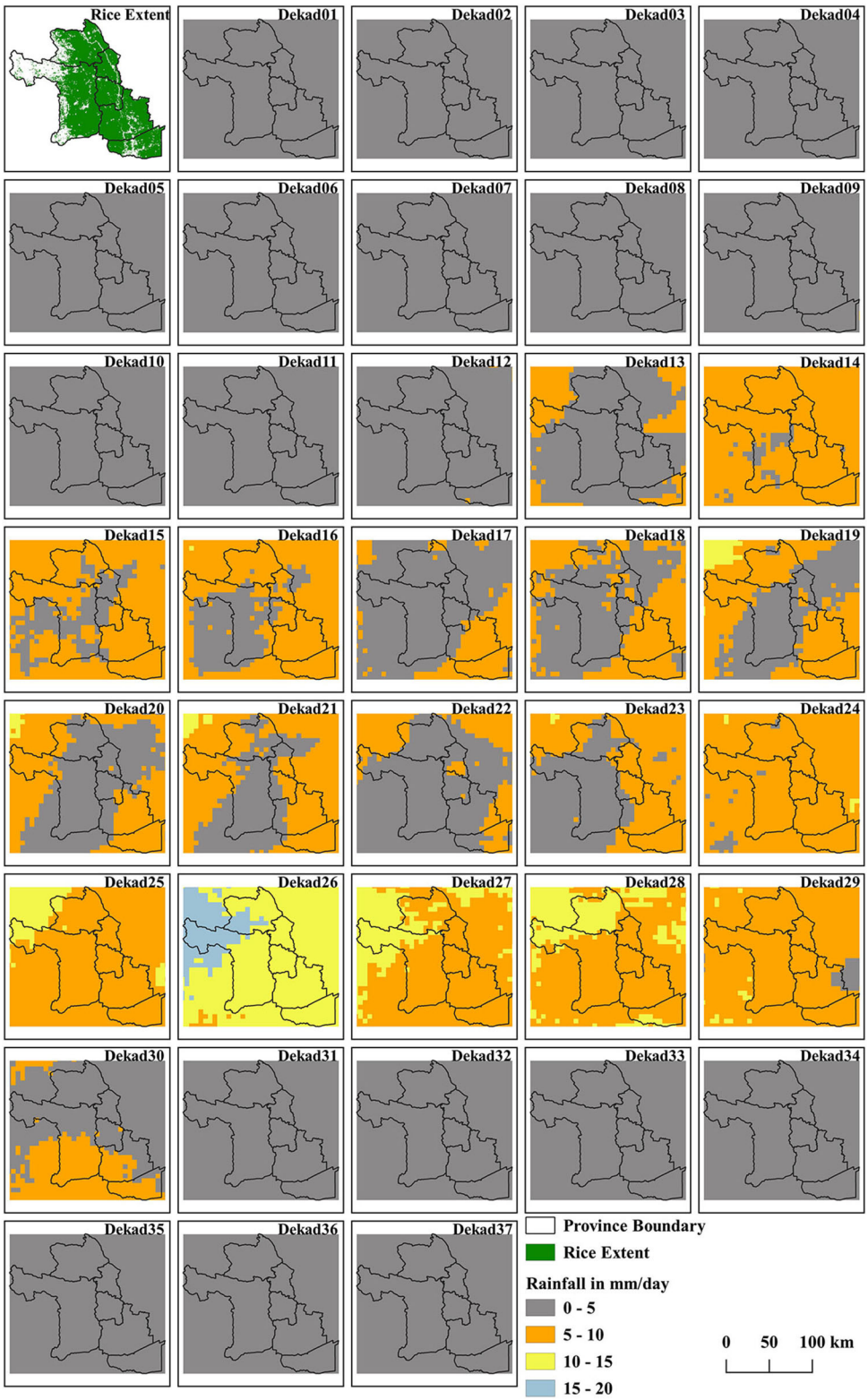

FIGURE 3 | Eleven-years mean rainfall in the target area per dekad.

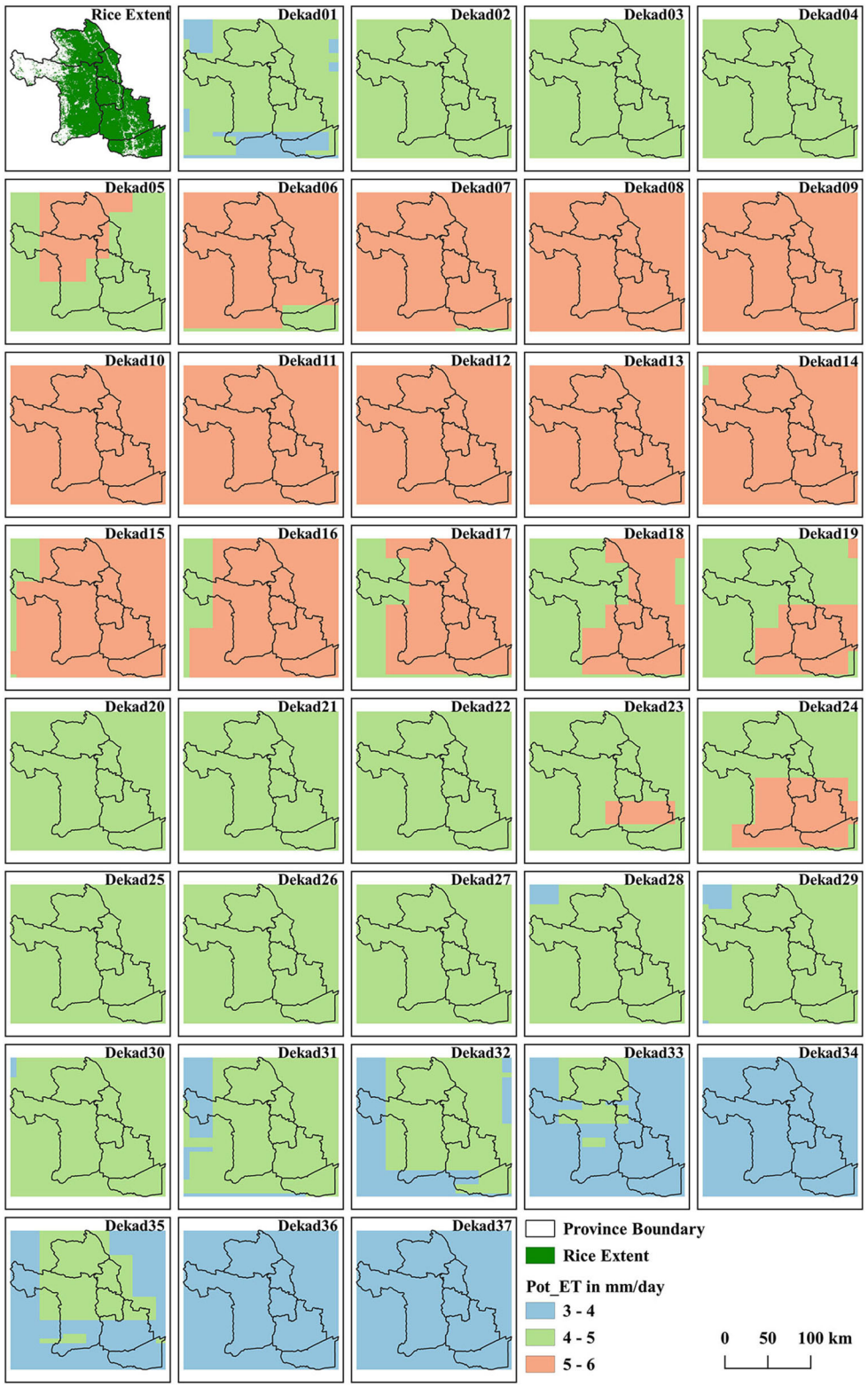

FIGURE 4 | Eleven-years mean potential evapotranspiration (Pot_ET) in the target area per dekad.

TABLE 3 | Potential percolation rate (Pot_Pc) for rice soil related to soil texture.

Soil texture	Pot_Pc (mm day^{-1})		
	Lower setting	Basic setting	Upper setting
Clay	1.5	3.0	4.5
Silty clay	1.5	3.0	4.5
Sandy clay	2.5	5.0	7.5
Clay loam	1.75	3.5	5.25
Silty clay loam	1.5	3.0	4.5
Sandy clay loam	3.5	7.0	10.5
Sand	6.0	12.0	18.0
Loamy sand	5.0	10.0	15.0
Sandy loam	4.5	9.0	13.5
Loam	2.0	4.0	6.0
Silty loam	2.0	4.0	6.0

Step 3. Scoring maps per dekad were provided according to the results from step 2. If the pixel had more Pot_ET plus Pot_Pc than the amount of rainfall (Rf), it was recognized as the pixel of deficit (DEF) water balance. Whereas, if the pixel had less Pot_ET plus Pot_Pc than Rf, it was recognized as the pixel of excess (EXC) water balance:

$$\text{Rf} < (\text{Pot\_ET} + \text{Pot\_Pc}) = \text{Water balance deficit (DEF)}$$
$$\text{Rf} \geq (\text{Pot\_ET} + \text{Pot\_Pc}) = \text{Water balance excess (EXC)}$$

Step 4. After step 3, the 37 dekadal maps were obtained. The suitability map was determined from the proportion of the DEF scored per rice season. The index for scoring from such proportions was set at 0–1, where 0 means no dekad was DEF and 1 means that it was DEF for all dekads. Classification of areas that showed low suitability, moderate suitability, and high suitability were ranked by scoring according to the index shown in **Table 4**. A sensitivity analysis was carried out to evaluate the uncertainty related to the setting of different "breakpoints."

CH_4 Emission Calculation

We estimated the potential of mitigating CH_4 emissions by following the IPCC guidelines, using Tier 1 and Tier 2 approaches [IPCC (Intergovernmental Panel on Climate Change), 2019]. For calculating the adjusted daily emission factor for the baseline water management with continuous flooding (EF_{i-CF}), the default baseline emission factor for Southeast Asia (EF_c: 1.22 kg CH_4 ha^{-1} day^{-1}) was applied. For the scaling factors for organic amendment (SF_o), the value of 1 was applied in the calculation of EFi-CF, according to the methodology used in the Third National Communication (TNC) of Thailand [ONREPP (Office of Natural Resources and Environmental Policy and Planning), 2018] with reference to the expert who calculated GHG emission inventories for rice cultivation in the TNC (personal communication: Prof. Patthra Pengthamkeerati, Kasetsart University). For the scaling factors for water regime before the cultivation period (SF_p), the aggregated default value (SF_p =1.22) was applied. For calculating the adjusted daily emission factor for AWD water management (EF_{i-AWD}), the scaling factors for multiple drainage water management in the wet season (SF_w =0.76) and the dry season (0.59) were applied to the major and second rice seasons, respectively, based on the result of the meta-analysis by Yagi et al. (2020), while the value of 1 was applied for the baseline water management with continuous flooding. For the cultivated period of rice, 120 and 122 days were applied for the major and second rice seasons, respectively, according to the information contained in the OAE report for the average growth period of major rice varieties planted [OAE (Office of Agricultural Economics), 2018].

RESULTS AND DISCUSSION

Dekadal Scoring Maps for Water Balance

Using data sets A–H and processing steps 1–3, we developed dekadal scoring maps, which showed spatial and temporal patterns of water balance in the target provinces throughout the year by averaging the data of each year between 2007 and 2017 as shown in **Figure 6**. A total of 37 maps for each dekad depicted the pixels of water balance either as DEF or EXC criteria throughout 1 year. The results showed that most of the pixels in the target provinces had a water balance of DEF throughout the year due to a lower amount of rainfall than the sum of Pot_ET and Pot_Pc, except for periods with a higher level of rainfall. The pixels under the EXC criterium appeared at dekads 16, 21, and 23 (at the end of August to the middle of September) in Pathum Thani province where higher rainfall (more than 5 mm day^{-1}, **Figure 3**) was recorded in the soil with relatively smaller Pot_Pc (<3 mm day^{-1}, **Figure 5**). After that, EXC pixels extended to other provinces, in response to the increased rainfall until dekad 29 (mid of October). Then, water balance switched to DEF again in all six target provinces after dekad 30 (at the end of October). As a result, most of the pixels in the target provinces had the score of DEF for more than 30 dekads (300 days) per 1 year due to lower rainfall than the sum of Pot_ET and Pot_Pc.

Climatic Suitability Maps

The climatic suitability maps for AWD in the major and second rice seasons with basic settings for the suitability breakpoint are shown in **Figure 7**. In the major rice season (wet season, **Figure 7A**), a large extent of the target provinces was assessed to be highly suitable for AWD, while the rest was moderately suitable. The area of high suitability was extended to the whole of Ang Thong, Ayuthaya, Chai Nat, and Sing Buri provinces, most of Pathum Thani, and about half of Suphan Buri. In all target provinces, the areas assessed to have high and moderate suitability accounted for 470,000 ha and 113,000 ha, respectively, corresponding to 80.5 and 19.5% of the total area of rice harvested, respectively. There was no pixel assessed to have low suitability in the target provinces. The difference between the rice calendars of Suphan Buri and the other provinces increased the proportion of EXC scored dekads per rice season, resulting in the appearance of pixels assessed to have moderate suitability in Suphan Buri. In addition, the spatial distribution of soil with relatively smaller Pot_Pc (**Figure 5**) overlapped with pixels of moderate suitability pixels.

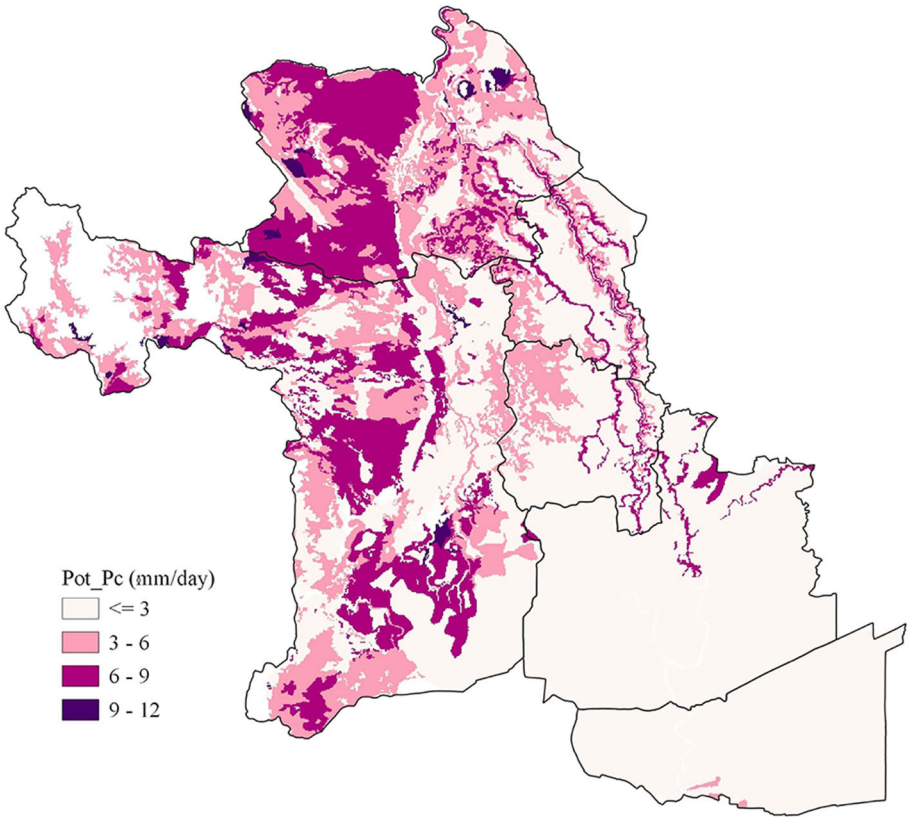

FIGURE 5 | Spatial distribution of potential percolation rates (Pot_Pc) at basic setting in the target area.

TABLE 4 | Breakpoints for the DEF score index as used in the sensitivity analysis on low, moderate, and high suitability for AWD.

Breakpoints	Seasonal suitability for AWD based on DEF score		
	Low	Moderate	High
1 (33–33–33, basic setting)	0.0–0.33	0.34–0.66	0.67–1.00
2 (20–60–20)	0.0–0.20	0.21–0.80	0.81–1.00
3 (25–50–25)	0.0–0.25	0.26–0.75	0.76–1.00
4 (30–40–30)	0.0–0.30	0.31–0.70	0.71–1.00
5 (50–30–20)	0.0–0.50	0.51–0.80	0.81–1.00

In contrast to the wet major rice season, the entire area of the target provinces was assessed to have high suitability for AWD in the second rice season (dry season, **Figure 7B**). This result was predominantly attributable to the relatively small amount of rainfall in the target areas during the season by the northeast monsoons.

According to the previous study for the Philippines (Sander et al., 2017), AWD suitability was mostly ranked as low for the wet season throughout the country; however some areas of moderate or high suitability were found on Mindanao island, the central Visayas, and in Bicol and Cagayan provinces in Luzon island. The authors explained those regional differences in suitability, particularly in the wet season, with differences in the amount of rainfall during the season. Here, in the target provinces of this study, most of the pixels had 11-years averaged dekadal rainfall of <15 mm day^{-1}, even during the wet major rice season, except for only one dekad (dekad 26, **Figure 3**). This resulted in the dominance of DEF scores throughout the year and high AWD suitability for the whole target area.

Sensitivity Analysis

The results of the sensitivity analysis on soil percolation rates are shown in **Figure 8**, where we applied the basic setting of breakpoint for scoring AWD suitability (**Table 4**). Differences in the suitability appeared during the major rice season. The area assessed to have high suitability increased as Pot_Pc values increased. The sizes of areas of high suitability were 284,000, 470,000, and 582,000 ha for the lower, basic, and upper settings of Pot_Pc values, corresponding to 48.6, 80.5, and 99.7% of the total area, respectively. All or most of the areas were classified as suitable for AWD (moderate and high suitability) and only a small area (<2.4% of the total area) was classified to have low suitability in the case of the lower Pot_Pc rate setting. Hence, we conclude that a change in Pot_Pc rate settings for each soil texture (±50% from the basic setting) did not affect the results of this suitability assessment in the target provinces. However, it changed the distribution of moderate and high suitability areas

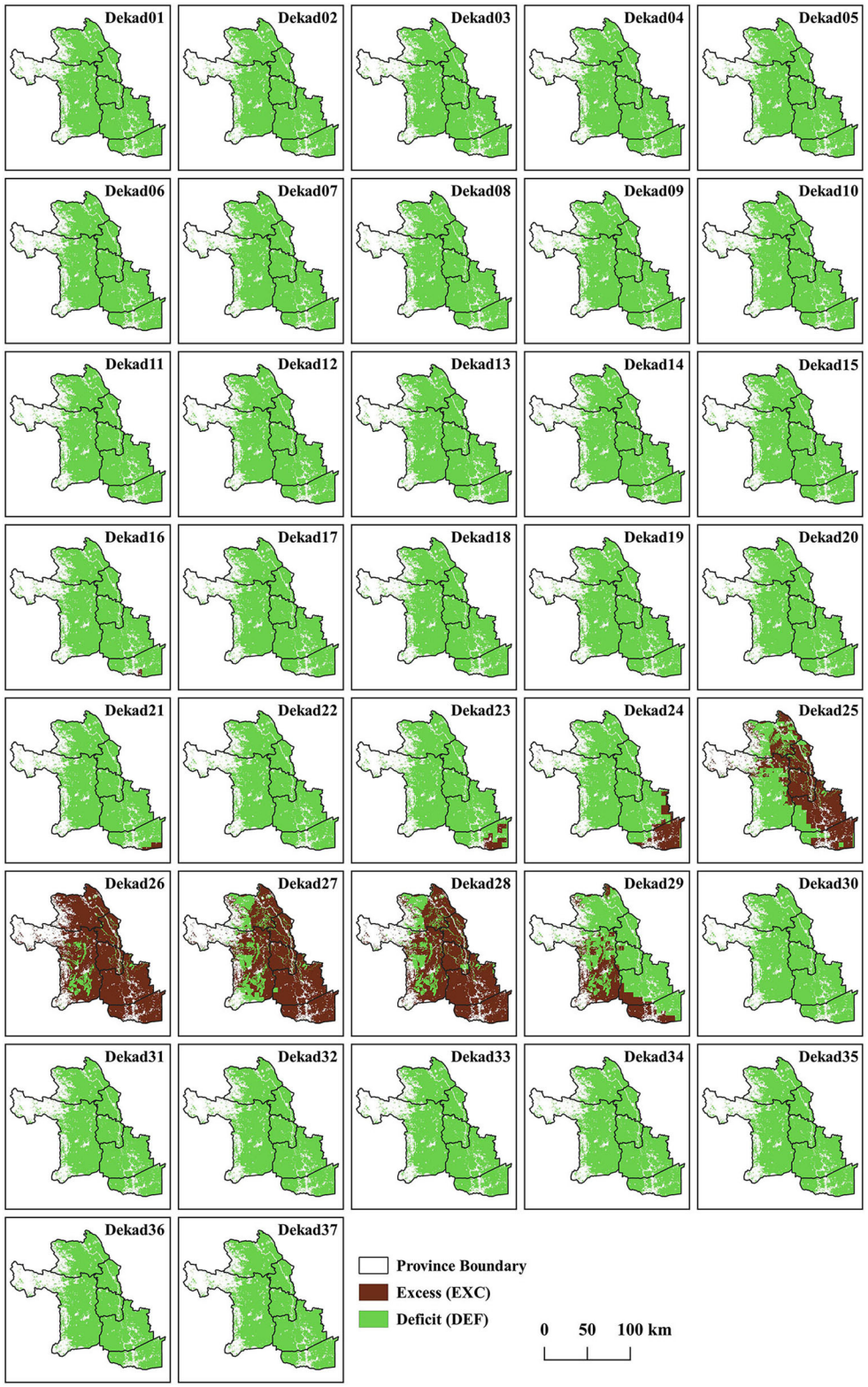

FIGURE 6 | Dekadal scoring maps for water balance.

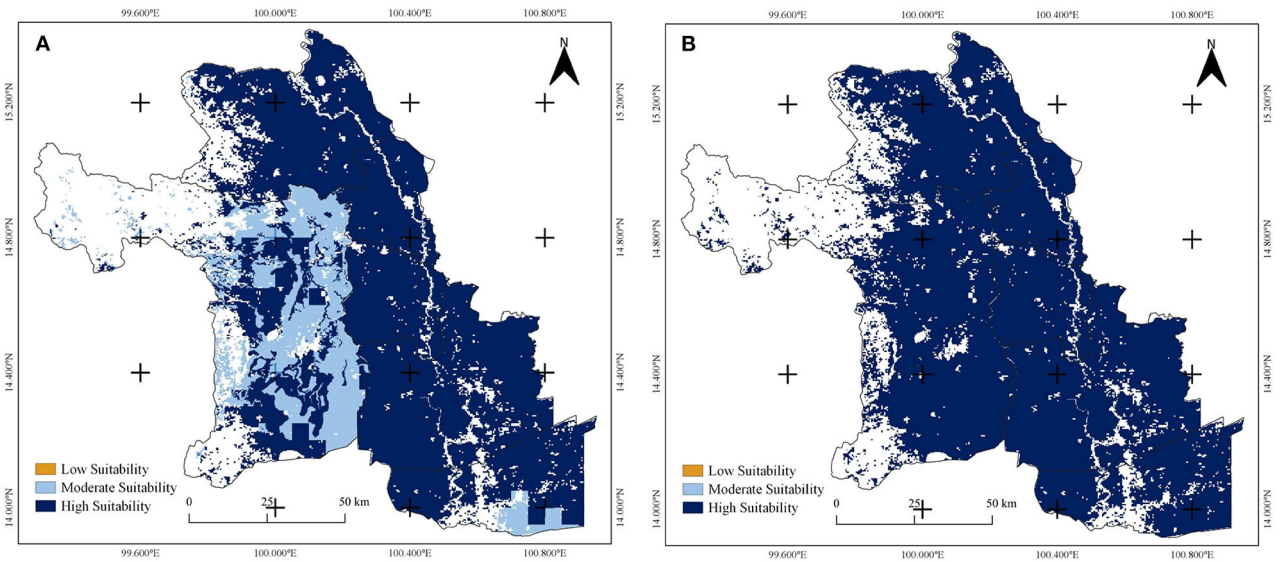

FIGURE 7 | Suitability maps (basic setting on the suitability breakpoint) for the major **(A)** and second **(B)** rice seasons.

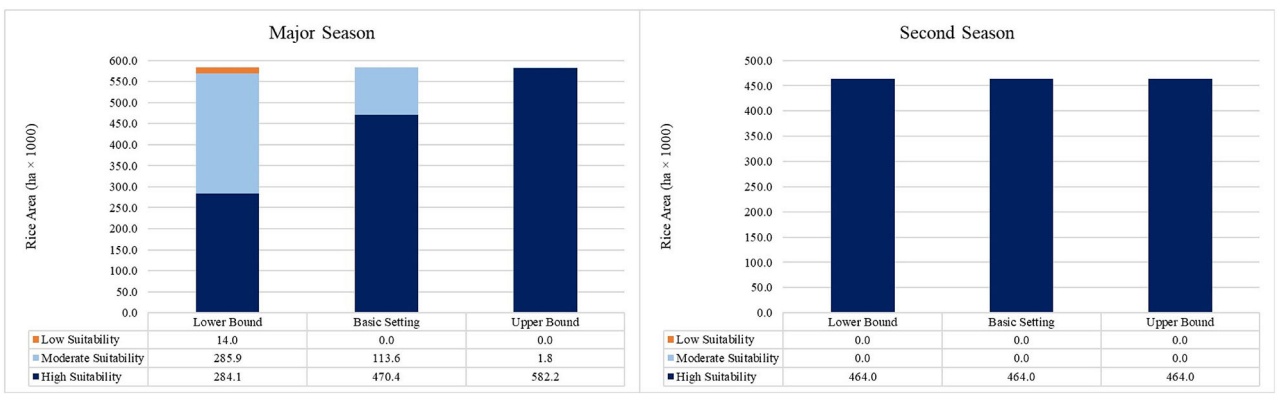

FIGURE 8 | Sensitivity analyses on the settings of potential soil percolation rates (Pot_Pc) in the major and second rice seasons (the case for breakpoint 1).

to some extent. On the other hand, a change in Pot_Pc rate settings did not affect the suitability area during the second rice season since the entire area was classified as highly suitable for implementing AWD.

The results of our sensitivity analysis on breakpoint settings for scoring AWD suitability are shown in **Table 5**. In the major rice season, the entire target area appeared to be suitable for AWD (moderate and high suitability). The area of high suitability decreased and that of moderate suitability increased once the breakpoint range of high suitability decreased and that of moderate suitability increased (breakpoints 1 4 3 2). The area of low suitability did not appear even with increasing the breakpoint range for low suitability up to 50% (breakpoint setting 5). The difference in the area of high suitability between breakpoints 1 and 2 was 306,000 ha, corresponding to 52.4% of the total rice area. Changing the breakpoint settings did not affect the suitability of the area during the second rice season since the entire area was classified to be highly suitable for implementing AWD.

Inter-annual Variation of Suitability

The results of the AWD suitability assessment in the major rice season for each year between 2007 and 2017 are shown in **Figure 9**. **Table 6** summarizes the statistics of the inter-annual variation, showing the average, standard deviation, maximum, and minimum values. The results indicated that the areas of different suitability classes varied in response to inter-annual climatic variability in the major rice season, but not in the second rice season. In the major rice season, some pixels with low suitability appeared in 2010 and 2011. Those exceptional years were characterized by high rainfall during the season, while moderate suitability areas appeared to be linked to relatively low Pot_Pc values. Larger extents of low and moderate suitability areas appeared in the years 2010 and 2017, while almost the entire

TABLE 5 | Sensitivity analyses on the settings of breakpoints for the major and second seasons.

Break point settings	Major rice season						Second rice season					
	Low		Moderate		High		Low		Moderate		High	
	1,000 ha	(%)	1,000 ha	(%)	1,000 ha	(%)	1,000 ha	(%)	1,000 ha	(%)	1,000 ha	(%)
1 (33–33–33, basic setting)	0	(0)	114	(19)	470	(81)	0	(0)	0	(0)	464	(100)
2 (20–60–20)	0	(0)	438	(75)	146	(25)	0	(0)	0	(0)	464	(100)
3 (25–50–25)	0	(0)	212	(36)	372	(64)	0	(0)	0	(0)	464	(100)
4 (30–40–30)	0	(0)	132	(23)	452	(77)	0	(0)	0	(0)	464	(100)
5 (50–30–20)	0	(0)	438	(75)	146	(25)	0	(0)	0	(0)	464	(100)

*The basic setting for potential soil percolation rates (Pot_Pc: **Table 3**) was used for the analysis.*

target area was classified as high suitability in 2007 and 2015. The coefficients of variation (CVs) for the inter-annual variation were calculated to be 82.4% and 36.5% for the areas of moderate and high suitability, respectively.

CH_4 Emission Mitigation

We calculate the potential for CH_4 emission mitigation by assuming that all irrigated rice areas were continuously flooded (baseline scenario) and AWD was applied to areas of high and moderate suitability (AWD adoption scenario). Estimates for emissions of CH_4 are given for the basic settings of soil percolation rates and breakpoint ranges in **Table 7**. The results showed that higher CH_4 emissions during the major rice season were due to a larger rice area and higher seasonal EF.

Under the AWD adoption scenario, CH_4 emissions were reduced by 24 and 41% in the major and second rice seasons, respectively, compared with those in the baseline scenario. Totally, annual emissions were reduced by 32%. As a result, the potential of seasonal CH_4 emission reduction was estimated to be 34,000 and 23,600 t CH_4 for the major and second rice seasons, respectively. Hence, we estimated that the six target provinces in the central plain of Thailand have the potential for reducing annual CH_4 emissions by 57,600 t CH_4 $year^{-1}$ if they implemented AWD water management in paddy fields. This emission reduction is equivalent to 1.61 Mt CO_2-eq $year^{-1}$, assuming the global warming potential (GWP) of CH_4 to be 28.

As a party to the Paris Agreement for mitigating climate change, Thailand has submitted its nationally appropriate mitigation actions (NAMA) in 2014 and the nationally determined contribution (NDC) in 2015, aiming ambitiously to reduce its GHG emissions by 20–25% from the projected business as usual level by 2030 [ONREPP (Office of Natural Resources and Environmental Policy and Planning), 2018]. To ensure the continuity in the mitigation actions from NAMA to NDC, Thailand approved the NDC roadmap on mitigation (2021–2030) in May 2017. One of the on-going activities in the roadmap is the Thai Rice NAMA project that aims at supporting farmers to switch to low-emission farming systems (including the implementation of AWD in the six target provinces included in this study) and estimates the potential emission reduction of 1.664 Mt CO_2-eq cumulative over the 5-years lifespan of the project (NAMA Facility, 2020). The potential of CH_4 emission reduction estimated in this study supports the feasibility of the Thai Rice NAMA project.

Limitations and Outlook

In line with the objectives described above, our study applies the methodology used for the Philippines to assess the climate-based suitability for AWD in paddy fields of Thailand. Therefore, the study may have inherent limitations and possible biases in its assessment results as discussed previously (Nelson et al., 2015; Sander et al., 2017).

One critical factor is the uncertainty in the water balance model resulting from the presence of a compacted "hard-pan" in the sub-surface layer of paddy soils caused by the long-term cultivation of crops. As the sensitivity analysis on Pot_Pc values indicated, ±50 percent changes in the values influenced the distribution of moderate and high suitability areas to some extent. It is necessary for reducing this uncertainty to compile and validate the monitoring data for soil percolation rates in the target area. Possible flood events are another influencing factor, particularly to the target areas in this study. According to the Office of Natural Calamity and Agricultural Risk Prevention of the LDD, Thailand, most of the target areas in this study had repeated flood events of more than three times during the year between 2009 and 2018 [LDD (Land Development Department), 2016]. Such flood events usually occur in the wet major rice season, but big ones such as the case in 2011 may prevent drainage of paddy fields even in the following second rice season.

Another important related aspect appears to be the structure of the irrigation system itself; in particular, the elevation of rice fields relative to the main irrigation canal or within the surrounding terrain. A previous study in Vietnam highlighted that higher-lying paddy fields tend to dry up earlier than lower-lying fields, influencing the suitability for AWD (Yamaguchi et al., 2017). Recent field observations by the Thai Rice NAMA Project in the target provinces of this study appear to confirm these observations: flooding of paddy fields during the wet season of 2019 could have been well-managed and AWD could have been applied if the rice field was located well above the level of the irrigation canal (Atthawit Watcharapongchai, personal observation). Preliminary analysis of CH_4 emissions of demo and control plots in all target provinces suggested that AWD shows good potential for emission reduction in the wet season

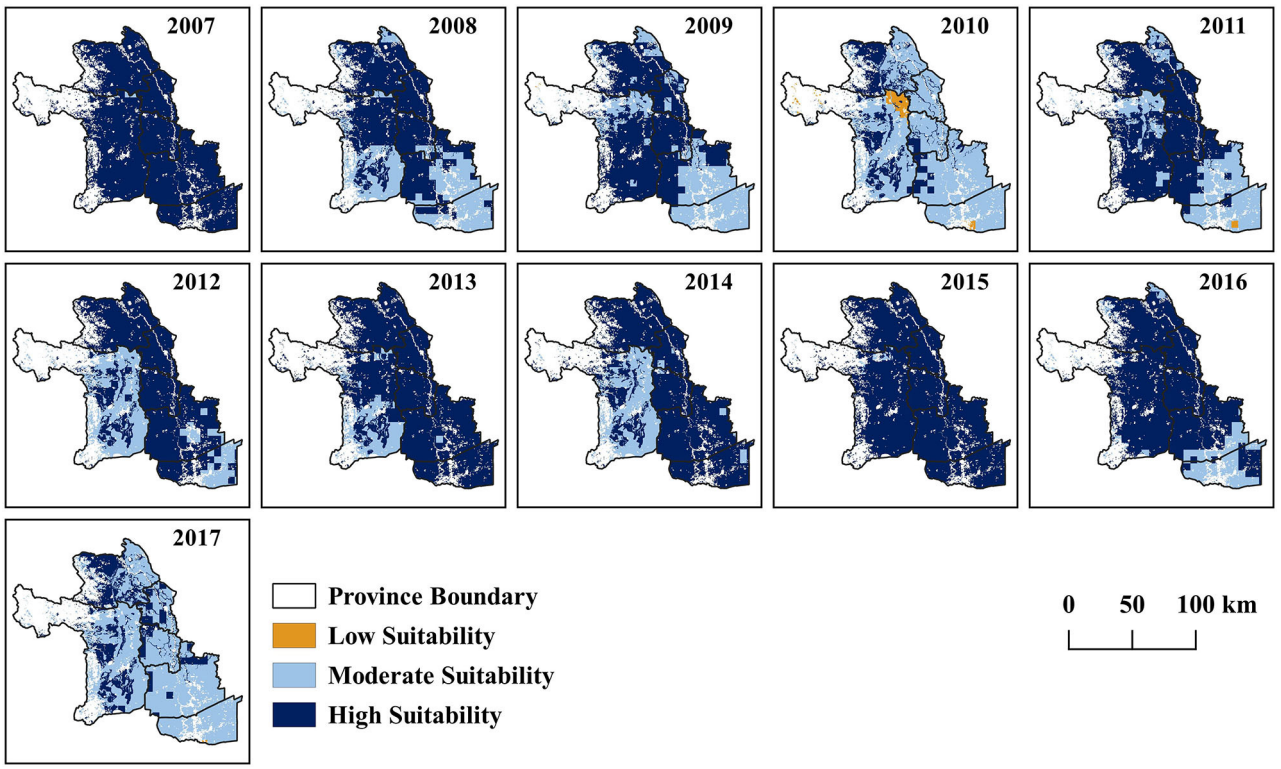

FIGURE 9 | Inter-annual variation of AWD suitability in the major rice season. Colors for each suitability score are same as **Figures 7**, **8**.

TABLE 6 | Statistical summary of the inter-annual variation of AWD suitability assessment.

Year	Major rice season						Second rice season					
	Low		Moderate		High		Low		Moderate		High	
	1,000 ha	(%)	1,000 ha	(%)	1,000 ha	(%)	1,000 ha	(%)	1,000 ha	(%)	1,000 ha	(%)
Average	2	(0)	177	(30)	406	(69)	0	(0)	0	(0)	464	(100)
Standard deviation	5	(1)	146	(25)	148	(25)	0	(0)	0	(0)	464	(100)
Maximum	16	(3)	431	(74)	581	(99)	0	(0)	0	(0)	464	(100)
Minimum	0	(0)	3	(1)	137	(23)	0	(0)	0	(0)	464	(100)

Basic settings for soil percolation rates and breakpoint were used for the analysis.

TABLE 7 | Estimated CH_4 emissions in the baseline and AWD adoption scenarios.

Suitability	Estimated CH_4 emissions (1000t CH_4)											
	Major rice season				Second rice season				Total			
	Baseline	AWD	Reduction	(%)	Baseline	AWD	Reduction	(%)	Baseline	AWD	Reduction	(%)
High	79.2	60.2	19.0	(24)	82.9	48.9	37.9	(41)	162.1	109.1	53.0	(33)
Moderate	19.2	14.6	4.6	(24)	0.0	0.0	0.0	(0)	19.2	14.6	4.6	(24)
Low	0.0	0.0	0.0	(0)	0.0	0.0	0.0	(0)	0.0	0.0	0.0	(0)
Total	98.3	74.7	23.6	(24)	82.9	48.9	34.0	(41)	181.2	123.6	57.6	(32)

Basic settings for potential soil percolation rates (Pot_Pc) and breakpoint were used for the analysis.

(T.J., unpublished results). In view of these observations, the information obtained by the climatic suitability assessment could be especially useful for water management practitioners and irrigation authorities and for the future improvement of the water management system.

Socioeconomic factors for the adoption of water management by farmers may influence the suitability for AWD significantly, as shown by this study which made the assessment based on natural factors that influence the water balance of paddy fields. Water management options by farmers within a wider irrigation system are characterized by the physical separation of adopter and benefiter as pointed out by Sander et al. (2015). Also, the option for AWD is not attractive for farmers who pay a fixed irrigation fee for each season. On the other hand, the result of the suitability assessment in this study can be used for changing rice cultivation management systems to promote effective AWD. As indicated, the number of dekads in the DEF and EXC criteria in a rice cultivation season affected the climatic suitability for AWD. Therefore, if farmers choose the period of rice planting to avoid the DEF periods, for example, earlier major rice planting in Suphan Buri province, they would have more chances to implement AWD in their rice fields. The water balance maps can help farmers to decide whether or not to implement AWD with the support of extension services and when and where to disseminate water-saving technologies.

Regarding the potential of GHG emission reduction, this study focused only on CH_4, because it is the dominant GHG that contributes to the net global warming potential (GWP) emitted from paddy fields. However, it is pointed out that the drainage of paddy fields may promote nitrous oxide (N_2O) emissions. This increase, however, only offsets a small amount of the reduction in CH_4 (Akiyama et al., 2005; Sander et al., 2015, 2020). Nevertheless, the change of N_2O emissions by applying AWD should be additionally considered for an overall assessment of GHG emission reduction, although its contribution to net GWP is much smaller and more variable than that of CH_4 (Yagi et al., 2020).

It is also noted that AWD and other water management options can be combined with other GHG mitigation options for enhancing GHG emission reduction. As indicated by Yagi et al. (2020), some other options like the application of biochar and sulfate-containing fertilizer can reduce GHG emissions while increasing yield in some rice-growing areas in Southeast Asia. These options can be applied to rice cultivation in the target provinces together with AWD. Another effective option is soil drying in the fallow season (Sander et al., 2014) which would be promising by using the dekadal scoring maps for water balance (**Figure 6**) for the decision of the timing of soil drying before starting rice cultivation.

Because this study focused on six adjacent provinces in the central plain, spatial differences in the climatic suitability for AWD among the provinces were not much significant. However, the climatic suitability and resulting estimates for the potential of CH_4 reduction may have a larger effect in other regions of Thailand due to the differences in climate and soil properties among the regions. Therefore, it is encouraged to extend the assessment made by this study over the country to help in formulating national policies for climate change actions.

CONCLUSIONS

This is the first GIS-based study on the mitigation potential of a technology that has been assessed and quantified with regards to GHG emissions in rice cultivation in Thailand. The results of this suitability assessment may guide future research into other aspects of AWD suitability or provide useful technical information for practitioners of water management. We hope to inform the dissemination process of AWD and other forms of water-saving techniques in the region in pursuit of switching to low-emission rice farming. Finally, the methodologies presented here could be applied to other regions of Thailand to help in formulating national policies for reducing CH_4 emissions and other climate change actions.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

KY, RW, AC, and ST designed the research. PP and JA conducted GIS analysis. PP, KY, JA, BS, RW, and CB interpreted GIS analysis. PP and KY had primary responsibility for writing the paper, with significant contributions to the Results and Discussion section from BS, RW, TJ, and AC. All authors reviewed and approved the final manuscript.

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Apparent Nitrogen Recovery in Milk and Early Dry Season Nitrous Oxide Emission Factors for Urine Deposited by Dual-Purpose Cattle on Different Soil Types

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Pasture conditions influence the nutrients use efficiency and nitrogen (N) losses from deposited excreta. Part of the N is lost as nitrous oxide (N_2O), a potent greenhouse gas. The objective of this study was to characterize apparent N recovery in milk of dual-purpose cattle and to quantify N_2O emissions from the urine they deposit following grazing on *Megathyrsus maximus* cv. Mombasa. The N content in the grass and the milk produced by the cattle and the milk urea N (MUN) content were quantified in two contrasting regions of Colombia (Casanare and Atlántico). Dry matter intake (DMI) by the cattle was estimated using the Cornell Net Carbohydrate and Protein System. We used a closed static chamber technique to measure N_2O emissions from soils in areas with and without urine patches (21 days in Atlántico and 35 Days in Casanare). Estimated DMI values were 11.5 and 11.6 kg DM day^{-1}, milk production was 6.5 and 5.9 L day^{-1}, apparent N recovery in milk was 24 and 23%, and the MUN content was 4.4 and 17.2 mg N dl^{-1} in Casanare and Atlántico, respectively. N applied to soil in the form of urine corresponded at rates of 20 and 64 g N m^{-2} and net cumulative N_2O emissions were 350 and 20 mg N_2O-N m^{-2} in Casanare and Atlántico, respectively. Despite low digestibility of offered diet, N recovery in milk was above the values reported at dairy cattle in tropical conditions. High urine-N inputs at Atlántico site did not result in high N_2O emissions suggesting that the default Tier 1 emission factor (EF) which is based on N inputs would have overestimated urine-based N_2O emissions in Atlántico. Comparing previous studies conducted in Colombia, we observed inter-regional differences by urine-based N_2O emissions. This observation suggests that to increase certainty in estimating urine-based N_2O emissions, Colombia needs to move toward more region-specific Tier 2 EF and reduce its dependence on the default IPCC Tier 1 EF. In addition, the adoption of Tier 2 EF in the cattle sector will facilitate accounting for the effect of animal diets on N_2O inventories.

Keywords: cattle systems, milk urea nitrogen, cattle urine-based nitrogen, *megathyrsus maximus*, greenhous gases

INTRODUCTION

Cattle production occupies 37 million ha and represents a major land-use option, which is a source of income and livelihood for small- to large-scale farmers in Colombia (FEDEGAN, 2018). In the case of Colombia's national greenhouse gas (GHG) inventories, total emissions are estimated to be 214 M tons CO_2-eq of which the AFOLU sector is the main emitter that is responsible for 55% of these GHG emissions (IDEAM, 2018). Despite the significant contribution of the cattle sector to Colombia's national GHG emissions, the country still relies on the IPCC's global Tier 1 emission factors (EF) to estimate nitrous oxide (N_2O) emissions from excreta deposited on pastures during cattle grazing.

Colombia's national cattle herd is estimated at 27 million animals (ICA, 2019), 41.4% dedicated to breeding, 37.3% to the dual-purpose (beef and milk) production, 21.2% to solely beef production, and 0.1% to milk production only (FEDEGAN, 2018). The cattle production systems are mainly extensively grazed systems based on native and naturalized pastures that are characterized by drastically low biomass production during the dry season (Mahecha et al., 2002). Extensive grazing systems are associated with low rates of live-weight gain and low milk outputs, which translate to lower profitability for livestock farmers (FAO, 2013). Since pasture management is generally absent in extensively grazed pastures, overgrazing, and signs of pasture degradation are common occurrences (Murgueitio and Ibrahim, 2001; Bacab et al., 2013). Animal excreta (i.e., urine) are randomly deposited in extensively grazed pastures, resulting in the formation of patches where N turnover and potential losses are high.

The loss of N from urine patches as NH_3 or N_2O depends on the pasture type, which influences N uptake, the urine composition, especially the amounts of excreted N (Voglmeier et al., 2018), which depends on efficiencies in the use of dietary N (Lessa et al., 2014; Rivera et al., 2019a). Part of the dietary N is retained in milk and other part is excreted in urine and dung. The amounts on N retained in milk depend on N intake; in systems characterized by high N intake the percentage of retained N in milk relative to the total consumed is low (Correa et al., 2012). Therefore, inefficiencies in apparent N recovery in milk will result in higher amounts of N being lost in urine and dung (Dijkstra et al., 2011). According to the IPCC (2019), N losses from excreta are between 40 and 70 kg of N $head^{-1}$ $year^{-1}$ for cattle, and the N_2O-N EF for excreted N is correspondingly 0.2 and 0.6% under dry (<1000 mm rainfall) and wet (>1000 mm rainfall) climates. Cattle urine is a source highly soluble N, that stimulates nitrification processes and their subsequent transformation into N_2O (Sordi et al., 2014). At the same time, it is also a volatile source of N depending on the ambient temperature and soil texture (Oenema et al., 2005). Several previous studies conducted in Latin America show considerable variations in N_2O emissions from urine patches ranging between 0.7 and 1.16% (Kelliher et al., 2014; Lessa et al., 2014; Byrnes et al., 2017; Chirinda et al., 2019). However, in most of these studies, the peak emissions were reported to occur within the first 10–20 days after urine application and the emissions usually extend up to 30 days post-urine application. The pattern of urine-based N_2O emissions is mainly regulated by microbial activity, mineral N, and oxygen dynamics (Rubol et al., 2013; García et al., 2014). Soil moisture is also a determining factor in the activity of soil nitrifying microorganisms (Oenema et al., 2005; Du et al., 2008; Laudone et al., 2011; Laville et al., 2011; Alves et al., 2012; Li et al., 2015; Marsden et al., 2016). In a study conducted under temperate conditions, Bell et al. (2015) reported that high soil moisture resulted in increased denitrification rates, and elevated N_2O emissions from deposited animal excreta.

Globally, the general aims of the current forage genetic improvement programmes are to increase the quantity and quality of forage production and, consequently, increase cattle productivity in different agro-ecological regions. An example is *Megathyrsus maximus (M. maximus)*, which produces between 20 and 30 t of DM ha^{-1} per year, has a protein level that fluctuates between 10 and 14% and a digestibility level between 60 and 70% (Arango et al., 2016). *M. maximus* has the potential to adapt to climate change, as it is a versatile pasture that adapts to intensive grazing and can be subjected to forage conservation processes in times of intense rainfall or prolonged periods of drought. However, information on greenhouse gas emissions in urine patches and bovine manure associated with this pasture is scant. Several studies have demonstrated the potential of *M. maximus* to increase dry matter consumption and increase animal productivity (Mahecha et al., 2007; Suárez et al., 2011; Gaviria et al., 2012; Rivera et al., 2019b). However, *M. maximus* is produced optimally under rotational compared to continuous grazing as, under the latter, the levels of insoluble fiber (lignin) can dramatically increase. An increase in the fiber content of grass reduces its digestibility and increases the amounts of excreted N in urine and manure (Barahona and Sanchez, 2005). A recent study (Villegas et al., 2020) showed that *M. maximus* has the potential to reduce nitrification rates by 50% compared to bare soil under controlled conditions. This finding suggests that *M. maximus* has the potential to increase cattle productivity and reduce the environmental impact of waste from animal production.

Due to the lack of data to generate local EF, Colombia relies on the IPCC's Tier 1 N_2O-N EF to quantify emissions in this key emissions category. Regional differences in offered diets, plant N uptake rates, edaphic factors, and climatic conditions (Bell et al., 2015) generate variations in the primary drivers of soil N_2O emissions. The determination of national N_2O-N EF is required to advance toward the Tier 2 method of N_2O emission estimation. We hypothesized that urine deposited on grazed pastures in the wetter Casanare region will have higher cattle urine-based N_2O emissions compared to urine deposited on pastures in the drier Atlántico region. We also hypothesized that the low quality of the diet offered in dual-purpose production systems results in low concentrations of N in the urine of grazing cattle.

MATERIALS AND METHODS

Study Sites

This study was conducted on two farms located in the Casanare (5°12'43"N−72°20'19.6"W 350 m altitude) and Atlántico (10°45'04.95"N−74°55'06.96"W 123 m altitude) regions of

Colombia. According to the Köppen and Geiger classification (Kottek et al., 2006), the Atlántico and Casanare localities, correspondingly have Aw–Equatorial climate with dry winter and Am–tropical monsoon climatic conditions (Climate-data.org., 2008a). These regions are two of the most important cattle-producing regions of Colombia. The Atlántico has an average relative humidity of 78%, a mean air temperature of 27.4°C and a mean annual precipitation of 1,074 mm (Climate-data.org., 2008a). While, the Casanare region has an average relative humidity of 66%, a mean air temperature of 26.3°C, and a mean annual precipitation of 3,009 mm (Climate-data.org., 2008b). In 2015, when the current study was conducted, the Atlántico region experienced a severe drought attributed to the "El Niño" climate phenomenon (IDEAM, 2016), (IDEAM. Code: 29040020-Montebello Baranoa-Atlántico). During the measurement period, rainfall data was obtained from direct measurements conducted at the sites or from weather stations of the IDEAM Institute of Hydrology (Taluma site: code 35010010-Puerto López; Patía site: 21050220-San Luis).

Soil Properties

According to Gardi et al. (2014), the soils corresponding to the study sites are classified as Cambisols. Specifically, soils at the Atlántico site are classified as Vertic-Cambisols (CMVr) and those at Casanare are classified as Ferralic-Cambisols (CMFI).

At both sites, soil samples were collected at 0–20 cm depths and characterized for soil pH in 1:1 soil: water solutions, bulk density using a ring of known volume (5cm high × 5cm diameter), total carbon (C) and N using dry a combustion technique, and soil texture using the hydrometer method (Bouyoucos, 1962) at the analytical laboratory in CIAT. The water-filled pore space (WFPS) was calculated using the gravimetric water content, the soil bulk density, and a particle density of 2.65 Mg m^{-3}.

Characteristics of Animals

In each region, 10 dual-purpose cows with commercial Zebu crosses were selected to be sources of the urine and milk used in the current study. These cows which were not permanently with their calves were in the second phase of lactation (100–200 days post-calving), with an average body weight of 475 ± 7.2 and 445 ± 4.6 kg in Casanare and Atlántico sites, respectively.

Dry Matter Intake

Dry matter intake of the cows in each region was estimated using the Cornell Net Protein and Carbohydrate System-CNCPS® version 6.0 model (Tylutki et al., 2008), which estimates beef and dairy cattle requirements and nutrient supply for different animal types under specific environmental conditions (i.e., climatic factors), management, and feeding regimes, through the computational engine. Cattle in Casanare were fed with tropical grass *M. maximus* cv. Mombasa (CIAT 6962), and those at the Atlántico site also fed on *M. maximus* cv. Mombasa (CIAT 6962) and a supplement that was based on barley, wheat, and corn grains and palm kernel cake. The supplement that was used at the Atlántico site was offered at a rate of 1 kg $animal^{-1}$ day^{-1}.

Chemical Composition and Digestibility of Pastures

For the collection of forage samples, the methodology described by Haydock and Shaw (1975) was used. In this method three reference points were established within the grassland. The first point corresponded to an area (0.5 m^2 quadrats) where there was the lowest level of forage (quantity and height), point two was an area with intermediate forage production and point three was an area with the highest amount of forage. The grass samples were collected at the beginning of the experiment, before the animals accessed the grassland. Samples from the three reference points were mixed and the homogeneous sample was taken to the laboratory for the characterization of its chemical composition. The *M. maximus* pasture was harvested at 55 and 45 days of regrowth at the Casanare and Atlántico site, respectively. This regrowth age corresponds to the rest period of the pasture determined by the farmers in each study region, based on local experience with variables such as soil fertility, cattle stocking rate, and environmental parameters such as precipitation and temperature. At the Casanare and Atlántico sites, the pastures had an average height of 107 and 93 cm, respectively. To simulate grazing events pastures were cut at height of 30 cm from the ground.

For collected grasses were characterized for different nutritional quality parameters. Specifically, N content was quantified using the Kjeldahl method [Kjeldahl AN 3001 FOSS Association of Official Analytical Chemists (AOAC, 1990: method 984.14)] and the amount of N obtained from this analysis was converted to crude protein (CP) content, using the following equation: CP = N × 6.25; neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined as proposed by Van Soest et al. (1991) adapted to an Ankom Fiber Analyzer AN 3805 (Ankom® Technology Corp USA). Forage digestibility was determined as described by Goering and Van Soest (1970). The insoluble protein in neutral and acid detergent was determined through the N content in the residue of the NDF and ADF as described by Goering et al. (1972). Dry matter was determined through the thermogravimetric method by drying at 60°C for 72 h in a forced ventilation oven. These analyses were conducted at the Forage Quality and Animal Nutrition Laboratory of the International Center for Tropical Agriculture (CIAT).

Chemical Composition of Milk

At both study sites, we collected 50 ml milk samples from each of the 10 cows once every 8 days, for a period of 35 days. The milk was characterized for N content using the Kjeldahl method, CP was calculated using the following equation: CP = N × 6.38, and milk urea nitrogen (MUN) content was determined using Infrared Spectrometry (MilkoScan, ISO-9622 standard method) (Milk I. S. O., 2013) . Apparent nitrogen recovery in milk was estimated from the efficiency of incorporation of consumed N in

milk using the relationship described by Van Horn et al. (1994) which we expressed follow (Equation 1).

$$Apparent\ nitrogen\ recovery\ in\ milk\ (\%) = \frac{Amount\ of\ N\ in\ milk}{Total\ N\ intake} x\ 100 \quad (1)$$

Experimental Set-Up for Soil N_2O Measurements

At both sites, a representative area of 160 m^2 was selected to conduct GHG measurements. In line with common farmer practice, no fertilizer was applied to the selected areas in recent years. To simulate grazing, grass in each plot was cut to ∼30 cm sward height, seven days before the initial collection of gas and soil samples. In both regions, urine samples were collected through stimulation of the vulva zone from 10 dual-purpose cows. The samples obtained from each cow were immediately mixed to obtain a homogeneous sample. Urine sub-samples (500 mL) were immediately applied uniformly into the respective chambers (isolated area 0.5 m^2). Cylindrical PVC static chamber bases with a 26-cm internal diameter and 10 cm height demarcated the area and the insertion depth was 5 cm. The urine-N application rates were 20 and 64 g N m^{-2} at the Casanare and Atlántico, respectively. These rates correspond to the N concentrations in urine resulting from different diets consumed at the two sites. At both study sites eight bases were used. Four bases with urine and four bases without urine as control treatments, therefore eight bases were installed in the soil at each site. The chamber bases were distributed in a completely randomized design with four replicates that were established on land with a slope ∼2%.

On the different gas sampling dates, chamber tops with a 25-cm height were connected to the chamber bases and sealed with a thick custom-made rubber band to prevent gas leakage during the sampling period (Alves et al., 2012). Gas samples were collected from 9:00 to 10:00 am as described by Byrnes et al. (2017). The first gas sample was taken immediately after connecting the chamber top and the chamber base, subsequent samples were taken every 15 min resulting in sampling times of 0, 15, 30, and 45 min after chamber closure. Based on dynamic of urine-based N_2O emissions reported by several studies carried out in Latin America (Kelliher et al., 2014; Lessa et al., 2014; Byrnes et al., 2017; Chirinda et al., 2019), in our study, the gas samples were collected on five consecutive days following urine application followed by 3–4 additional sampling campaigns within the 35-day monitoring period. Due to logistical challenges we were unable to conduct the gas monitoring during the planned 35 day period at the Atlántico site. We were only able to conduct measurements over 21 days.

The N_2O concentrations were analyzed by gas chromatography (GC–Shimadzu 2014). The daily gas fluxes were calculated by regressing N_2O concentrations with the corresponding sampling times and correcting for temperature and pressure. All flux data were checked for linearity by visual inspection during data analysis. Cumulative fluxes were calculated from mean N_2O emissions by interpolation between measurement days (Dobbie et al., 1999). Specifically, we determined the mean N_2O emissions between two consecutive sampling days and them the sum of the calculated averages for the monitoring period. The cumulative N_2O-N EF for the Atlántico and Casanare sites were correspondingly calculated for periods of 21 and 35 days, using the following Equation:

$$EF\ (\%) = \frac{(N2O - Nemitted\ from\ urine\ treatment) - (N2O - Ncontrol)}{N\ applied} x\ 100 \quad (2)$$

At each site, a sub-sample of urine (50 mL) was acidified with 0.5 mL of 1 M sulphuric acid and frozen at −20°C to avoid N loss before analysis. Total N in the urine was quantified using the Kjeldahl method [Kjeldahl AN 3001 FOSS; Association of Official Analytical Chemists (AOAC, 1990: method 984.14)]. Urine N contents were used in the calculation of N_2O EF (Equation 2).

Statistical Analyses

A completely randomized design was used, with four repetitions, where the experimental unit corresponded to the static chamber, where there were two treatments equivalent to soil with urine and without urine. Statistical analyses were conducted using generalized linear model (GLM) procedure of the statistical Software Statistical Analysis Systems (SAS ®, version 9,4) (SAS Institute, 2012). In the GLM model the response of cumulative N_2O emissions to urine application was tested. Relationships between N_2O emission and percentage of water-filled pore space (WFPS) as well as N_2O emissions and rainfall were explored. The PROC REG procedure was used for regression analyses. The figures were constructed with python 2.7.14, Numpy 1.18.1, Scipy 1.4.1, and Motplotlib 3.2.1.

RESULTS

Expected Dry Matter Intake and Feed Quality

Similar levels of dry matter intake were estimated for animals at the Casanare (11.5 kg DM d^{-1}) and Atlántico site (11.6 kg DM d^{-1}). The estimated energy balance from the CNCPS model was 2.44 and 3.34 Mcal in Casanare and Atlántico sites, respectively. The CP content of the *M. maximus* at Casanare and Atlántico site was similar (8%). However, due to the drought experienced at the Atlántico site, the animals received a supplement that had a high CP content (21%) (**Table 1**), which increased the amount of N intake around 20% by the animals at this site (**Table 2**). At both study sites, pasture digestibility was low (<50%) and NDF was high (>70%).

Apparent Nitrogen Recovery in Milk and N Content in Urine

The 8-day average of N content measured in milk was similar at the Casanare and Atlántico sites. The relationship between milk N/ intake N showed that both regions had optimal apparent N recovery in milk values. The MUN concentrations of animals at the Atlántico site was above the range suggested by Peña (2002) and Cerón et al. (2014) (>16–18 mg dl^{-1}). While at the Casanare

TABLE 1 | Chemical composition of pastures and feed supplements collected in Casanare and Atlántico localities.

Parameter	Casanare	Atlántico	Atlántico
	Megathyrsus maximus		*Feed supplement*
IVDMD (%)	42.8	47.7	83
Protein (%)	8.00	8.12	21.0
NDF (%)	76.6	71.7	14.9
ADF (%)	48.3	37.1	28.7
Crude fat (%)	1.4	1.6	2.0
Ash (%)	10.0	9.1	9.8
Moisture (%)	84.5	85.0	10.3
Lignin (%)	3.2	5.0	-
ADIP (%)	-	<1.4	-
NIDP (%)	2.75	4.1	-

NDF, Neutral detergent fiber; ADF, Acid detergent fiber; ADIP, Acid detergent indigestible protein; NIDP, Neutral detergent indigestible protein; ME, metabolizable energy; IVDMD, In vitro digestibility of dry matter.

TABLE 2 | Nitrogen intake, apparent nitrogen recovery in milk, and nitrogen content in urine and dung in dual-purpose cows in Casanare and Atlántico regions of Colombia.

Parameter	Casanare	Atlántico
Nutrient intake		
Predicted DMI (kg cow^{-1} d^{-1})	11.5 ± 0.08	11.6* ± 0.11
N intake (g N d^{-1})	147 ± 1.0	171 ± 1.44
Diet energy contents		
Predicted ME (Mcal kg DM^{-1})	2.07	1.93
Balance ME (Mcal d^{-1})	2.44 ± 0.13	3.34 ± 0.16
Nitrogen output and retention		
Milk production (L d^{-1})	5.9 ± 0.1	6.5 ± 0.1
Milk CP (g d^{-1})	226 ± 1.7	246 ± 1.5
MUN (mg N dl^{-1})	4.37 ± 3.4	17.16 ± 2.4
Milk N (g N d^{-1})	35.4 ± 0.26	39 ± 0.23
Apparent N recovery in milk (%)	24	23
Total urine N output (g L^{-1})	2.1 ± 0.1	6.8 ± 0.3
Total dung N output (g kg^{-1})	22 ± 2.1	±1.3

**The dry matter intake in the Caribe site corresponds to the sum of the dry matter intake predicted by the CNCPS and the amount of supplement offered to the cows. ME = predicted metabolizable energy. Values are means ± S.E n = 10.*

site, the mean MUN concentration of animals was below the threshold values (<12 mg dl^{-1}). The N content in urine at the Atlántico site was over three-fold higher than values reported at the Casanare site (**Table 2**).

Soil Properties

Soil pH was 5.2 and 6.7, bulk density was 1.3 and 1.4 g cm^{-3}, and soil texture was clayey and loamy in the Casanare and Atlántico sites, respectively. Total N and total C content were similar at both sites (0.11 and 1.1%, respectively).

Nitrous Oxide Emissions

At both study sites, the N_2O emission peaks were observed 3 days after the urine application, which also coincided with precipitation events (**Figure 1**). The application of urine in both regions generated an increase in the cumulative N_2O fluxes compared to soil without urine (389 mg N_2O m^{-2}, $p < 0.0001$ and 27.6 mg N_2O m^{-2}, $p < 0.0002$ for Casanare and Atlántico sites, respectively) (**Figure 1**). A positive exponential relationship between N_2O fluxes and %WFPS was found in the Casanare site ($p = 0.027$ $R^2 = 0.45$). While in the Atlántico site this relationship was not significant ($p = 0.176$ $R^2 = 0.19$).

N_2O-N Emitted From Urine Treatment

Overall, the different EF calculated in the present study and those from other studies conducted in Colombia showed variations in EF under different rainfall conditions (**Table 3**). Taking into account the results of this study and additional data available for Colombia (**Table 3**), we find evidence for a positive, non-linear relationship between rainfall and EF ($p < 0.0001$; $R^2 = 0.95$) (**Figure 2**).

DISCUSSION

It is important to note that this study was conducted in an El niño year which was characterized by low rainfall. The unique climatic conditions associated with the El niño phenomenon influenced feed availability resulting in a need to supplement the grazing systems in the drier Atlántico region. Despite the fact that the El niño phenomenon was experienced across the country, differences in feed composition and rainfall intensities observed at the two study sites appear to have influenced the observed N_2O emission patterns. The N concentration in applied urine would certainly influence N_2O emissions, but calculating emission factor enabled us to normalize for N content. Another factor that may have affected our cumulative N_2O emissions is the fact that due to logistical challenges we were unable to conduct N_2O monitoring for the same period at the two study sites. However, since most of the peak emissions in the current and previous studies were reported to occur within the first 10–20 days (Kelliher et al., 2014; Lessa et al., 2014; Byrnes et al., 2017; Chirinda et al., 2019), our monitoring period allowed us to gain important insights on N_2O emission patterns during this short-term monitoring period.

Expected Dry Matter Intake and N Intake

At both Casanare and Atlántico, the DM intake was similar. The similarity in DM intake may have been due to the similar NDF contents in the diet, a major determinant of intake in grazing cattle (Barahona and Sanchez, 2005). The digestibility values suggest that DM intake reported in this study is high, which may be due to a selectivity response of the animals, given the height of the forage that in both regions was >90 cm and the cutting height of the forage for the chemical composition analysis was 30 cm. The estimated N intake by cattle at both study sites (147 and 171 g N day^{-1} at the Casanare and Atlántico, respectively) were within the range frequently observed for open-grazing tropical cattle (40–170 g N day^{-1}) (Cole and Todd, 2008; Nha et al., 2008).

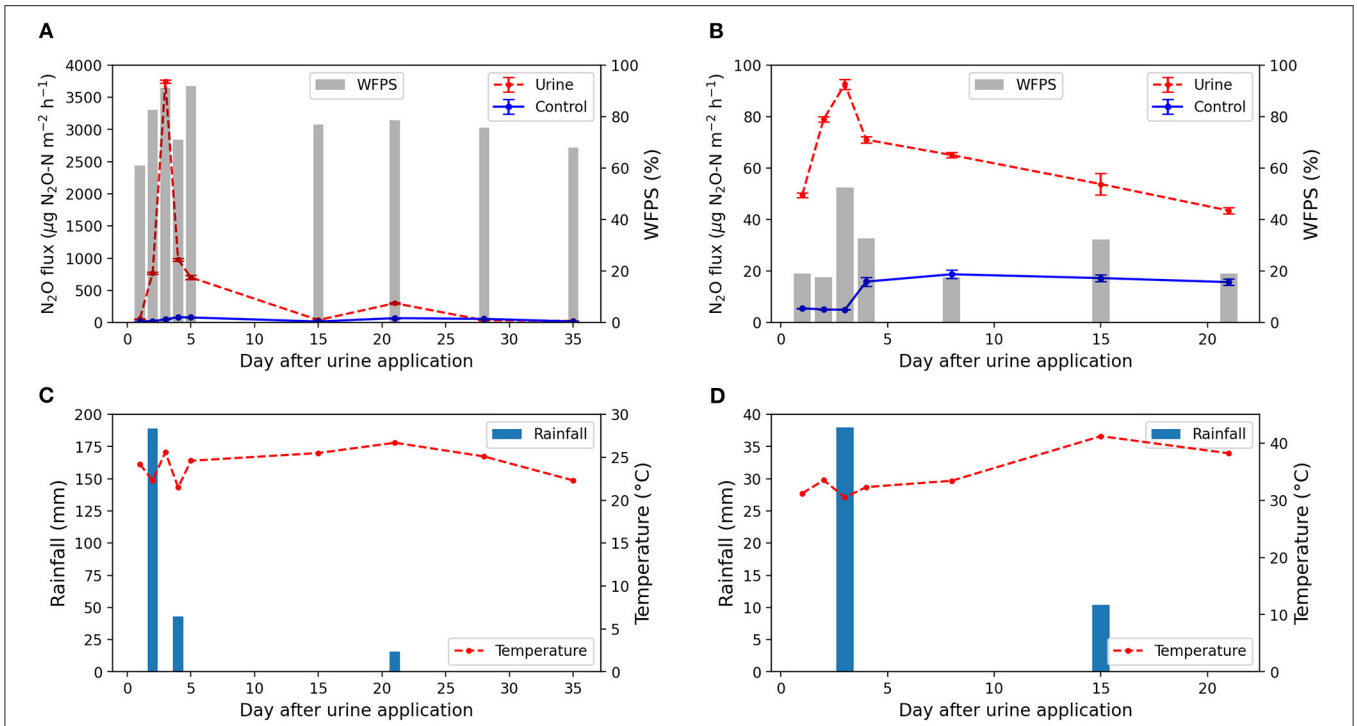

FIGURE 1 | Soil nitrous oxide emission following urine application and percent water-filled pore space saturation (WFPS) (**A**-Casanare and **B**- Atlántico), and Rainfall and temperature (**C**-Casanare and **D**-Atlántico). Solid arrows indicate time of urine application. Vertical bars in **(A)** and **(B)** show the standard error ($n = 4$).

TABLE 3 | Soil urine derived N_2O-N EF for studies conducted at different sites in Colombia.

Region	Forage type	Nitrogen in applied urine (g N m^{-2})	EF (%)	Rainfall (mm)	Reference
Atlántico	*Megathyrsus maximus* + feed supplement	69.3	0.03	48.4	Current study
Casanare	*Megathyrsus maximus*	21.4	1.76	248.3	Current study
Meta	*Brachiaria humidicola*	46.3	0.02	36	Durango (unpublished)
Meta	*Brachiaria humidicola* + *Arachis pintoi*	40	0.05	127.4	Durango (unpublished)
Valle del Cauca	*Brachiaria hybrid cv Cayman*	65.3	0.05	41	Durango (unpublished)
Valle del Cauca	*Brachiaria hybrid cv Cayman* + *Leucaena leucocephala*	128.5	0.17	41	Durango unpublished)
Valle del Cauca	*Brachiaria hybrid cv. Mulato*	123	0.07	4.6	Byrnes et al., 2017
Valle del Cauca	*Brachiaria humidicola cv.Tully*	123	0.00002	4.6	Byrnes et al., 2017
Patía	*Brachiaria hybrid Mulato II, Brachiaria brizantha and Megathyrsus maximus*	78.9	0.28 0.24	83 83	Chirinda et al., 2019 Chirinda et al., 2019
Patía	*Canavalia brasiliensis and Dichanthium aristatum*	78.9	0.164	31	Chirinda et al., 2019
Meta	*Brachiaria humidicola*	11.2	0.02	7	Chirinda et al., 2019
Meta	*Brachiaria humidicola*	11.2	0.01	7	Chirinda et al., 2019

Higher N intakes (>300 g d^{-1}) have been reported by other authors in cattle fed on fertilized pastures and feed supplements (Correa et al., 2012; Dickhoefer et al., 2018; Castro et al., 2019). Pastures in the tropics are typically characterized by low digestible protein and high fiber content (Mahecha and Rosales, 2005), partly due to poor agronomic management (Pelster et al.,

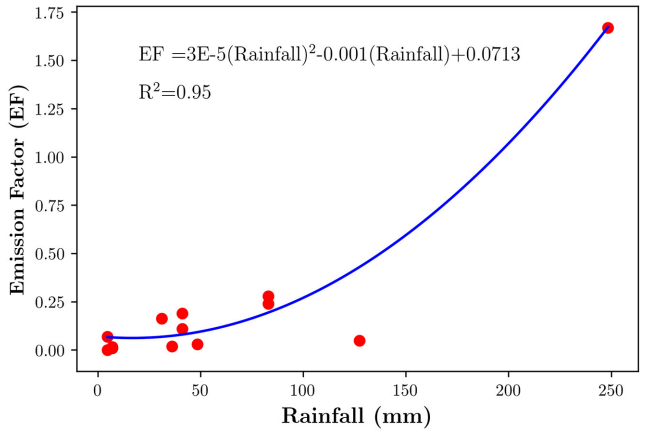

FIGURE 2 | Relationship between EF and rainfall obtained using Byrnes et al. (2017), Chirinda et al. (2019), Durango (unpublished) data and measurements conducted in the current study. At each site, rainfall data includes recordings of precipitation events from 8 days before greenhouse gas measurement commenced to the end at the gas measurement period. The model was chosen based on statistical fit (R^2).

2016), and this may be corrected through the use of protein supplementation. However, the low dry matter digestibility of pastures can exacerbate nutritional imbalance resulting in greater excretion of nutrients (Broderick, 2003; Barahona and Sanchez, 2005). Under the pasture conditions in our study, moderate N intake can be considered an advantage over excess protein in relation to the available energy that commonly occurs in tropical diets and can have a positive impact on N inputs to the soil, since according to Dijkstra et al. (2013), lowering intakes of CP is a strategy effective in reducing total and especially urinary N excretion.

Apparent Nitrogen Recovery in Milk

The MUN results reported for the Casanare site (4.37 mg dl^{-1}) suggest that there was an inadequate amount of degradable protein ingested and that soluble carbohydrates may not have been balanced in the rumen. As MUN is the result of the diffusion of urea from the blood serum in the secretory cells of the mammary gland, MUN values are indicative of excess or deficiency of rumen available soluble carbohydrates, relative to dietary N (Peña, 2002). Conversely, at the Atlántico site, where cows received supplementation with a protein source, MUN values were above the range frequently observed in lactating cows under tropical conditions (Acosta and Delucchi, 2002; Peña, 2002). However, the MUN value reported for Atlántico does not suggest an excess of protein in the diet in comparison to the 10 and 19 mg dl^{-1} range reported by Hess et al. (1999), for dual-purpose cows in Colombia. Further research is required to understand the metabolic processes associated with the increased MUN observed at the Atlántico site. At both sites, the pastures contributed low amounts of N to the animal diets, which probably led to the inadequate synthesis of microbial protein, which in turn is the main metabolizable protein source for the animal (Agricultural Food Research Council, 1992; Ma et al., 2010).

Based on the average milk protein content observed in the current study, The amount of N retained from the total N ingested in the diet is on average 24 and 23% for at the Casanare and Atlántico, respectively, while 76 and 77% is excreted by feces and urine. This is consistent with the values reported by Colmenero and Broderick (2006) using multiparous cows 120 days post-calving, receiving low N diets. Correa et al. (2012) in a study carried out in Colombia with dairy cows, reported an apparent recovery of N in milk of 20% under following N intake of 389 g d^{-1}. According to Steinshamn et al. (2006), the higher the N intake in the diet, the greater the excretion of N through the urine and the lower the percentage retained. León et al. (2008), reported N retention percentages in milk of 15.6% with respect to N intake of 667 g d^{-1}. The N intake at both study sites was much lower than was reported by other authors. Consequently, we have found a higher percentage of N retained. Based on this information, the results found in this study suggest that the moderate protein intake of typical tropical diets such as *M. maximus*, can favor N retention in milk for cows in dual-purpose systems. However, other variables could have influenced the apparent N recovery in milk observed in the current study, such as the number of days since calving and the low level of milk production. In both regions, the cows had passed their peak lactation period, which has a higher energy requirement for milk synthesis. According to Lapierre and Lobley (2001), the recycling of urea N synthesized in the liver can substantially contribute to N availability in the intestines which mainly occurs when sufficient metabolizable energy is available in the diet. Kennedy and Milligan (1980) also indicated that urea transfer to the rumen is inversely proportional to the ammonium concentration in the rumen. It is likely that under these circumstances, the activation of urea N recycling mechanisms toward the rumen was stimulated, which may explain the high apparent nitrogen recovery in milk observed for both regions. In the same sense, the excretion of N in urine in both groups of animals did not exceed the range reported by Bolan et al. (2004) (1–20 g L^{-1}).

Our results suggest that during this physiological stage (∼100–200 days in lactation), the use of protein supplementation, which farmers consider as an important practice, may not have been necessary. This is in agreement with previous reports in the tropics, where energy availability can be a more limiting dietary factor for adequate animal productivity than CP availability (Barahona and Sanchez, 2005). Perhaps nutritional strategies such as reducing the levels of structural carbohydrates (e.g., ADF) in the diet (**Table 1**) through improved grazing management, (e.g., reducing grazing periods in rotational grazing systems) could increase feed digestibility as reported by previous studies evaluating pasture quality at different regrowth stages (Chacón and Vargas, 2009; Valles de la Mora et al., 2016). This would also contribute to an increase of milk yield as well as its compositional quality (Rabelo et al., 2003). These results suggest that with the diets offered in these milk production systems, and with cows in the second stage of lactation, urinary excretions of N were low.

This implies a reduction in the substrate for soil nitrifying and denitrifying microorganisms.

N_2O Fluxes Emissions

The cumulative net N_2O emissions and the transformation rate of urinary N into N_2O-N was very different at the Casanare (350 mg m^{-2}; 1.76% EF) and Atlántico (20 mg m^{-2}, 0.03% EF) sites. This supports the proposal that region-specific EF could greatly improve the accuracy of national GHG inventories. In addition, data generated during the different sampling campaigns aimed at developing region-specific EF could also be used to validate and improve Tier 3 models that are capable of integrating different factors regulating N_2O emissions from urine patches such as the pasture type, climatic and soil conditions (Saggar et al., 2004; Mazzetto et al., 2014). At the Atlántico site, the cumulative N_2O fluxes were lower compared to those reported in other previous studies conducted in the Latin America region [Sordi et al., 2014, (3,198 μg N_2O-N m^{-2} h^{-1}, 1,934 g N m^{-2}); Simon et al., 2018 (3,700 μg N_2O-N m^{-2} h^{-1}, 256 g N m^{-2}); Chirinda et al., 2019 (1,125 μg N_2O-N m^{-2} h^{-1}, 78.9 g N m^{-2})]. Several factors could have led to the lower N_2O peaks including the low N application rate in the urine patched (**Table 3**), low precipitation regime and the low amounts of total carbon and N in the soil.

The peak N_2O emissions observed at the Casanare site (3,745 μg N_2O-N m^{-2} h^{-1}) (**Figure 1A**) were lower than those reported by Byrnes et al. (2017) (25,000 μg N_2O-N m^{-2} h^{-1}) under similar tropical conditions. Rivera et al. (2019a) reported a higher peak N_2O emissions value (9,450 μg N_2O-N m^{-2} h^{-1}) for intensively managed pastures that received external N inputs through chemical fertilization in the Andean region of Colombia. According to Anger et al. (2003), the activity of nitrifying and denitrifying microorganisms is much greater in soils with high N inputs compared to those where N availability is low; the latter was the case in the regions evaluated in the present study where the pastures did not receive N fertilizers prior the study. On the other hand, the high values of bulk density (≥1.3 g cm-3, Jaramillo et al., 2002), suggest lower aeration conditions, which according to Klefoth et al. (2014) reduce the diffusivity of N_2O and increase the probability of its transformation to molecular N or dinitrogen (N_2) possibly explaining the observed low N_2O emission values. Skiba and Ball (2002), reported high N_2O emissions in clay soils that were characterized by poor aeration (high %WFPS) and high bulk densities, conditions similar to those at the Casanare site.

Villegas et al. (2020) reported that the accession *M. maximus* cv. Mombasa can reduce nitrification rates by 50% when compared to a bare-soil control. They suggest that this reduction in nitrification rates is facilitated through the release of enzymatic complexes that inhibit the activity of bacterial nitrifiers. *Brachiaria humidicola* is another forage pasture species that has been reported to reduce soil nitrification by 60% (Subbarao et al., 2009). The ability of forage species to inhibit nitrification represents a potential N_2O reduction mechanism. *Megathyrsus*' ability to inhibit nitrification, could have influenced the low cumulative fluxes observed in the current study.

Although in both regions the evaluation period corresponded to the end of the rainy season, in the Casanare site, a higher rainfall regime was experienced compared to the Atlántico region (**Figures 1C,D**). Consequently, soils at the Atlántico site had low vegetation cover. According to Oenema et al. (2005), between 3 and 15% of N in urine is lost as NH_3 by volatilization, under conditions of high soil compaction, high temperatures, and low moisture; as prevalent at the Atlántico site. Additionally, Voglmeier et al. (2018), found a greater loss of N from urine as NH_3 when the urine-N concentration was higher. These alternative N loss pathways probably resulted in lower amounts of N being available for nitrification and denitrification processes. On the other hand, according to Porre et al. (2016), the connectivity between pores and the size of the pore influence the N_2O diffusivity and ultimate emission to the atmosphere. In our study, the soil texture was different between the sites. Specifically, whereas the Casanare soil had a clay texture, the soil at the Atlántico site was a loam. A clay texture supposes a combination of medium pores and micropores, which favors the water retention in the soil and inhibits the diffusion of O_2 from the atmosphere, creating anaerobic microsites in the soil profile (Porre et al., 2016). While a loam soil would have higher macroporosity, with less moisture retention capacity due to higher sand and silt content and more aerobic conditions. Thus, the more clayey soil and frequent rainfall events at the Casanare site probably resulted in long periods with optimum moisture conditions for microbe-mediated N transformation processes in the deposited urine (Bateman and Baggs, 2005; Laudone et al., 2011; Dijkstra et al., 2013) and induced the higher N_2O emissions observed at this site. This is consistent with the study by Adhikari et al. (2020), who reported less N_2O emissions in soils with coarse (sandy) textures and in soils with free drainage compared to clay soils that were characterized by high bulk densities/compaction and subsequent elevated water-filled pore space. The positive relationship between the N_2O emissions and % WFPS at the Casanare site ($P = 0.027$ $R^2 = 0.45$) further provides evidence for the influence of water in driving the high N_2O emissions observed at the Casanare site (**Figure 1A**). previous studies (Oenema et al., 2005; Laudone et al., 2011; Marsden et al., 2016) also reported the influence of %WFPS on denitrification processes. Specifically, N_2O emissions associated with denitrifier activity are typically highest at values between 60 and 80% WFPS (Sey et al., 2008; Klefoth et al., 2014) with higher moisture contents leading to complete denitrification that produced dinitrogen as the end product.

There was no relationship between % WFPS and N_2O emissions at the Atlántico site ($P = 0.176$ $R^2 = 0.19$). The coarse-textured soil at the Atlántico site was more compacted which probably reduced water infiltration and moisture retention. This low infiltration and water retention rates probably explain the poor relationship between N_2O emissions and % WFPS at the Atlántico site. These findings corroborate with Sordi et al. (2014) who suggested that the % WFPS, does not closely reflect the rain pattern, because the moisture in the

soil depends on the evapotranspiration and the drainage between the rain event and the moment of taking the soil sample.

Based on the information from previous studies [Byrnes et al., 2017; Chirinda et al., 2019; Durango (unpublished)], as well as findings in the current study under tropical conditions, the rate of transformation of N in urine patches to N_2O was found to be mainly influenced by soil moisture, associated with precipitation events (**Figure 2**). The loss N as N_2O is affected by several factors, however, as has been previously discussed, these results suggest that under Colombian tropical condition, higher losses of N, in the form of N_2O, appear to occur during the wet season. This result is consistent with studies where higher emissions have been found following high precipitation events (Bolan et al., 2004). In contrast, Sordi et al. (2014) obtained lower EF during the wet season, which they attributed to the lower temperatures recorded during the study period (13.9°C). However, in the evaluated regions, the average temperatures recorded are above (≥23°) those reported by Sordi et al. (2014).

The N_2O-N EF obtained in the current study, corroborate with those reported in other studies developed under tropical conditions in unfertilized grasslands in Colombia [0.0002–0.471% EF Chirinda et al., 2019) and Brazil (0.2% EF. Barneze et al., 2014); 0.1–1.93% EF Lessa et al., 2014)]. The N_2O-N EF obtained in the Atlántico region (0.03%) was lower than Tier1 EF given by the IPCC (2019) for these climatic regions (0.6% of N in bovine excreta).

CONCLUSIONS

The apparent nitrogen recovery in milk was similar at both sites and despite the low digestibility and high fiber content of the offered diets, the recovery of N in milk was above the values reported at dairy cattle under tropical conditions. The N concentrations in the applied urine were low compared to those reported in previous studies. This suggests that local cattle production systems may be characterized by low urine-N inputs which may imply potential low environmental impacts. On the other hand, the transformation rate of urinary N into N_2O-N differed at the two locations although the same *M. maximus* grass cover was used. Our study provides further evidence on the need to determine site-specific EF as N_2O emissions are affected by multiple biotic and abiotic factors.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study did not require approval by the ethics committee because the animals were not directly intervened, diets were not changed and only external samples such as milk, urine, and dung were taken. None of the activities compromised their animal health and welfare. The measurements carried out can be considered routine and qualified personnel were always present to take samples.

AUTHOR CONTRIBUTIONS

SD conceived and designed the experiments, conducted laboratory analyses, and led the writing of the manuscript. SD, NC, RB, DB, JA, and LV, analyzed the data. All authors provided editorial advice and revised manuscript. All authors contributed to the article and approved the submitted version.

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frontiers in Sustainable Food Systems

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Field Application of Organic Fertilizers Triggers N_2O Emissions From the Soil N Pool as Indicated by ^{15}N-Labeled Digestates

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Anaerobic digestion (AD) can generate biogas while simultaneously producing digestate which can be used as fertilizer. Feedstocks used for AD influence digestate composition, which in turn may affect carbon (C) and nitrogen (N) turn-over in soils and subsequently influence nitrous oxide (N_2O) emissions after soil application. Assessment of greenhouse gas emissions from digestates can help to evaluate the overall sustainability of an agricultural production system. The objective of this study was therefore to evaluate and understand the effect of differences in digestate composition on *in situ* N_2O emissions within the 1st weeks after application of seven digestates. The digestates were derived from different feedstocks and ^{15}N-labeled, either in total N or only in ammonium-N. Therefore, the experimental design enabled us to differentiate between potential N_2O-N sources (i.e., digestate N or soil N). Furthermore, it allowed to distinguish to some extent between organic-N and ammonium-N as potential N sources for denitrification. Digestates were homogeneously incorporated into the upper 5 cm of microplots in an arable Haplic Luvisol in South Germany at a rate of 170 kg N ha^{-1}. After application, N_2O fluxes were measured for ~60 days (May-July) using the closed chamber method in 2 experimental years. Mainly due to higher precipitations in the 1st year, cumulative N_2O emissions were higher (312–1,580 g N_2O-N ha^{-1}) compared to the emissions (133–690 g N_2O-N ha^{-1}) in the 2nd year. Between 16–33% (1st year) and 17–38% (2nd year) of N_2O emissions originated from digestate N, indicating that digestate application triggered N_2O production and release mainly from soil N. This effect was strongest immediately after digestate application. It was concluded that the first (short term) peak in N_2O emissions after digestate application is largely related to denitrification of soil-N. However, the experimental setup does not allow to differentiate between the different denitrification pathways. Weather conditions showed a substantial effect on N_2O emissions, where the correlation between N_2O and CO_2 flux rates hinted on denitrification as main N_2O source. The effect of digestate composition, particularly organic N from the digestate, on soil N_2O emissions seems to be of minor relevance.

Keywords: anaerobic digestion, biogas slurry, organic fertilizer, greenhouse gas emissions, stable isotope, field experiment

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INTRODUCTION

In the EU, about 180 million tons of anaerobic digestate are estimated to be produced per year, most of which is used as organic fertilizer (Corden et al., 2019). Digestates have been shown to have the potential to substitute mineral fertilizers and contribute to a sustainable soil management (Gutser et al., 2005; Cavalli et al., 2016; Verdi et al., 2019). However, application of organic as well as mineral nitrogen (N) fertilizers is also known to increase greenhouse gas (GHG) emissions from soils. Globally, agriculture contributes up to 20% to carbon dioxide equivalents (CO_2-eq.) from all human activities (2010–2017), with nitrous oxide (N_2O) and methane (CH_4) as main GHGs (FAO, 2020). About 60% of anthropogenic N_2O emissions are emitted by agricultural soils (Ciais et al., 2013), thus it is of high relevance to assess N_2O in relation to fertilizer application.

Studies have shown that digestates might lead to a higher risk of N_2O formation than manures, which is related to the higher share of ammonium (NH_4^+-N) after AD (Möller and Stinner, 2009). Ammonium is quickly nitrified to nitrate (NO_3^-), which can further be denitrified to dinitrogen gas (N_2). Both processes, as well as nitrifier denitrification, bear the risk of producing N_2O and are considered as main N_2O source from soils (Granli and Bøckman, 1994; Bremner, 1997; Koola et al., 2010). Application of liquid manures like slurry or digestates provides available N and carbon (C), which in turn promotes heterotrophic activity (oxidation of C, N, S, etc.), depleting oxygen (O_2) availability in soil, and thus favors creation of anaerobic microsites that ultimately trigger N_2O production and release via denitrification (Chadwick et al., 2000; Petersen et al., 2003). Hence, N_2O emissions largely depend on the availability of labile organic C (C_{org}), mineral N, O_2 and water in the soil and their subsequent effect on soil microbial processes (Flessa and Beese, 2000; Ruser et al., 2001). However, AD has also been reported to reduce the N_2O potential compared to the initial feedstock e.g., by decreasing slurry viscosity or increasing the recalcitrance of organic matter (OM) (Petersen, 1999; Möller, 2015).

The different organic substrates that are used as feedstock for anaerobic digestion (AD) affect the physico-chemical characteristics of the digestate (Fouda et al., 2013; Zirkler et al., 2014). For example, comparing food wastes and maize silage, food wastes are already processed goods with a high degradability and high protein content. Thereby, food waste-based digestates tend to have a higher OM degradability and a higher share of NH_4^+-N than maize silage, that could enhance soil microbial activity (Möller and Müller, 2012; Guilayn et al., 2020).

Based on compositional differences, such as N content, C/N ratio and OM degradability [C_{org}/organic N (N_{org})], it can be assumed that digestates from different feedstocks will show differences in N_2O emissions after field application. However, a differentiated consideration of the GHG emission potential for digestates from different feedstocks is currently scarce, and therefore will be the main research focus of this study.

The largest share of N_2O release during the growing season usually occurs shortly after field application, with further peaks correlated to rainfall-events (Guzman-Bustamante et al., 2019; Herr et al., 2019) or freeze-thaw periods (Flessa et al., 1995; Rochette et al., 2008). For this reason, the following experiment was conducted to evaluate digestates regarding short-term N_2O emissions on fallow land. To calculate the amount of N_2O derived from the digestate, ^{15}N-stable isotope labeling was used. The following hypotheses were tested:

(1) Digestates with varying physical and chemical properties will show different temporal N_2O and ^{15}N-N_2O flux patterns.
(2) Application of these digestates will also result in different cumulative N_2O emissions and N_2O emission factors.
(3) The amount of N_2O-N directly derived from the digestate will differ among the digestate types.

MATERIALS AND METHODS

^{15}N Labeling and Digestate Production

Labeled anaerobic digestates were prepared by cultivation of ^{15}N-enriched plants in a comparable approach as applied by Schouten et al. (2012). Maize (*Zea mays* L. cv. Ronaldinio), ryegrass (*Lolium perenne* L. cv. Kentaur), and sugar beet (*Beta vulgaris* subsp. vulgaris, Altissima Group) were ^{15}N labeled, by addition of ^{15}N ammonium sulfate ($(NH_4)_2SO_4$) as fertilizing solution. Ryegrass was cultivated in sand culture in 12 kg boxes of 10 cm height. For fertilization, 96.2 mg N kg $^{-1}$ as $(NH_4)_2SO_4$ (30 atom% ^{15}N) solution was applied before sowing ryegrass. We cut the ryegrass three times in 30-days intervals. Sugar beet was grown in Mitscherlich pots with 12 kg sand. After pre-growing sugar beet seedlings in peat, two plants were set for each pot. Four rates of $(NH_4)_2SO_4$ (50 atom% ^{15}N) solution were applied during growth (in total 1.5 g N per pot). Maize was grown in a hydroponic system with two plants per 10-liter pot. Nutrient solution adapted after Engels (1999) with modified N concentration was exchanged twice a week. Within the first 5 weeks of growth, NH_4^+-N concentration was gradually increased, while NO_3^--N supply was decreased to acclimate maize plants to primary NH_4^+-N nutrition. After this adaption phase for the plants, the N concentration was kept stable at 0.5 mM NO_3^--N and 3 mM NH_4^+-N, in the form of calcium nitrate and ammonium sulfate. For ^{15}N labeling, four additions of NH_4^+-N were substituted by 50 atom% ^{15}N- NH_4^+ and applied at BBCH stages: 16–19, 30–33, 51–55, and 71. As commonly done for maize, as energy crop, it was harvested at the dough-ripe stage. After harvest, ryegrass, maize sugar beet, as well as sugar beet leaves were immediately cut and homogenized by short blending (Thermomix TM31, Wuppertal, Gemany). The ^{15}N enrichment of crops and harvest residues was determined by IRMS with previous freeze-drying, leading to 19.3 atom% ^{15}N in maize, 26.1 atom% in ryegrass, 43.8 atom% in sugar beet, and 45.3 atom% in sugar beet leaves. After weighing the ^{15}N-plant biomass into small portions, they were frozen at −20°C until anaerobic digestion in a batch reactor as previously described by Brulé (2014), Mönch-Tegeder et al. (2014).

Anaerobic digestion of the ^{15}N-labeled plants and plant residues was carried out at the State Institute of Agricultural Engineering and Bioenergy, at the University of Hohenheim. Before, digestates from maize, grass silage and sugar beet were

TABLE 1 | Physico-chemical digestate properties, ^{15}N-labeling and ^{15}N abundance.

	DM (%)	pH (water)	EC ($\mu S\ cm^{-1}$)	C_t (% DM)	C_{org} (% DM)	^{15}N labeling	^{15}N (atom%)	N_t (g kg^{-1} FM)	NH_4^+-N/N_t (%)	C/N	C_{org}/N_{org}
Maize (M)	6.7	8.3	4,370	37.8	37.1	N_t	7.4	6.77	67.9	3.7	11.3
Grass (G)	8.2	9.1	4,250	28.0	27.3	N_t	11.7	4.41	58.8	5.2	12.4
Sugar beet (SB)	3.9	8.5	1,380	16.9	15.9	N_t	6.3	2.03	63.6	3.3	8.5
Sugar beet leaves (SBL)	4.8	8.8	1,640	18.5	17.2	N_t	12.2	1.97	65.8	4.5	12.2
Organic waste (OW)	13.7	7.7	3,100	31.2	30.0	NH_4^+-N	5.36	6.33	56.9	6.8	15.1
Food waste (FW)	4.3	8.1	3,650	36.7	36.3	NH_4^+-N	5.36	6.79	67.6	2.3	7.1
Cattle slurry (CS)	9.3	7.9	3,380	38.3	37.9	NH_4^+-N	5.36	4.06	56.7	8.8	20.1

TABLE 2 | Soil characteristic at the beginning of the experiment spring 2016 (1st year) and 2017 (2nd year), mean mineral N (N_{min}) (± standard deviation ($n = 2$).

	pH ($CaCl_2$)	Total C (%)	Total N (%)	C/N	NH_4^+-N (kg N ha^{-1})	NO_3^--N (kg N ha^{-1})	N_{min} (kg N ha^{-1})
1st year	7.0	1.25	0.14	8.9	3.52 ± 1.70	27.5 ± 3.4	31.0 ± 5.1
2nd year	6.8	1.13	0.13	9.0	1.84 ± 0.41	30.2 ± 1.4	32.1 ± 1.8

collected from biogas plants in southern Germany to be used as inoculum for AD of the ^{15}N-feedstocks (maize, ryegrass, sugar beet, and leaves). Digestates were "starved" for 10 days according to the German standard VDI 4630 guideline (2016) to minimize residual gas production. During this starvation phase, the vessels were kept open and stirred to volatilize ammonia (NH_3) from the inoculum. By decreasing NH_4^+-N in the digestate, hence total N concentration, a high ^{15}N-signature could be assured, with only marginal N dilution of the added ^{15}N-feedstock. Prior to AD, the inoculum was sieved to produce a homogeneous slurry. ^{15}N-labeled ryegrass, maize, sugar beet, and sugar beet leaves were separately added to the substrate-specific inoculum in a ratio of 1:2.5 organic total solids (oTS) (VDI 4630, 2016). Anaerobic digestion was carried out in 2 liter fed-batch systems under mesophilic temperature at 37.5 ± 1°C for 60 days. During digestion, three feeding portions of ^{15}N enriched plant substrates were added: at the start of the experiment, after 20, and after 40 days, respectively. Due to the amount of oTS added by the digestates, the ^{15}N amount of the feedstocks was diluted by N contained in the inoculum, leading to a lower labeling of the ^{15}N-digestates compared to the initial plant feedstock (**Table 1**).

Additionally, digestates from existing biogas plants were included and the mineral NH_4^+-N fraction was labeled: organic waste digestate, food waste digestate, and cattle slurry digestate. The digestates were analyzed for total N and NH_4^+-N. Each digestate was filled into a glass beaker and put into a rotating water bath for 12 h at 37°C, to volatilize a small amount of NH_3. Afterwards the digestates were analyzed again for total N and NH_4^+-N to assess the amount of N that was emitted. The lost N was substituted by addition of ^{15}N-enriched $(NH_4)_2SO_4$ solution to 5 atom% ^{15}N excess. If more N was lost than resupplied by ^{15}N-NH_4^+, ammonium chloride solution was added.

Experimental Design

The experiment was performed at the research station "Heidfeldhof" at the University of Hohenheim, 13 km south of Stuttgart, in South-Germany. The research station has a mean annual precipitation of 686 mm and a mean annual temperature of 8.8°C, monitored by a local meteorological station. The soil type of the arable field was a Haplic Luvisol (IUSS Working Group, 2015) with a silty loam soil texture (2% sand, 68% silt, and 30% clay), a bulk density of 1.24 g cm^{-3} in the upper 30 cm. Soil analytical results are presented in **Table 2**. The micro-plot field experiment (1 × 1 m plot size) was conducted as randomized block design with four replicates per treatment in 2 years 2016 (1st) and 2017 (2nd year) from May to July. The treatments consisted of one unfertilized control and seven ^{15}N-labeled digestates based on maize (M), grass (G), sugar beet (SB), sugar beet leaves (SBL), organic waste (OW), food waste (FW), and cattle slurry (CS) (**Table 1**).

Gas Measurements and Analysis

The closed chamber system was used to monitor N_2O, CO_2, and methane (CH_4) soil fluxes (Hutchinson and Mosier, 1981). The system consisted of a polyvinyl chloride (PVC) base ring (30 cm inner diameter) and a corresponding chamber (Pfab et al., 2011). Within the center of the 1 m^2 micro plot, the PVC base ring was embedded 10 cm deep in the soil. The ^{15}N-labeled digestates (**Table 1**) were applied at a rate of 170 kg N ha^{-1}, meaning 1.2 g N per base ring and quickly incorporated into the upper 5–10 cm of the soil. In order to do so, a 10 cm deep furrow was dug across the ring, using a spade. Digestate was filled into the furrow, covered by soil and mixed. The same procedure was done for the unfertilized control using water. The amount of water (290 ml) corresponded to the average volume of digestate application. Directly after application, the first gas measurement was performed. For gas sampling between 8.00 and 12.00 am, the base ring was covered with the dark, vented PVC chamber, sealed by a rubber ring to collect the trace gas. The chambers were closed for 45–60 min. The first gas sample was directly taken after closure, followed by additional sampling every 15–20 min using a syringe and transferred into evacuated 20 ml gas vials. At the same time, two additional gas samples were collected into 100 ml vials at the start and end of each measurement for

^{15}N-N_2O determination. Soil and chamber temperature were recorded within each block from two random plots at beginning and end of sampling. Within the 1st month, gas samples were taken 3–4 times a week. Afterwards the sampling frequency was reduced to once or twice per week, with additional samplings after strong rainfall events. In both years 20 gas samplings were performed and measured for N_2O, CO_2, and CH_4, whereas 13–15 out of 20 ^{15}N-N_2O gas samples could be measured due to cost and time reasons in the 1st and 2nd year, respectively.

Gas samples were measured with a gas chromatograph (GC 450 Greenhouse Gas Analyzer, Bruker Daltonic, Bremen, Germany) equipped with electron capture detector (ECD) and flame ionization detector (FID) and an automatic sampler (GX-281, Gilson, Limburg, Germany). During GC measurements, concentrations of N_2O and CO_2 were analyzed with a ^{63}Ni ECD and CH_4 concentrations were determined with the FID. Fluxes of N_2O, CO_2, and CH_4 were calculated by an extended version of the R (R Core Team, 2016) package "gasfluxes" (Fuß and Asger, 2014).

Analysis of Digestate and Soil

Digestates were dried at 105°C for dry matter (DM) analysis. Total C (C_t) was measured by Dumas combustion via elemental analysis (Elementar vario MAX CN, Analysensysteme GmbH, Hanau, Germany). Carbonate content was determined volumetrically using the Scheibler method according to DIN 10693 (2014). Thus, organic C can be calculated as the difference between C_t and Carbonate-C. Total N (N_t) and NH_4^+ of fresh matter (FM) digestate sample was determined by Kjeldahl method. Organic N was derived by the difference of NH_4^+ from N_t. The pH value was measured in FM digestate using 0.01 mol L^{-1} calcium chloride solution (1:10 w/w).

Soil mineral N (N_{min})was determined by extraction with 0.5 M potassium sulfate solution (1:4) and measured colorimetrically with a photometer (Flow-injection-analyzer 3 QUAAtro, SEAL Analytical, UK). Bulk density of the top soil was determined using 100 ml stainless steel cylinders in the field.

Total N and C, and ^{15}N-signature of ^{15}N-labeled plant substrates, soil and digestate was measured with a CN-elemental analyzer (EuroVector, HEKAtech, Wegberg, Germany) with Isotope Ratio Mass Spectrometer (IRMS) (Delta plus Advantage, Thermo Finnigan, Bremen, Germany). For the determination of the ^{15}N abundance in N_2O we used an IRMS delta plus (Finnigan MAT, Bremen, Germany) coupled with an automated PreCon-Interface (Brand, 1995).

Statistics and Calculations

Trapezoidal linear interpolation of daily gas fluxes (N_2O and CH_4) was used to calculate total cumulative emissions for the 55–58 days of the experiment in the 1st and 2nd year, respectively. The percentage of N_2O-N originating from digestate N (Nd) was calculated by equation (1), with digestate *i* at sampling time *t*. Atom%^{15}N excess was calculated by subtraction of the natural abundance of N_2O in the atmosphere (0.369 atom%) from the measured ^{15}N. The daily N_2O flux rate ($\mu g\ N_2O$–$N\ m^{-2}\ h^{-1}$) was multiplied with *Nd* in equation (2) to determine the amount of N_2O derived from digestate (^{15}N-N_2O) as reported by Senbayram et al. (2009). We calculated the recovery (%) of ^{15}N applied by summing up ^{15}N content of the soil at the end of the experiment and cumulative ^{15}N-N_2O loss. This sum was then divided by the amount of ^{15}N applied by the digestate as described by Pfab (2011).

$$Nd_{i,t}\ (\%) = \frac{atom\%^{15}N\ excess\ digestate_i}{atom\%^{15}N\ excess\ N_2O_{i,t}} \quad (1)$$

$$^{15}N - N_2\ O_{i,t}\ \ \left(\mu g\ N_2\ O - N\ \ m^{-2}\ h^{-1}\right) = Nd_{i,t}\ (\%) * N_2O\ flux_{i,t} \quad (2)$$

Digestate derived fluxes (^{15}N-N_2O) were also linearly interpolated to calculate cumulative (cum) ^{15}N-N_2O emissions. The total share of N derived from digestate (total Nd) in cumulative N_2O was calculated by Equation (3). As suggested by Schleusner et al. (2018), the amount of primed N_2O-N lost by fertilizer application was calculated by a simplified approach accounting for cumulative N_2O-N emissions of the unfertilized control treatment (Equation 4), without considering other gaseous losses *via* NH_3 or N_2.

$$total\ Nd_i\ (\%) = \frac{cum\ ^{15}N - N\ _2O_i\ (g\ N_2O\ ha^{-1})}{cum\ N_2O_i\ (g\ N_2O\ ha^{-1})} * 100 \quad (3)$$

$$Primed\ N_2O_i\ (g\ N_2O\ ha^{-1}) = \left(cum\ N_2O_i - cum\ ^{15}N_2O_i\right) - cum\ N_2O\ control \quad (4)$$

$$EF_i\ (\%) = \frac{\left(cum\ N_2O_i - cum\ N_2O\ control\right)\ g\ N_2O\ ha^{-1}}{170\ kg\ ha^{-1} * 1000} * 100 \quad (5)$$

N_2O emission factors (EFs) were calculated according to the IPCC guidelines for direct emissions (Equation 5), meaning total cumulative N_2O-N emissions accounted for the control, per applied N (IPCC, 2019). The disaggregated IPCC N_2O EF for "other N inputs in wet climates" with the default value of 0.6% was applied for comparison, where other N inputs refer to organic amendments such as digestates. Field conditions of the experimental site showed a positive water balance and fit with IPCC conditions for wet climates (IPCC, 2019). Greenhouse gas emissions of N_2O and CH_4 were transformed to CO_2-eq. to assess the total global warming potential (GWP) of each digestate. The default values of 296 g g^{-1} CO_2 for N_2O and 24 g g^{-1} CO_2 for CH_4 were applied to the measured emissions. Ammonia (NH_3) volatilization within the first 72 h was derived from the ALFAM2 model to calculate potential indirect N_2O emissions (Hafner et al., 2019). The model is used to predict NH_3-N losses within the first 72 h from animal slurry and therefore holds a higher uncertainty for digestates. Digestate NH_3-N losses mainly served as an indicator for the amount of indirect N_2O emissions. Indirect emissions from NO_3^- leaching were not

accounted for. According to IPCC (2019) 1% of NH_3-N losses was assumed to be re-deposited as N_2O-N. Soil organic C stocks were presumed to be stable over the experimental period, thus, CO_2 fluxes were not considered for the calculation of total GWP (Herr et al., 2020).

Water filled pore space (WFPS) was calculated by Equation (6) using the measured volumetric water content (WC_{vol}) and porosity (P),

$$WFPS\ (\%) = \frac{WC_{vol}}{P} * 100 \quad (6)$$

where P is depicted as soil bulk density (ρd) and solid particle density (ρs) (Equation 7). For ρs the density of quartz (2.65 g cm^{-3}) was assumed.

$$P\ (\%) = \left(1 - \frac{\rho d}{\rho s}\right) * 100 \quad (7)$$

For each year, a regression analysis of N_2O fluxes was calculated, using a stepwise forward selection in a multiple linear regression approach. Air temperature (2 m height), WFPS and CO_2 fluxes were included as independent variables within the model (8). Only significant variables remained in the model ($\alpha = 0.05$) and the square root of the partial R^2 was determined. Same regression procedure was applied for cumulative N_2O and ^{15}N-N_2O emissions within each year separately. For this approach the effect of digestate composition was determined, using NH_4^+-N share, and the ratios C/N and C_{org}/N_{org} in model (9).

$$y_{it} = \mu + \beta_1 temp_t + \beta_2 WFPS_{it} + \beta_3 CO_{2it} + b_{it} + e_{it} \quad (8)$$

$$y_i = \mu + \beta_1 CN_i + \beta_2 C_{org} N_{org_i} + \beta_3 NH_{4i} + b_i + e_i \quad (9)$$

where y_i is the observation of the i^{th} digestate treatment, μ represents the average response, β_n are the parameters of fixed effects, b_i is the complete block effect and e_i is the error of y_i.

Significant differences among treatments for cumulative N_2O, CH_4, CO_2, as well as ^{15}N-N_2O and total Nd (%) were determined by the Proc MIXED procedure and the Tukey test ($\alpha = 0.05$). The MIXED procedure can fit various mixed linear models to data and produces the appropriate statistics (SAS Institute Inc, 2015). All statistical analyses were performed with SAS 9.4 (SAS Institute, Cary NC, USA). Graphics were produced with SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany).

RESULTS

Meteorological Conditions

Weather conditions showed distinct differences in precipitation between the 2 experimental years. Over the 1st and 2nd year, 183 and 178 mm of precipitation were measured during the 55 and 58 days when the experiment lasted, respectively (**Figure 1**). Within

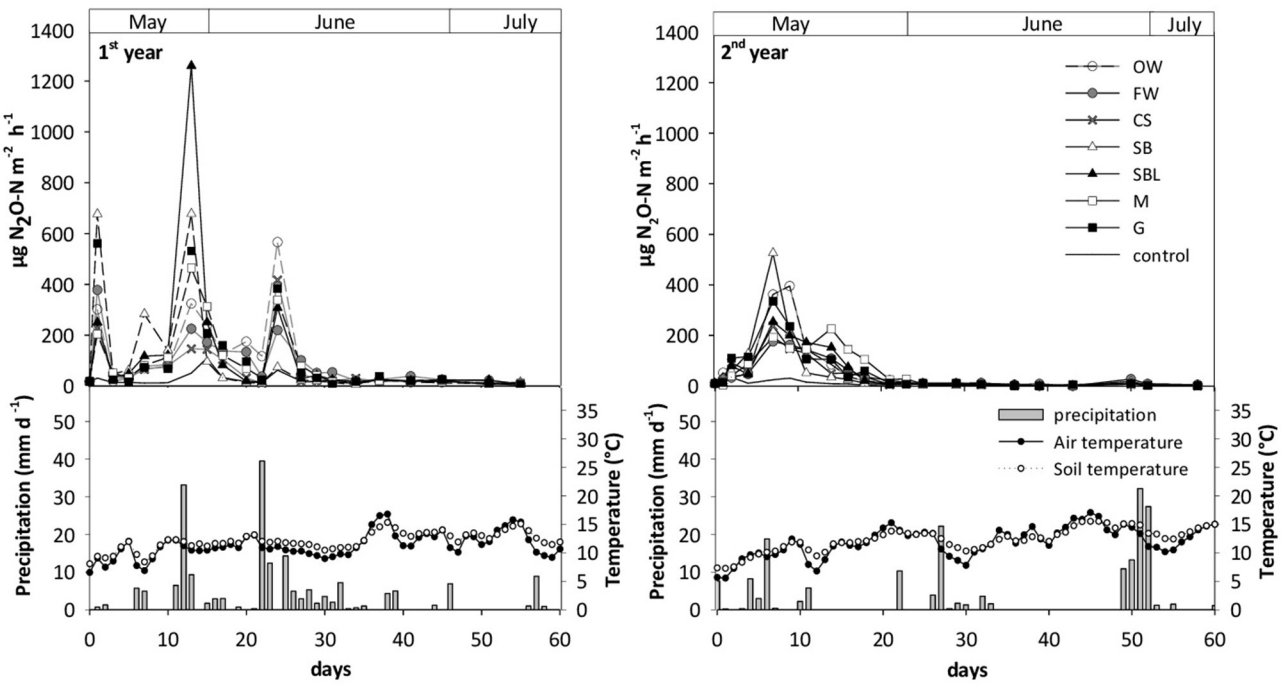

FIGURE 1 | Mean daily N_2O fluxes after application of different digestates based on organic waste (OW), food waste (FW), cattle slurry (CS), sugar beet (SB), sugar beet leaves (SBL), maize (M), grass (G), and unfertilized soil (control) measured from May to July 2016 and 2017 (1st and 2nd year) at Heidfeldhof. Digestate application of 170 kg N ha^{-1} on day 1. Standard error of measurements was excluded due to improved clarity, but shown in ^{15}N fluxes (**Figures 2**, **3**). Climate data with mean daily precipitation on the left y-axis and mean daily temperature on the right y-axis.

TABLE 3 | Output of (stepwise forward) regression analysis for N_2O fluxes in the 1st and 2nd year, testing for the inclusion of parameters CO_2 flux, water filled pore space (WFPS), and soil temperature into the model (model 8).

Year	Partial R^2			R^2	Model R^2	*F*-value	*p*-value
	WFPS	Soil temp	CO_2	$\sum$			
1st year	0.2064			0.281	0.207	160	<0.001
			0.0693		0.277	58.7	<0.001
		0.0048			0.282	4.08	0.0438
2nd year	0.1574			0.209	0.166	117	<0.001
		0.0459			0.213	36.1	<0.001
			0.0053		0.218	4.22	0.0404

the first 30 days of measurements the rainfall pattern differed, showing 155 mm in the 1st compared to 86.2 mm precipitation in the 2nd year. In the 1st year, two strong rainfall events occurred on day 12 (33.2 mm) and day 22 (39.5 mm). In contrast, the 2nd year showed lower rainfall events on day 6 and 27 with 18.8–22.2 mm, and two stronger events at the end of the experiment on day 51 and 52 (32.2–27.4 mm). The mean air temperature over the experimental period was 17.1°C in the 1st and 18.0°C in the 2nd year.

Temporal N_2O Fluxes

Nitrous oxide fluxes measured in the 2 experimental years showed distinct differences in peak number and flux magnitude. In both years N_2O pulses occurred directly after digestate fertilization and after strong rainfall events (**Figure 1**). Three major peaks were detected in the 1st year: one directly after digestate application, the second and third peak after 13 and 24 days, and a minor peak after 1 week, following strong rainfall events on day 12 and 23. Highest N_2O flux rate in the 1st year was measured with the SBL treatment on day 13 (1,260 μg N_2O-N m^{-2} h^{-1}). In the 2nd year, the N_2O pulse developing directly after N fertilization did not reach the same magnitude as in the 1st year and appeared 1 day later. Highest N_2O flux in the 2nd year followed a rainfall event 1 week after digestate application reaching up to 424 μg N_2O-N m^{-2} h^{-1} with SB (**Figure 1**). The peak decreased sharply in case of SB, and gradually until day 14 for the other digestates. After another strong rainfall event on day 22 (2nd year), only FW showed a slight N_2O rise (38 μg N_2O-N m^{-2} h^{-1}). Approaching the end of the experiment, 50 days after digestate application, a small peak (5.22–21.9 μg N_2O N m^{-2} h^{-1}) was noted within 4 days of continuous rainfall (**Figure 1**).

In both years, WFPS showed a significant positive linear correlation with N_2O flux rates, $r = 0.400$ ($p < 0.001$) and $r = 0.454$ ($p < 0.001$) in the 1st and 2nd year, respectively. Similarly, CO_2 fluxes correlated with N_2O fluxes, exhibiting $r = 0.233$ ($p < 0.001$) in the 1st year, and a weaker coefficient of correlation in the 2nd year ($r = 0.144$, $p < 0.001$). Soil temperature showed a negative correlation with N_2O fluxes ($r = -0.340$; $p < 0.001$) in the 2nd year, but no significant correlation in the 1st year. All parameters (WFPS, soil temperature and CO_2) combined in a linear regression model (model 8) could account for 28.1 to 20.9 % of the prediction of N_2O fluxes in the 1st and 2nd year, respectively (**Table 3**).

Temporal ^{15}N-N_2O Fluxes

Total N_2O and digestate derived ^{15}N-N_2O fluxes in the 1st year are shown in **Figure 2** and the 2nd year data are shown in **Figure 3**. A comparable trend was observed for both $^{15}N_t$- and $^{15}NH_4^+$-N-labeled digestates in each year, with variations in flux magnitude of ^{15}N-N_2O among digestates (**Supplementary Tables 2, 3**).

In the 1st year, the emerging peak directly after digestate application showed a low ^{15}N signature, indicating that 92.4–96.5% of N_2O was derived from soil internal N sources (**Supplementary Table 2**). Within the first 10 days after digestate application, ^{15}N-N_2O fluxes showed no significant differences among treatments (**Supplementary Table 2**). Only on day 7, a small peak in ^{15}N-N_2O (13–87 μg ^{15}N-N_2O-N m^{-2} h^{-1}) appeared, and SB showed significantly higher emissions than M, OW, FW, and CS. On that day, ∼18–30% of N_2O-N was derived from digestates (**Figure 2** and **Supplementary Table 2**). During the highest peak on day 13, a significant proportion of digestate-based N_2O-N (31–59%) was emitted. Highest ^{15}N-N_2O among digestates was measured with SBL (539 μg ^{15}N-N_2O-N m^{-2} h^{-1}), not significantly different from G (338 μg ^{15}N-N_2O-N m^{-2} h^{-1}). Following the two peaks on day 7 as well as on day 13, lower total N_2O and ^{15}N-N_2O fluxes were measured, but the share of digestate-derived N was still relatively high (**Supplementary Table 2**). The last major peak appeared on day 24 (10–114 μg ^{15}N-N_2O-N m^{-2} h^{-1}), with 10–27% N_2O-N originating from digestates. Flux rates peaking on day 24 were comparable among most treatments, and only OW exhibited significantly higher flux rates than SB. Prior to peaks of day 24, OW already indicated a rising flux rate on day 20, being significantly higher than all digestates, except G and FW. The flux rate further increased on day 22, where OW significantly exceeded all other treatments (**Figure 2** and **Supplementary Table 2**). Within the first 3 weeks of measurements, flux pattern of CS digestate significantly differed from the other treatments, where ^{15}N-N_2O gradually increased after 7 days and reached its maximum on 13 (**Figure 2**). At both peaks, on day 13 and 24, ^{15}N-N_2O flux rates of CS were in a comparable range (48–40 μg ^{15}N-N_2O-N m^{-2} h^{-1}). The 2nd year showed a similar temporal pattern in ^{15}N-N_2O abundance over the duration of the experiment. The first pulse after digestate application was observed 2 days after application with more than 80% soil-borne N_2O-N. Only CS showed lower soil-borne N_2O-N, thus highest digestate derived N_2O-N (45%) among digestates (**Supplementary Table 3**). The major peak in total N_2O and ^{15}N-N_2O appeared after 1 week (**Figure 3**), with highest ^{15}N-N_2O flux measured in G (189 μg ^{15}N-N_2O-N m^{-2} h^{-1}) showing 67% digestate-derived N. In contrast, 28–36% of N_2O-N was emitted from the other digestate treatments on that day (**Supplementary Table 3**). From day 7 to day 18, ^{15}N-N_2O gradually decreased for all digestates to 0.3–20 μg ^{15}N-N_2O-N m^{-2} h^{-1}, except for M being significantly higher (66.1 μg ^{15}N-N_2O-N m^{-2} h^{-1} on day 18). From day 18 through 29, the M

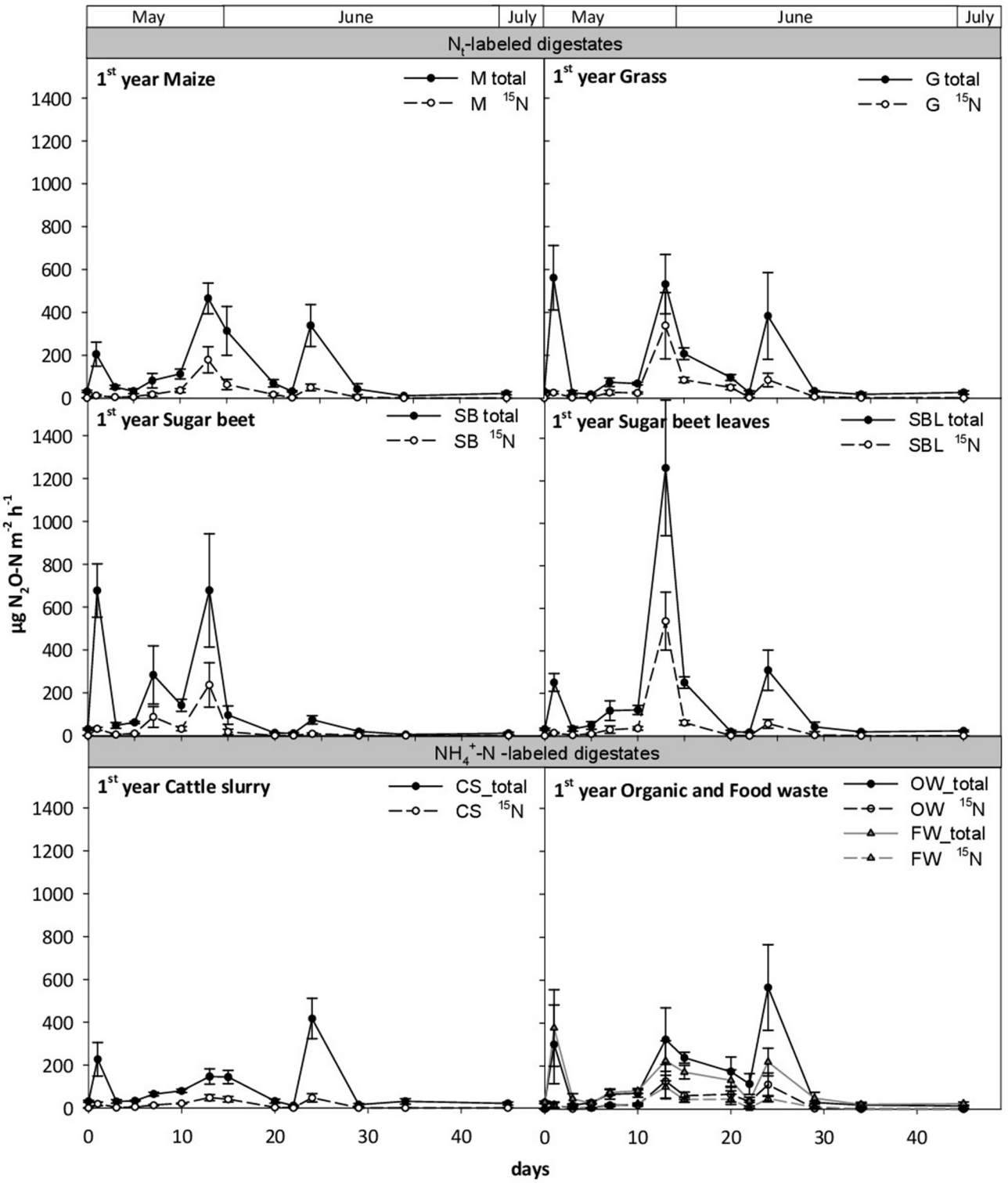

FIGURE 2 | Mean daily N_2O fluxes (total) and digestate-derived ^{15}N-N_2O fluxes (^{15}N) within the 1st year Digestates from maize (M), grass (G) sugar beet (SB), and sugar beet leaves (SBL) were $^{15}N_t$-labeled (mineral and organic N) and digestates based on cattle slurry (CS), organic waste (OW), and food waste (FW), were ^{15}N-labeled only in the NH_4^+-N pool. Error bars indicate the standard error ($n = 4$).

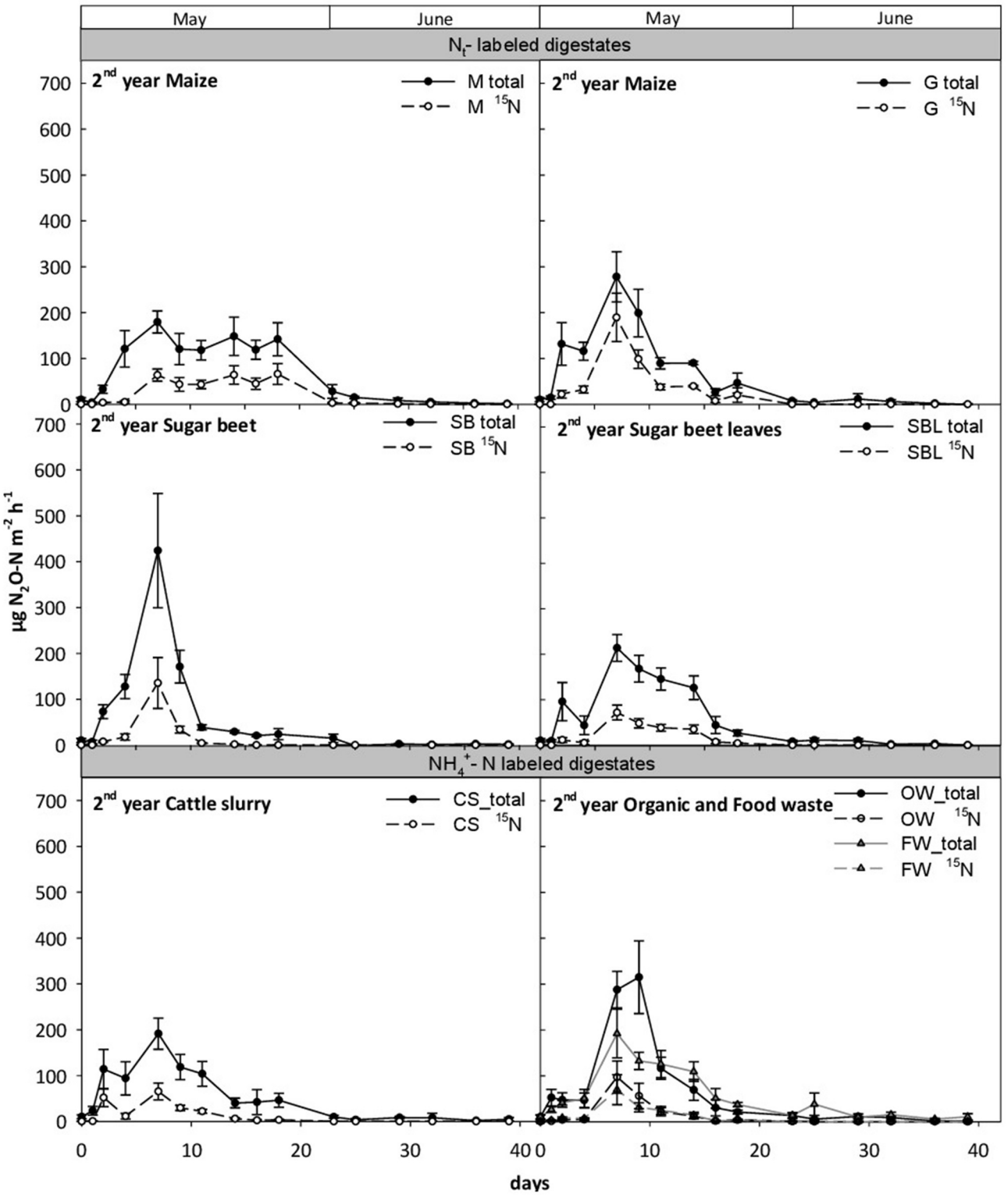

FIGURE 3 | Mean daily N_2O fluxes (total) and digestate-derived ^{15}N-N_2O fluxes (^{15}N) within the 2nd year Digestates from maize (M), grass (G) sugar beet (SB), and sugar beet leaves (SBL) were $^{15}N_t$-labeled (mineral and organic N) and digestates based on cattle slurry (CS), organic waste (OW), and food waste (FW), were ^{15}N-labeled only in the NH_4^+-N pool. Error bars indicate the standard error ($n = 4$).

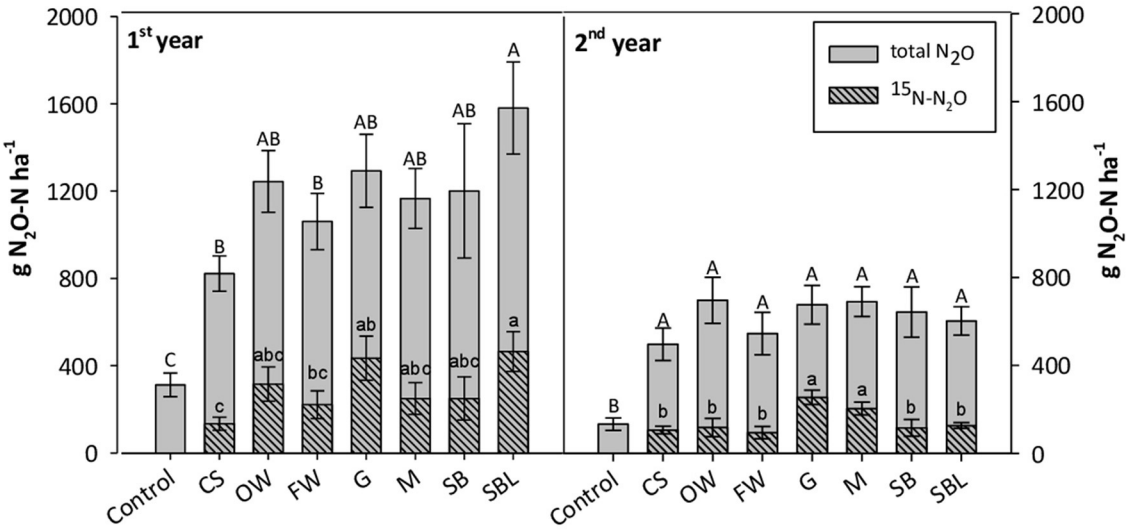

FIGURE 4 | Total cumulative N_2O-N emissions derived from digestate (^{15}N-N_2O) 55 (1st year) and 58 days (2nd year) after digestate application. In both years, measurements were conducted from May to July. Unfertilized soil served as control. Digestates from cattle slurry (CS), organic waste (OW), food waste (FW) were ^{15}N-labeled only in NH_4^+-N; digestates based on grass (G), maize (M), grass (G), sugar beet (SB), and sugar beet leaves (SBL) were $^{15}N_t$-labeled (mineral and organic N). Error bars show standard error ($n = 4$). Different letters indicate significant differences at $p < 0.05$ (Tukey Test); Capital letters refer to total N_2O and small letters to ^{15}N-N_2O.

TABLE 4 | Total cumulative N_2O-N, ^{15}N-N_2O-N emissions, and primed N_2O-N emissions after 55 (1st) and 58 days (2nd year).

Year	Total cumulative N_2O-N		Cumulative ^{15}N-N_2O-N		Primed N_2O-N	
	1st	2nd	1st	2nd	1st	2nd
			g N ha^{-1}			
Control	312 ± 54 c	133 ± 28 b				
N_t -labeled digestates						
Maize	1,166 ± 137 ab	690 ± 68 a	250 ± 73 abc§	203 ± 29 a AB	604 ± 82 ab	354 ± 48 ns
Grass	1,293 ± 167 ab	676 ± 88 a	434 ± 102 ab§	255 ± 33 a A	547 ± 119 ab	289 ± 56 ns
Sugar beet	1,201 ± 308 ab	643± 114 a	251 ± 99 abc§	116 ± 38 b B	638 ± 209 ab	394 ± 78 ns
Sb-leaves	1,580 ± 211 a	602 ± 65 a	465 ± 92 a§	127 ± 13 b B	804 ± 121 a	343 ± 55 ns
NH_4^+-N -labeled digestates						
Organic waste	1,244 ± 142 ab	697 ± 105 a	315 ± 79 abc§§	118 ± 42 b§§	617 ± 77 ab	446 ± 84 ns
Food waste	1,060 ± 129 b	545 ± 97 a	221 ± 63 bc§§	94.2 ± 27.8 b§§	528 ± 95 ab	318 ± 121 ns
Cattle slurry	822 ± 81 b	496 ± 74 a	133 ± 30 c§§	106 ± 17 b§§	376 ± 57 b	257 ± 60 ns

Unfertilized soil as control and application of Nt -labeled or NH_4^+-N -labeled digestates. Mean values ± standard error (n = 4). Different letters indicate significant differences at $p < 0.05$ (Tukey Test), ns = no significant differences. Small letters represent statistical differences among all treatments. For cumulative ^{15}N-N_2O-N, large letters refer to significant differences only among N_t-labeled or NH_4^+-N-labeled digestates.

§no significant differences among N_t -labeled labeled digestates, when excluding NH_4^+-N-labeled digestates.

§§no significant differences among NH_4^+-N-labeled digestates, when excluding N_t -labeled digestates.

treatment continued to show higher ^{15}N fluxes compared with the other digestates, even though these emission rates were quite low (from 1.1 to 2.7 μg ^{15}N-N_2O-N m^{-2} h^{-1}).

Cumulative N_2O and ^{15}N-N_2O Evolution and Emission Factors

Total cumulative N_2O emissions in the 1st year (302–1,345 g N_2O-N ha^{-1}) were more than twice as high as in the 2nd year (124–613 g N_2O ha^{-1}) (**Figure 4** and **Table 4**). In both years, digestates lead to significantly higher N_2O emissions than the unfertilized control. Differences among digestates were observed only in the 1st year, with significantly higher N_2O emissions for SBL compared to CS and FW (**Figure 4**). Compared to total N_2O emissions, digestate-based ^{15}N-N_2O emissions indicated larger differences between the different treatments in both years (**Figure 4**). In the 1st year, G and SBL emitted significantly more ^{15}N-N_2O than CS, while all other treatments did not differ significantly. In the 2nd year, highest ^{15}N-N_2O emission was

TABLE 5 | Share of digestate derived N_2O-N on total N_2O emissions (Total Nd) and N_2O-N emission factors after 55 (1st) and 58 days (2nd year).

Year	Total Nd		N_2O emission factor	
	1st	2nd	1st	2nd
		%		
N_t -labeled digestates				
Maize	20.3 ± 4.2 bc B	29.3 ± 2.6 ab B	0.50 ± 0.08 ab	0.33 ± 0.05 ns
Grass	32.9 ± 5.5 a A	37.8 ± 1.1 a A	0.58 ± 0.10 ab	0.32 ± 0.05 ns
Sugar beet	18.2 ± 3.8 bc B	16.8 ± 2.8 bc C	0.52 ± 0.18 ab	0.30 ± 0.07 ns
Sb-leaves	28.8 ± 1.8 ab B	21.2 ± 1.3 c C	0.75 ± 0.12 a	0.29 ± 0.04 ns
NH_4^+-N -labeled digestates				
Organic waste	24.2 ± 4.1 abc[§]	16.5 ± 4.8 c[§]	0.55 ± 0.08 ab	0.33 ± 0.06 ns
Food waste	20.1 ± 4.1 bc[§]	20.7 ± 7.0 bc[§]	0.44 ± 0.08 ab	0.24 ± 0.06 ns
Cattle slurry	15.7 ± 2.4 c[§]	21.6 ± 1.9 bc[§]	0.30 ± 0.05 b	0.21 ± 0.04 ns

Mean values ± standard error (n = 4). Different letters indicate significant differences at $p < 0.05$ (Tukey Test), ns = no significant differences. Small letters represent statistical differences among all treatments For total Nd, large letters refer to significant differences only among N_t-labeled or NH_4^+-N-labeled digestates.
[§]no significant differences among NH_4^+-N-labeled digestates, when excluding N_t -labeled digestates.

measured with G and M, while all NH_4^+-N-labeled digestates OW, FW and CS, as well as SB and SBL were comparable. Calculated amounts of primed N_2O-N showed that significant higher N_2O-N losses were induced by SBL compared with CS in the 1st year (**Table 4**). In the 2nd year, there were no significant differences among digestates. Total Nd emitted by the digestates was 16–33% in the 1st, and 17–38% in the 2nd year (**Table 5**). Grass digestate tended to show the highest share of digestate derived Nd in both years.

There was no correlation of digestate properties (C/N, C_{org}/N_{org}, NH_4^+-N/N_t) with N_2O emissions. The respective digestate characteristics did not help to predict cumulative N_2O or ^{15}N-N_2O emissions in a multiple (stepwise forward) linear regression model (model 9).

According to IPCC guidelines, ∼0.6% of the annual amount of total N of organic amendments applied as fertilizer is lost as N_2O-N in wet climates (IPCC, 2019). In the 1st year, most digestates approached this IPCC EF within only 55 days and SBL even exceeded it with 0.75% (**Table 5**). Only FW and CS indicated lower EFs than the IPCC default value in the 1st year, with 0.44 and 0.30%, respectively. Related to the overall lower cumulative N_2O emissions of the 2nd year, mean N_2O EFs were below 0.33% and in a comparable range for all digestates.

Total ^{15}N recovery within cumulative N_2O and soil N at the end of the experiment was 10–57% and 27–64% in the 1st and 2nd year, respectively (**Supplementary Table 4**). The largest share of digestate ^{15}N remained in the soil.

Total Global Warming Potential

Cumulative CH_4-C emissions were significantly higher in the 1st year compared to the 2nd. In both years, unfertilized soil served as CH_4 sink (−147 to −184 g CH_4-C ha^{-1}) (**Table 5**). Within the 1st year, emissions among digestates ranged between 0.26 and 1.82 kg CH_4-C ha^{-1} and decreased in the following order SBL ≥ CS ≥ FW, OW, SB ≥ M, G ≥ control. In the 2nd year, digestates as well as unfertilized soil were comparable and digestates indicated CH_4-C emissions close to zero (**Table 6**).

TABLE 6 | Modeled NH_3-N losses over the first 72 h after application (ALFAM2 model) and total cumulative CH_4 fluxes of digestates and unfertilized soil (control) after 55 (1st) and 58 days (2nd year).

	NH_3-N		Total cumulative CH_4	
	1st year	2nd year	1st year	2nd year
	kg NH_3-N ha^{-1}		kg CH_4-C ha^{-1}	
Control			−0.184 ± 0.202 d	−0.147 ± 0.106[ns]
Maize	6.2	0.5	0.360 ± 0.231 cd	−0.0369 ± 0.044[ns]
Grass	5.3	0.4	0.257 ± 0.133 cd	0.0171 ± 0.122[ns]
Sugar beet	2.8	0.5	0.848 ± 0.068 bc	−0.0608 ± 0.067[ns]
Sugar beet leaves	3.2	0.5	1.82 ± 0.394 a	0.0778 ± 0.129[ns]
Organic waste	3.9	0.4	0.767 ± 0.142 bc	−0.0518 ± 0.075[ns]
Food waste	4.8	0.5	0.671 ± 0.106 bc	−0.0876 ± 0.057[ns]
Cattle slurry	5.1	0.4	1.29 ± 0.369 ab	0.0550 ± 0.079[ns]

For CH_4, mean values ± standard error (n = 4). Different letters indicate significant differences in CH_4 emissions at $p < 0.05$ (Tukey Test); ns = no significant differences.

In both years, the release of CO_2-eq after digestate application was significantly higher than in the control (**Table 7**). Significant differences among digestates were only noted in the 1st year, where SBL caused significantly higher total CO_2-eq. than M, FW, and CS. In both years, N_2O emissions made up the largest share in total GHG emissions, based on CO_2-eq, above 85.6% in the 1st and almost 100% in the 2nd year.

DISCUSSION

Temporal N_2O and ^{15}N-N_2O Fluxes

The high temporal variability of N_2O fluxes in this study, with increased flux rates after application of crop residues or organic fertilizers and after rainfall events, was similarly documented in

TABLE 7 | Carbon dioxide equivalents (CO_2-eq.) of unfertilized soil (control) and soil after application of different digestates, originating from maize, grass, sugar beet, sugar beet leaves, organic waste, food waste, and cattle slurry, based on cumulative N_2O and CH_4 emissions (kg ha^{-1}) after 55 (1st) and 58 days (2nd year); and indirect N_2O emission as NH_3-N volatilization over 72 h after application.

Year	Treatment	Share of total CO_2_eq.			Total CO_2-eq.
		N_2O direct	N_2O indirect[§]	CH_4	
		%			kg ha^{-1}
1st	Control	103	-	−3.45	139 c
	Maize	92.9	5.17	1.91	583 b
	Grass	94.7	4.13	1.16	634 ab
	Sugar beet	91.9	2.60	5.48	599 ab
	Sugar beet leaves	90.7	1.92	7.34	808 a
	Organic waste	93.5	3.06	3.42	618 ab
	Food waste	91.1	4.32	4.62	540 b
	Cattle slurry	85.6	5.51	8.87	447 b
2nd	Control	104	-	−3.88	62.3 b
	Maize	100	0.746	−0.55	322 a
	Grass	101	0.632	−1.44	314 a
	Sugar beet	98.6	0.831	0.56	303 a
	Sugar beet leaves	101	0.867	−1.59	278 a
	Organic waste	100	0.606	−0.14	325 a
	Food waste	98.3	0.981	0.76	258 a
	Cattle slurry	101	0.877	−1.49	231 a

Different letters indicate significant differences at $p < 0.05$ (Tukey Test).
[§]*indirect emissions only based on NH_3 loss, nitrate leaching was not accounted for.*

other experiments (Pfab et al., 2012; Herr et al., 2019). Ultimately, N_2O fluxes leveled off 30 days after digestate application in both years with drying of the soil during warm periods with low rainfall. Dry conditions with low WFPS have often been reported to result in low N_2O emissions from arable soils even if these soils were well-provided with microbial easily degradable C and available N (Möller and Stinner, 2009; Pezzolla et al., 2012). However, in the 2nd year strong rainfall events were recorded 50 days after digestate application and only caused a minor increase in N_2O fluxes (**Figure 1**). Hence, digestate-related effects were short-term and had the highest impact on N_2O release within the first 30 days after application.

First Peak After Digestate Application

Peaks evolving shortly after organic N fertilizer application, such as digestates or manures, have been reported by several studies (Wulf et al., 2002; Johansen et al., 2013; Holly et al., 2017). As indicated by ^{15}N measurements in both years, the first N_2O peak after digestate application showed a low ^{15}N abundance, demonstrating that more than 90% of N_2O-N was derived from soil N (**Figures 2**, **3**). However, the experimental setup does not allow for a differentiation between nitrification and denitrification. Therefore, we can only conclude that the first N_2O peak was mainly derived from soil N. The high share of soil-borne N suggests that the addition of OM positively affected microbial activity which further enhanced the turnover of native soil-N, as also stated by Schleusner et al. (2018). Furthermore, digestate or slurry application moistened the soil close to the applied fertilizer, another factor that has been shown to promote denitrification of NO_3^--N (Comfort et al., 1990). Moreover, CO_2 flux rates were elevated directly after digestate fertilization (**Supplementary Figure 1**), supporting the assumption of increased microbial activity which further stimulated denitrification of NO_3^- by O_2 depletion (Buchen-Tschiskale et al., 2020). However, these are only speculations, as soil N_{min} and its ^{15}N-signature was not measured during the experiment. It should also be considered that digestates contain carbonate-C (HCO_3^- and CO_3^{2-}): the higher the total N content, the higher the carbonate-C content (Georgacakis et al., 1982). Carbonate-C in the digestates can also contribute to soil CO_2 release within the 1st days after application (Chen et al., 2011). For example, carbonate-C release from digestates can occur after application to acidic soils (Chen et al., 2011), which is not the case in present study, or due to microbial turnover processes (Tamir et al., 2013). Therefore, the immediate effects of digestate application on soil microbial activity and the related CO_2 release might be masked by decomposition of carbonate-C to CO_2. In order to elucidate the driving processes related to the N turnover processes in the soil shortly after digestate application, a more detailed measurement of the pathways of the different fractions of soil and digestate N (NH_4^+, N_{org}), as well as digestate C (C_{org}, carbonate-C), is necessary.

Rainfall-Induced Peaks

The emission pattern found in present study strongly coincided with the precipitation pattern, providing a major indication that the environmental conditions are the main driving factor for soil N_2O fluxes. Also the unfertilized control showed a significant increase in N_2O flux rates after rainfall, whereas almost no fluxes were observed in dry periods. The occurrence of increased N_2O fluxes in conjunction with heavy rainfall events, hence a high soil WFPS, is typical for arable fields and has extensively been described in the literature (Pfab et al., 2011; Senbayram et al., 2014; Ruser et al., 2017).

Contribution of Digestate N and Soil N Pool to N_2O Emissions

The largest rainfall-induced N_2O peaks in both years, had also the highest ^{15}N abundance, with up to 56–66% of N_2O-N derived from the digestate (**Figures 3**, **4** and **Supplementary Tables 2, 3**). Although it was shown that even at a high soil moisture of 70% WFPS nitrification may also contribute to the N_2O-release from soils (Ruser et al., 2006), the positive correlations between N_2O flux rates and CO_2 flux rates as well as between N_2O fluxes and soil moisture (**Table 3**) indicate that denitrification is the driving process releasing N_2O after rainfall. The contribution of denitrification to the N_2O release generally increases with increasing soil moisture (Davidson, 1991). When compared to soil air, the $\sim 10^{-4}$ lower diffusion coefficient for atmospheric O_2 in soil water (Heincke and Kaupenjohann, 1999) restricts O_2 delivery, the creation of anaerobic conditions is favored. Similarly, the turn-over of fresh OM, as indicated by the increased CO_2 fluxes, further depletes O_2 availability and thus fuels anaerobiosis (Flessa and Beese, 1995).

The largest peaks evolved 13 (1st year) and 7 days (2nd year) after digestate application, where presumably digestate NH_4^+-N was already nitrified (Johansen et al., 2013) and available for denitrification, thus, explaining the high share of digestate-based N_2O-N. Senbayram et al. (2009) observed that nitrification of ^{15}N-labeled digestate rapidly increased 1 week after application in a pot experiment initiating a rise in N_2O flux rates, as also noted in our study. It cannot be excluded that beside denitrification also nitrification contributed a share of the measured N_2O. Yet, Köster et al. (2011) measured the intramolecular ^{15}N distribution in N_2O within a 43-days incubation experiment, showing that bacterial denitrification was the main process emitting N_2O after application of food waste digestate, driven by C availability. This is in line with other studies, reporting that the largest N_2O-N contribution of digestates was caused by denitrification, even at 65% WHC (Senbayram et al., 2009). Later N_2O peaks (1st year, day 24) showed lower ^{15}N-N_2O fluxes, hence indicating an increasing share of N_2O from soil-internal N. This shift in ^{15}N-N_2O abundance over the measuring period indicates increased effects of the soil microbial processes, affecting N availability and N_2O emissions, and might result from mineralization of digestate $^{15}N_{org}$-N and subsequent processes. A comparable shift was observed by Senbayram et al. (2014).

For both, NH_4^+-N or N_t-labeled digestates, the low shares of fertilizer-derived N_2O-N supported the notion that the largest source of N_2O was native soil N (> 62%, **Table 5**). The open hypothesis of an "enhanced soil-derived N_2O" stated by Senbayram et al. (2014), regarding the low share of emitted digestate-N, can therefore be confirmed. This triggering effect on N_2O emissions due to digestate application was accounted for by a simplified calculation via equation 4. The amount of triggered N_2O-N reflects the high share of soil-derived N_2O, and was approximately half of total cumulative N_2O emissions (**Table 4**). Significant differences in primed N_2O-N among digestates followed the same trend as N_2O emissions. For NH_4^+-N-labeled digestates, N_2O-N losses which might originate from digestate N_{org}, were not accounted for. Therefore, the amount of primed N_2O-N might be overestimated.

In general, the rather comparable share of total digestate-derived N_2O-N losses among the digestates with different labeling approaches indicates that digestate-N_{org} plays only a minor role in short-term N_2O formation. Senbayram et al. (2014) labeled only the mineral N fraction of a digestate and found 31% of N_2O-N was derived from the digestate mineral fraction. The share of digestate-derived N_2O-N losses among NH_4^+-N-labeled digestates FW, OW and CS ranged from 15.7 to 24.2% over the 2 years. For these digestates as well as for the digestates in the study of Senbayram et al. (2014) it cannot be excluded that non-labeled organic N was mineralized and emitted as N_2O. However, the N_t-labeled digestates, M, G, SB, and SBL showed a rather comparable range with 18.2–37.8% digestate-N being emitted as N_2O over the 2 years. Similar to our findings, also other studies reported a higher share of N_2O-N originating from the soil N pool than from fertilizer N. For instance, only 22% of N_2O-N was derived from ^{15}N-labeled manure after 22 days (Ingold et al., 2018) or 40.4% from ^{15}N-urea after 35 days (Roman-Perez and Hernandez-Ramirez, 2020) in incubation experiments. In a field study, NH_4^+-N-labeled cattle slurry was applied, which produced higher fertilizer-derived N_2O emissions within the first 10 days, but higher soil-derived N_2O 11–22 days after application (Dittert et al., 2001). However, the study was carried out on grassland and using the injection technique (Dittert et al., 2001), which has been reported to increase N_2O emissions compared to trail hose application with immediate incorporation (Herr et al., 2019).

N_2O Emissions Affected by Fertilizer Type

As previously described, N_2O fluxes were shaped and influenced by weather conditions and soil microbial processes. Environmental conditions and soil type may play a more important role than the fertilizer type, as previously suggested by Senbayram et al. (2014): the authors noted no significant differences in N_2O emissions between mineral and organic N fertilization. However, in both years, significant differences among digestates were noted on several sampling dates, for N_2O as well as ^{15}N-N_2O fluxes (e.g., flux rates from M digestate vs. fluxes from SB digestate in **Figure 2** and **Supplementary Tables 1–3**), indicating that digestate composition affects N_2O emissions. This supports hypothesis (1), that digestates from different feedstocks will differ in N_2O flux rates.

However, regarding cumulative effects, there was no clear indication that the digestate type influenced total N_2O emissions. This was supported by the lack of a significant correlation between digestate composition (NH_4^+/N, C/N, C_{org}/N_{org}) and cumulative N_2O or $^{15}N_2O$. Only measurements of the 1st year showed significant differences among digestates. Therefore, hypothesis (2) had to be rejected for the 2nd year and could be partly accepted for the 1st year. Yet, when separating cumulative ^{15}N-N_2O data into N_t-labeled and NH_4^+-N-labeled digestates, there was a significant effect of C/N ratio in the 1st year, predicting 22.1% of ^{15}N-N_2O emissions of $^{15}N_t$-labeled digestates ($R^2 = 0.36$, F-value $= 3.81$, p-value $= 0.077$). For the 2nd year, C_{org}/N_{org} accounted for 39.2% ($R^2 = 0.56$, F-value $= 9.85$, p-value $= 0.0094$) of ^{15}N-N_2O emissions among $^{15}N_t$-digestates (M, G, SB, and SBL). Also Abubaker et al. (2013) noted significantly different cumulative N_2O emissions after 24 days between two types of urban waste digestates, which were low or high organic C. For NH_4^+-N-labeled digestates, there was no significant relation of digestate properties to ^{15}N-N_2O emissions. Hence, the correlation between digestate properties and N_2O emissions seems more strongly related to the total N and N_{org} content of digestates, than NH_4^+-N. Regarding the total share of digestate-derived N_2O-N (Nd), significant differences among digestates (**Table 5**) could support hypothesis (3).

Ultimately, the results of the present study suggest that the different digestate types influenced cumulative N_2O, flux rates and digestate derived N_2O-N only marginally. Hence, N_2O emissions were more strongly affected by environmental conditions (**Table 3**). The effect of digestate properties on total N_2O emissions was overlaid to some extent by the high amount of N_2O from the native soil N pool. Abubaker et al. (2013) incubated two digestates in three soil textures and noted considerable

differences regarding emission peaks and cumulative N_2O-N emissions among digestates, particularly in the sandy soil. In loam, digestates showed comparable total N_2O emissions (Abubaker et al., 2013). Therefore, N_2O emissions discussed in this study might differ on soils with different soil textures or amendment history (Rosace et al., 2020). In this context, soil texture, soil amendment history and fertility status, especially OM content, plays a crucial role, exceeding the effect of digestate properties.

Digestate Emission Factors and Practical Consequences

Digestate EFs determined in this study (0.21–0.75%) were all within the range of the IPCC default value, except for SBL in the 1st year (**Table 5**). However, these EFs will not cover the whole year and might underestimate the total EF of the digestates. Shang et al. (2020) determined 10–30% lower EFs when only the growing season and not the whole year N_2O emissions were considered. Moreover, the authors found that the differences between EFs of the whole year and growing season were higher with higher precipitation (Shang et al., 2020). The experimental design of the present study used bare soil, hence there were no crops removing the applied digestate N. Crop N uptake could have decreased available N from the soil as well as soil moisture, which could have lowered digestate-derived N_2O emissions and EFs. Thorman et al. (2020) determined annual N_2O EFs from different organic amendments, top-dressed to a cereal crop (0.15–0.73% in 2011 and 0.27–0.51% in 2012), which were in a comparable range with our EFs. Most digestate EFs did not show significant differences, except SBL compared with CS in the 1st year, thus hypothesis (2) cannot be fully confirmed.

Soil derived N_2O-N contributed to a large extent to digestate EFs. As a consequence of the high share of N_2O-N from the native soil pool within the first 30 days after digestate application, crop cultivation should be synchronized with available soil N. In particular, mineral N from the soil pool should be taken up by the crops, before digestates are applied. Thereby, the triggering effect of short-term soil-enhanced N_2O emissions by digestates could be decreased. For example, N_{min} supply in the present study would be sufficient for maize cultivation in the early growth stage. Digestates could then be top-dressed ∼1 month after emergence when most of soil M was already taken up by the crop. Also de Neve (2017) emphasized that in ideal cropping systems fertilizer availability and soil mineral N should be synchronized with crop demand, which could mitigate potential N losses.

Experimental Limitations

Determination of N_2O isotopomers in the present study, including the $\delta^{18}O$ and site preference of ^{15}N in the N_2O molecule, could have helped to understand the underlying soil microbial processes, differentiating between denitrification and nitrification (Köster et al., 2015). Yet, distinguishing nitrifier denitrification from nitrification is not possible using site preference (Köster et al., 2011). A dual isotope labeling approach of ^{15}N and ^{18}O-labeled water would be required (Koola et al., 2010), which is not feasible in field studies (Baggs, 2008). Also the $N_2O/N_2O+ N_2$ product ratio could have provided a better indication of denitrification in the study (Buchen-Tschiskale et al., 2020). However, measuring N_2 in the field is rather difficult due to the high N_2 background level in the atmosphere, as well as its spatial and temporal heterogeneity (Groffman et al., 2009). Instead N_2 is often studied in incubation experiments using an artificial helium–oxygen atmosphere (Scholefield et al., 1997). Regular soil N_{min} and $^{15}N_{min}$ analysis at the sampling dates could have given a hint for respective microbial processes, but would not have completely identified them. Thus, allocating the specific N_2O pathways after digestate application in the field is still challenging and needs further research and suitable methods to provide accurate measurements (Well et al., 2019).

CONCLUSION

The major finding of this study was the large share of N_2O-N from the soil pool, showing that digestate application triggers "enhanced soil-derived N_2O." The major driving forces of the emission pattern are the weather conditions, the specific chemical composition of digestates do have only minor effects on the denitrification. The different ^{15}N-labeling approaches of the digestates indicate that contribution of the organic fraction seems to be of very low significance for short-term N_2O emissions. The ^{15}N labeling approach helped to determine the source of N_2O emissions, but not the underlying processes (nitrifier denitrification or heterotrophic denitrification). Analysis of isotopomers and N_2 is needed to further identify the N_2O-releasing microbial processes in the soil. Emission factors were comparable for most digestates, but reached and even exceeded the default IPPC EF (0.6%) within only 60 days in the 1st year.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

FH prepared original draft, including statistical analysis, and graph production. RR supported calculation of global warming potential, provided guidance, and new input for determination of indirect N_2O emissions. The basis for the experimental idea was based on KM, with further contribution by RR regarding design of the field experiment, sampling frequency, and measurement technique. IC-M supported calculation of ^{15}N abundance in N_2O from IRMS data. FH and IC-M conducted the experiment. All authors reviewed and proofread the whole manuscript and gave critical feed-back to all sections.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.614349/full#supplementary-material

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N_2O Emissions From Residues of Oat and Grass Pea Cover Crops Cultivated in the US Southern Great Plains

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Grass pea (*Lathyrus sphaericus*) and oat (*Avena sativa*) are potential cover crops for spring periods of summer crop systems in the US Southern Great Plains (SGP). The main objective of this study was to compare nitrous oxide (N_2O) emissions from residues of grass pea and oat grown as green nitrogen (N) crops. The comparisons included responses from plots cultivated with oat, grass pea, and control (spring-fallowed) plots. Two management options were applied to grass pea: residues retained and aboveground biomass removed for forage. Crabgrass (*Digitaria sanguinalis*) was cultivated as a main summer crop immediately after termination of the cover crops. Fluxes of N_2O were measured by closed chamber connected to a portable gas analyzer on 23 dates during a 3 month growing period for crabgrass. At termination, oat produced more aboveground biomass than grass pea (2.17 vs. 3.56 Mg ha^{-1}), but total N in biomass was similar (102–104 kg ha^{-1}) due to greater N concentrations in grass pea than oat (4.80% vs. 2.86% of dry mass). Three month cumulative emissions of N_2O from grass pea-incorporated plots (0.76 ± 0.11 kg N_2O-N ha^{-1}; mean ± standard error, $n = 3$) were significantly lower than from oat-incorporated plots (1.26 ± 0.14 kg N_2O-N ha^{-1}). Emissions from grass pea plots with harvested biomass (0.48 ± 0.04 kg N_2O-N ha^{-1}) were significantly lower than those from grass pea-incorporated plots. Cumulative N_2O emissions from control plots were significantly greater than those from grass pea-harvested plots but were similar to the emissions from grass pea-incorporated plots. Yields produced by crabgrass were similar from all cover crop treatments (8.65–10.46 Mg ha^{-1}), but yield responses to the control (18.53 Mg ha^{-1}) were significantly larger. Nitrogen concentrations in crabgrass were greater in response to oat- and grass pea-incorporated plots (2.86–2.87%) than in grass pea-harvested (1.93%) and control (1.44%) plots. In conclusion, the results indicated that (i) post-incorporation emissions of N_2O can be greater from a non-legume green N crop than a legume green N crop due to greater biomass productivity of the cereal, and (ii) emissions of N_2O could be mitigated by removing biomass of the green N crop for use as forage.

Keywords: nitrogen mineralization, crabgrass, biomass decomposition, forage, cover crop, residue

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INTRODUCTION

Interest in including cover crops in production systems used in the US Southern Great Plains (SGP) has been increasing. Cover crops are seen to provide a number of environmental services, including reducing soil erosion, improving soil aggregation and infiltration, suppressing weeds, increasing the pool of nitrogen (N) in soils, reducing leaching and runoff, and increasing soil organic matter (Snapp et al., 2005; Foster et al., 2017). The predominant cropping systems used by producers in the SGP are different forms of winter wheat–summer fallow rotations and are generally applied in continuous rotations (Decker et al., 2009). However, warm-season crop–winter fallow rotations are also utilized in some years to increase income (Decker et al., 2009; Aiken et al., 2013). The warm-season–winter fallow systems can be used to cultivate cool-season legumes or grasses with short growing seasons (generally spanning March–May) as cover crops or green N sources to support a summer cash crop (Biederbeck et al., 1993; Singh et al., 2019a).

A major issue with growing green N crops to support subsequent cash crops is depletion of available soil moisture by the green N crops (Nielsen et al., 2015). Precipitation in the SGP is extremely erratic in terms of timing and amounts received (Singh et al., 2019b). Further, irrigation is limited to a small amount of the total land area of the SGP. Therefore, the selected N crop should not excessively deplete soil moisture, as this water use could reduce yield of following cash crops, such as corn (*Zea mays*), sorghum (*Sorghum bicolor*), or annual forage grasses (Singh et al., 2019b). Given the limitations of available water in rainfed systems, spring-planted crops with short growing seasons have potential as cover or green N crops within summer cropping systems of the SGP. Among the candidates are grass pea (*Lathyrus sphaericus*), a grain legume, and oat (*Avena sativa*), a cereal grass. Rao et al. (2005, 2007) reported grass pea as an adaptable crop suited to the dry conditions in the SGP. Legume-based cover crops can fix atmospheric N and serve as a N sources for following summer crops. However, both legume and non-legume species can contribute to increased N in soil pools by reducing losses through leaching, runoff, and gaseous emissions (White et al., 2017).

One key aspect for the success of cover crops grown as nutrient sources for following crops is the synchronization between nutrient mineralization from decomposing residues and the demand for nutrients by the following recipient crops (Myers et al., 1994; Kandel et al., 2019a,b). Due to short growing seasons, spring-planted cover crops have low carbon (C)/N ratios at termination as compared to fall-planted cover crops with long growing seasons. These low C/N ratios may result in rapid biomass decomposition and N mineralization after termination (Kandel et al., 2019b). However, rapid decomposition and N mineralization from residues of cover crops prior to establishment of the following crop may contribute to large losses of N as emissions of nitrous oxide (N_2O), a highly potent greenhouse gas (Huang et al., 2004; Basche et al., 2014; Kandel et al., 2018). Heavy or frequent rainfall events are common during the late spring in the US SGP, which is also the termination period of spring-planted cover crops. Such simultaneous increases in soil concentrations of mineral N and moisture after termination of cover crops could be conducive for denitrification, a major microbial pathway for N_2O production (Kandel et al., 2018). Therefore, a better understanding of how interactions between types of cover crops and their management, combined with patterns of precipitation during spring, impact N_2O emissions from decomposing biomass of cover crops is important to defining N losses to the atmosphere (Hoorman et al., 2009).

Nitrogen mineralization from decomposing biomass and resulting soil N_2O emissions can be influenced by the type of the cover crop (Basche et al., 2014). Legume cover crops in general contribute greater N_2O emissions compared to non-legumes due to increased pools of N from biological fixation (Baggs et al., 2000). Additionally, legume biomass generally has low C/N ratios, decompose rapidly, and deplete soil O_2 concentrations during decomposition, a condition conducive to denitrification (Aulakh et al., 1991; Thilakarathna et al., 2016). However, since spring-planted cereals used as cover crops are generally terminated before flowering, and C/N ratios and lignin concentrations can be low enough for rapid decomposition, thereby resulting in high N_2O emissions.

One potential strategy recommended for limiting N_2O emissions from residues of cover crops is removal of aboveground biomass as forage, instead of incorporating into the soil as N sources (Li et al., 2015; Kandel et al., 2019a). Beef cattle are a major agricultural commodity in the US SGP, so harvesting biomass for forage could be profitable by lowering risks of forage shortages in dry years, which are common in the region (Holman et al., 2018). However, biomass removal will also result in the removal of the major portion of biomass (Biederbeck et al., 1993; Kandel et al., 2019b). Therefore, biomass removal of cover crops may impact the growth of following crops due to reduced N supply, if N is not supplied from external sources (Kandel et al., 2019a).

Although interest in cover crops is increasing in the US SGP, there is limited information on influences of the residues of type of cover crops on N_2O fluxes after termination of the cover crops. Additionally, although large fluxes of N_2O after termination of legume-based cover crops have been reported (Kandel et al., 2018), there is limited information on how to mitigate post-incorporation soil N_2O emissions from residues of cover crops through management strategies. Therefore, we tested influences of type of cover crop (legume vs. cereal) and a management strategy (incorporation vs. harvest) for legume residues on post-incorporation fluxes of N_2O. The objective of this study was to compare post-incorporation emissions of soil N_2O from residues of spring-grown legume (grass pea) and non-legume (oat) cover crops within a summer-based system of forage production in the US SGP. We hypothesized that (i) emissions of N_2O would be lower from decomposing oat residue than grass pea residue, and (ii) emissions of N_2O could be mitigated by harvesting legume biomass for forage, compared to incorporating the biomass to supply N to following forage grass.

MATERIALS AND METHODS

Experimental Site and Soil Properties

This field study was conducted at the USDA-ARS Grazinglands Research Laboratory near El Reno, OK, USA (35°34′21″ N, 98°02′12″ W; 411 m elevation). The study was conducted during the March–August time period of 2018 and included growth periods of both spring green N and recipient summer hay crops. The study site was situated on an upper terrace of the bottomland area of the North Canadian River drainage basin (Goodman, 1977). The soils for the site were classified as Brewer silty clay loams (fine, mixed, superactive, thermic Udertic Argiustolls). The top soils had average pH of 6.9 and bulk density of 1.35 Mg m^{-3}. Brewer series are among the most fertile soils in the Canadian River basin. Average soil organic C and N contents of the sites were 1.31 and 0.10%, respectively (USDA-NRCS, 1999). The topsoil (0–0.15 m) had particle fractions of 18% sand, 52% silt, and 30% clay.

Experimental Design and Crop Management

The experiment consisted of 12 plots (4 m × 3 m) arranged in a completely randomized design. Grass pea was planted on six plots, and oat was planted on three plots on March 10, while three plots were left fallow during spring as a control treatment. Three oat and grass pea plots were terminated by tillage (disked once to ∼10 cm depth and roto-tilled once) on May 18 to incorporate biomass of green N crops. Grass pea biomass from the remaining three plots was harvested manually on the same day. Grass pea in these plots was harvested to 1.0 cm aboveground, and only the roots were incorporated. Crabgrass was then planted on all plots at a rate of 5 kg ha^{-1} at 0.03-m-spaced rows on May 19.

A long dry spell (>1 month) started 7 days after crabgrass was sown. This dry period restricted the growth of crabgrass, and plants showed severe symptoms of water shortage. Therefore, the plots were irrigated with 30-mm water on days 23 and 64 after termination of cover crops. The entire area of the plots, except that covered by collars placed to measure N_2O fluxes (described in the following section), was irrigated with a sprinkler system. Irrigation inside collars was subsequently applied using a watering can for application of precise amounts of water.

Measurements of Yield and Quality of Cover Crop Biomass

Total aboveground biomass of grass pea was determined by harvesting all biomass from the 12 m^2 areas of plots ($n = 3$) assigned to harvest treatments. Biomass produced by oat was determined by harvesting biomass from 1 m^2 areas of each plot ($n = 3$). Samples of root biomass of oat and grass pea were collected by shovel to a depth of 15 cm from the sampled areas of plots, and the soil was cleaned manually by washing samples through a 2.0 mm sieve under running water. Biomass samples (roots and shoots) from both green manures were oven-dried at 60°C to constant weight in a forced draft oven to determine the amount of dry matter. The major portion of dried biomass (except small subsamples retained for chemical analysis) was returned to the same 1 m^2 sampled areas after determination of biomass productivity. The 1 m^2 areas where biomass was sampled for productivity and quality were not used for measurement of gas fluxes, soil properties, or biomass productivity of the following summer crop. Samples used in chemical analysis were ground through a 1-mm screen by Wiley mill for analyses.

Biomass samples were analyzed for total C, N, and cell wall components (cellulose, hemicellulose, and lignin). Concentrations of C and N were assayed by flash combustion (900°C for 10 min) method (Model VarioMacro, Elementar Americas, Inc., Mt. Laurel, NJ, USA). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined by the Van Soest and Wine (1967) method. Concentrations of cellulose were calculated as the difference between ADF and ADL and hemicellulose as the difference between NDF and ADF. The ADL fraction was presented as lignin concentration.

Gas Flux Measurements

N_2O and carbon dioxide (CO_2) fluxes were measured on 23 dates at irregular intervals using a closed chamber system during May 19 to August 16, 2018. Fluxes were measured frequently (often daily) after rainfall (>5 mm) and irrigation events to capture N_2O emission peaks observed after moisture inputs but less frequently during dry periods (longest interval 15 days) when conditions conducive to N_2O emissions were not present, and emissions remained close to zero. In each plot (total $n = 12$), a white painted steel collar (0.65 m × 0.65 m = 0.42 m^2) was inserted to a 0.10 m depth immediately after tillage operations and planting of crabgrass. These collars had a 0.04 m-wide outer flange to support the top chamber used for flux measurements. During flux measurements, a white-colored PVC chamber (0.70 m × 0.70 m × 0.21 m) was placed on the permanently installed collars.

During chamber enclosure, air in the chamber headspace was mixed using two small battery-driven fans. Air in the chamber headspace was circulated through 3.0 mm inlet and outlet tubing to a portable Fourier transform infrared-based gas analyzer (DX4040; Gasmet Technology Oy, Helsinki, Finland). The concentrations of N_2O and CO_2 were recorded at 20-s intervals, resulting in 18–24 data points during 6–8 min enclosures for each measurement period. All fluxes were measured between 10:00 and 12:00 on days of measurement (Kandel et al., 2018).

Fluxes were calculated by linear regression using the routine developed by Kutzbach et al. (2007). Based on visual inspection of the CO_2 flux curve, the first few records after chamber enclosure were discarded as dead-band. Total cumulative emissions of N_2O during the measurement period were calculated using linear interpolation of measured fluxes between the measurement dates.

Measurements of Environmental Variables

Soil temperatures were recorded continuously at 1-h intervals in one of the control plots using TMC-6 soil sensors (Onset Computer Corporation, Bourne, USA). Three soil sensors were placed at 0.05-, 0.10, and 0.15 m soil depths, and the average temperature of the three depths is presented. Air temperature and precipitation data for the study period were obtained from a weather station (Oklahoma Mesonet, Oklahoma Climatological Survey) located roughly 1.0 km from the study site.

Volumetric water content (VWC) was continuously recorded at hourly intervals in the same control plot where soil temperature was recorded using soil moisture sensors (model EC-10; Meter Environment, Pullman, WA). Three sensors were inserted at soil depths of 0–0.05, 0.05–0.10, and 0.10–0.15 m, and an average of three sensors is presented. The VWC was presented as water-filled pore space (WFPS) calculated as relative VWC at saturation. Although some influence of applied treatments on the magnitude of soil moisture and temperature was expected, the dynamics of these variables were mostly expected to be similar. Therefore, temperature and moisture measurements at the control plot were presented to show the dynamics of these variables at the study site.

Analyses of Soil Samples

To determine the concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) in soils, samples were collected from all plots at the 0–0.15 m depth on all 23 dates of flux measurements. Two soil cores (diameter, 0.02 m) were taken within 0.10-m distance from opposite sides of the collars and pooled to form a composite sample for analyses. Aliquots of samples were extracted in 1.0 M KCl, and the flow injection method (Timberline Instruments, Boulder, CO, USA) was used to determine the concentrations of NO_3^- and NH_4^+.

Measurements of Plant Growth, Yield, and Quality of Crabgrass

Canopy reflectance was measured periodically inside the collars using a portable spectro-radiometer (PSR-3500; Spectral Evolution, Lawrence, USA) to monitor the growth of crabgrass non-destructively. Ratio vegetation index (RVI) was calculated as a ratio of canopy reflectance at red and near-infrared (656 and 779 nm, respectively) wavelengths.

All biomass of crabgrass inside the collars was harvested manually on August 16, 2018. The biomass was oven-dried at 60°C to constant weight, and the dried biomass was milled to pass through a 1-mm sieve. Concentration of N in biomass of crabgrass was determined by flash combustion (900°C for 10 min) method. The amount of N uptake per hectare in crabgrass biomass was calculated as a product of biomass yield and N concentration.

Statistical Analysis

The data are presented as averages and standard errors of three plots from a treatment. The differences of measured fluxes among the treatments were determined using a mixed model in SAS 9.4 (SAS Inc., Cary, NC, USA). The normality of data was tested using Shapiro–Wilk test, and homogeneity of variances was tested using Levene's test. The effect of sampling dates was included in the model and treated as repeated measurements for the measured dynamic variables. Effects of applied treatments on dynamics of measured N_2O were analyzed by the model:

$$Y_{tdb} = \mu + \alpha_t + \beta_d + (\alpha\beta)_{td} + C_b + E_{tdb}$$

where Y_{tdb} is the dependent variable for treatment t, day d, and block b; μ is the overall mean response; α_t, β_d, and $(\alpha\beta)_{td}$ are the fixed effect of treatment, measurement date, and interaction between day and treatment. The terms C_b and E_{tdb} are the random effects of block and residuals, respectively. For the variables without measured dynamics (cumulative emissions of N_2O and biomass yield, N concentrations, and N uptake by crabgrass), the effects of applied treatments were analyzed using a similar model without measurement date. Contrasts were used for pairwise comparisons at the 5% level.

Pearson correlation coefficients (R) were applied to test for relationships between accumulated CO_2 and N_2O emissions 7 days (prior to emergence of crabgrass) after cover crop incorporation and to test for correlations of N_2O emissions and soil variables (soil moisture, concentrations of NO_3^- and NH_4^+). Averages of the soil variables and N_2O emissions across treatments at 23 measurement dates were used for the test.

RESULTS

Cover Crop Yield and Biomass Properties

Grass pea produced 2.17 Mg ha^{-1} aboveground biomass with N concentrations of 4.81%, resulting in 104.37 kg N ha^{-1} (**Table 1**). Additionally, grass pea produced 0.30 Mg ha^{-1} root biomass containing 7.86 kg N ha^{-1}. Oat produced 1.6 times greater ($P < 0.05$) amounts of aboveground biomass (3.56 Mg ha^{-1}) than grass pea but had 1.6 times lower N concentration (2.86%), which resulted in similar ($P > 0.05$) amounts of N in aboveground biomass (101.81 kg N ha^{-1}). Yield of root biomass (0.40 Mg ha^{-1}) and its N content (1.81%) in oat were similar to amounts noted for grass pea. The amount of N in root biomass represented <7% of N in total biomass for both species. Cellulose concentrations were similar ($P > 0.05$) in both crops, but hemicellulose concentrations were significantly greater ($P < 0.05$) in oat biomass, and lignin concentrations were significantly ($P < 0.05$) greater in grass pea.

Environmental Conditions

Average daily air temperatures during the 90 day period of flux measurement ranged between 18 and 33°C, while average daily soil temperatures ranged between 21 and 35°C (**Figure 1A**). The average air temperatures for the months of May and June at the study site were 23.2 and 26.2°C, respectively, which was 2.8 and 1.1°C, respectively, higher than long-term (1977–2019) averages for both months (**Supplementary Figure 1a**). In contrast, the average air temperatures of July (27.4°C) and August (25.9°C) were 0.7 and 1.7°C lower than the long-term averages for those months.

A 15-mm rainfall was recorded within the first 2 days after soil incorporation of cover crops, followed by a prolonged period without precipitation (**Figure 1B**). Thereafter, ∼87 mm of rainfall was recorded in mid-June, while a long dry period occurred during the month of July, with ∼33 mm of rainfall received toward the end of the month. The remaining precipitation events were recorded toward the end of the study period in mid-August.

Due to the heavy rainfall event that occurred at the beginning of the study, WFPS was higher for the first few days but decreased

TABLE 1 | Average ($n = 3$) yield and chemical composition of grass pea and oat grown as green N crops.

Properties	Grass pea			Oat		
	Shoot	Root	Total	Shoot	Root	Total
Biomass yield (Mg ha^{-1})	2.17	0.30	2.20*b*[x]	3.56	0.40	3.60[a]
N concentrations (% of DM)	4.81	2.62	3.71[a]	2.86	1.81	2.33[b]
C concentrations (% of DM)	46.32	49.44	47.88[b]	53.67	57.06	55.36[a]
C/N	9.62	18.87	12.90[b]	18.76	31.52	23.75[a]
Total N in biomass (kg ha^{-1})	104.37	7.86	112.23[a]	101.81	7.24	109.05[a]
Cellulose (% of DM)	22.63	20.81	21.72[a]	23.01	21.15	22.08[a]
Hemicellulose (% of DM)	8.11	7.82	7.96[b]	19.46	16.90	18.18[a]
Lignin (% of DM)	8.71	14.01	11.36[a]	5.73	10.41	8.07[b]

[x]*different letters within a row are statistically different ($P < 0.05$).*
C, carbon; DM, dry matter; N, nitrogen.

thereafter due to a long drought. Amounts of soil moisture increased significantly after all major rainfall or irrigation events.

Total precipitation during the months of May, June, and July was 50, 93, and 33 mm, respectively, which was 93, 27, and 34 mm lower than the long-term (1977–2019) average precipitation for those months [143 (± 88), 120 (± 63), and 67 (± 45) mm for May, June, and July, respectively] (**Supplementary Figure 1b**). However, total precipitation in August (109 mm) was 30 mm greater than long-term average [79 (±149) mm]. In total, ∼230 mm of rainfall was recorded at the study sites when flux measurements were recorded during May through August compared to 409 (±63) mm for the long-term average. Therefore, this study was undertaken during a drought-affected summer (56% of long-term precipitation). However, the 60 mm of supplemental irrigation reduced some of this rainfall deficit.

Dynamics of N_2O and CO_2 Emissions

CO_2 emissions were greater from the oat- and grass pea-incorporated plots than the control or grass pea-harvested plots during the first week after incorporation (**Figure 1C**). The emission rates declined subsequently with declining WFPS but increased slightly after the first irrigation event. Greater CO_2 fluxes were recorded from control plots than the plots cultivated with cover crops during days 35–72 after biomass incorporation, as crabgrass grew better in response to the control treatment during the drought period.

Amounts of N_2O emissions were greater from plots with residue incorporated compared to emissions from the control and grass pea-harvested plots, indicating that the decomposing residues of green N crops contributed to N_2O emissions (**Figure 1D**). The N_2O emissions from control and grass pea-harvested plots approximated zero until 22 days after soil incorporation, but few rainfall-induced peaks were recorded thereafter. Emissions of N_2O were observed after rainfall or irrigation events until 85 days after soil incorporation, but emissions did not increase during the last measurement date, which occurred after two successive rainfall events that provided >25-mm moisture. Average emissions from oat-incorporated plots (14.02 g N_2O-N ha^{-1} d^{-1}) were significantly greater ($P <$ 0.05) than average emissions from the grass pea-incorporated plots (8.52 g N_2O-N ha^{-1} d^{-1}). Likewise, average N_2O emissions across measurement dates from grass pea-incorporated plots were significantly greater than the average emissions from grass pea-harvested plots (5.36 g N_2O-N ha^{-1} d^{-1}).

Dynamics of Soil Mineral N

Soil NH_4^+ concentrations remained low and stable in response to all treatments during the first 20 days of the study but increased after the first irrigation event on day 23 (**Figure 1E**). Effects of sampling dates on soil NH_4^+ concentrations were significant, while there were no significant differences between applied treatments except three sampling dates (days 12, 19, and 27). Soil NH_4^+ concentrations were significantly greater in oat- and grass pea-incorporated plots compared to concentrations in control and grass pea-harvested plots on days 12 and 19 after soil incorporation. On day 27 after soil incorporation, soil NH_4^+ concentrations were significantly greater in oat-incorporated plots than that in response to the other treatments.

There were decreases in soil NO_3^- concentrations on days 66 and 84 after soil incorporation (**Figure 1F**). Average soil NO_3^- concentrations across sampling dates remained statistically similar among the treatments, ranging between 10.84 and 13.38 mg kg^{-1} soil. Additionally, the average of weekly NH_4^+ and NO_3^- concentrations and amount of soil moisture were significantly ($P < 0.05$) correlated with average weekly N_2O emissions, with Pearson's correlation coefficient (*R*) of 0.28, 0.24, and 0.33, respectively (**Supplementary Figure 2**).

Cumulative N_2O Emissions

The cumulative N_2O emissions from the oat-incorporated plots were significantly greater ($P < 0.05$) than emissions from other treatments (**Figure 2**). Additionally, cumulative N_2O emissions from grass pea-incorporated and control plots were 58–66% greater ($P < 0.05$) than cumulative emissions from grass pea-harvested plots (**Figure 2**). There was no significant difference between cumulative N_2O emissions from grass pea-incorporated and control plots. The recorded CO_2 emissions during the first 7 days of the study primarily represented heterotrophic respiration due to the absence of green plants. Cumulative emissions of CO_2 and N_2O from individual collars at this time correlated (*R*

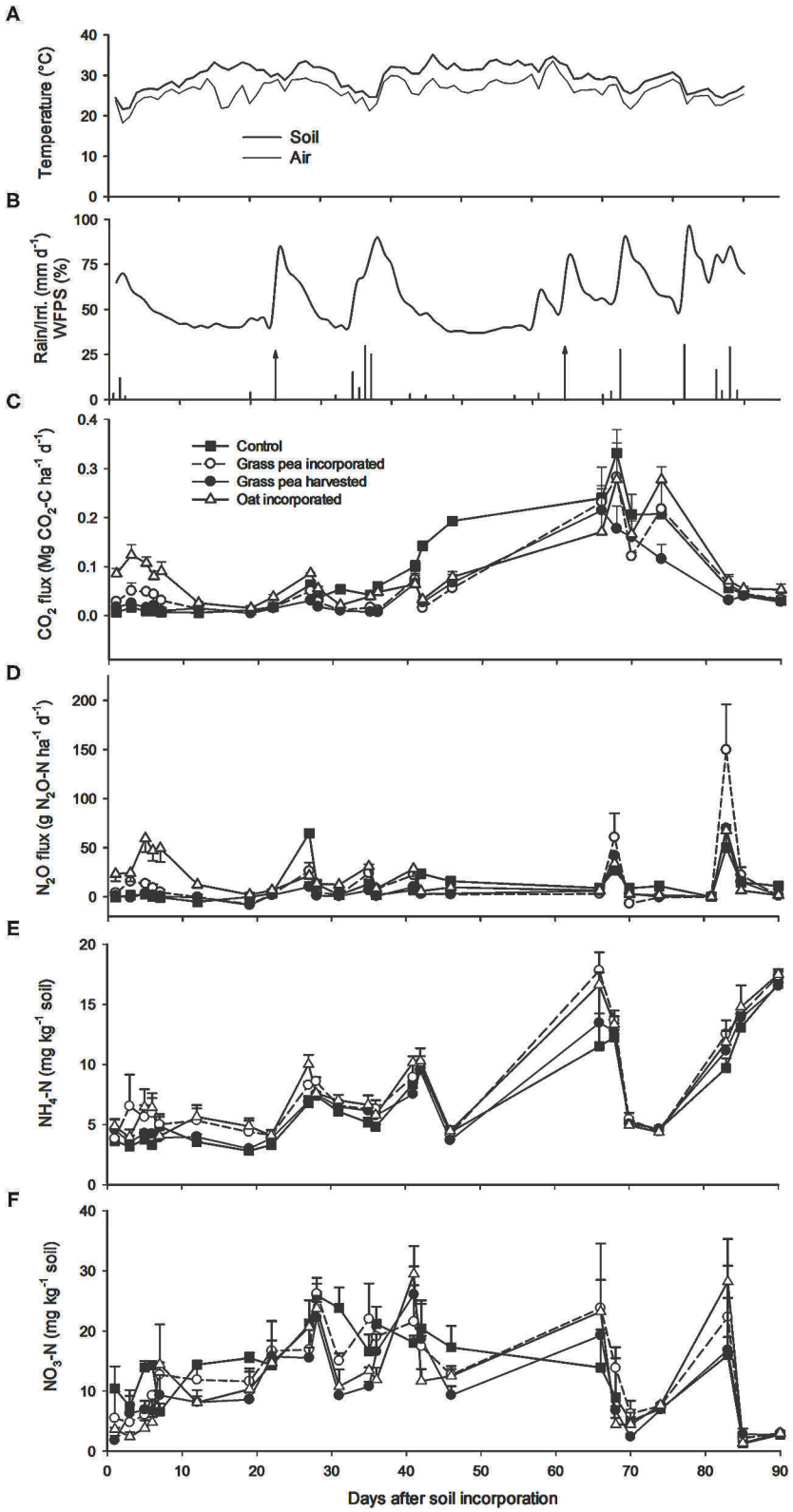

FIGURE 1 | (A) Average soil and air temperatures during flux measurements. **(B)** Dynamics of daily precipitation (black bars), irrigation amount (gray bars with arrows), and water-filled pore space (WFPS) measured at 0–0.15 m soil depth (black line) during flux measurement. Dynamics of **(C)** carbon dioxide (CO_2), **(D)** nitrous oxide (N_2O) emissions, **(D)** ammonium (NH_4^+), and **(E)** nitrate (NO_3^-) N in the 0–0.15 m soil depth. Unidirectional error bars are shown for clarity. Error bars **(C–F)** represent standard error ($n = 3$).

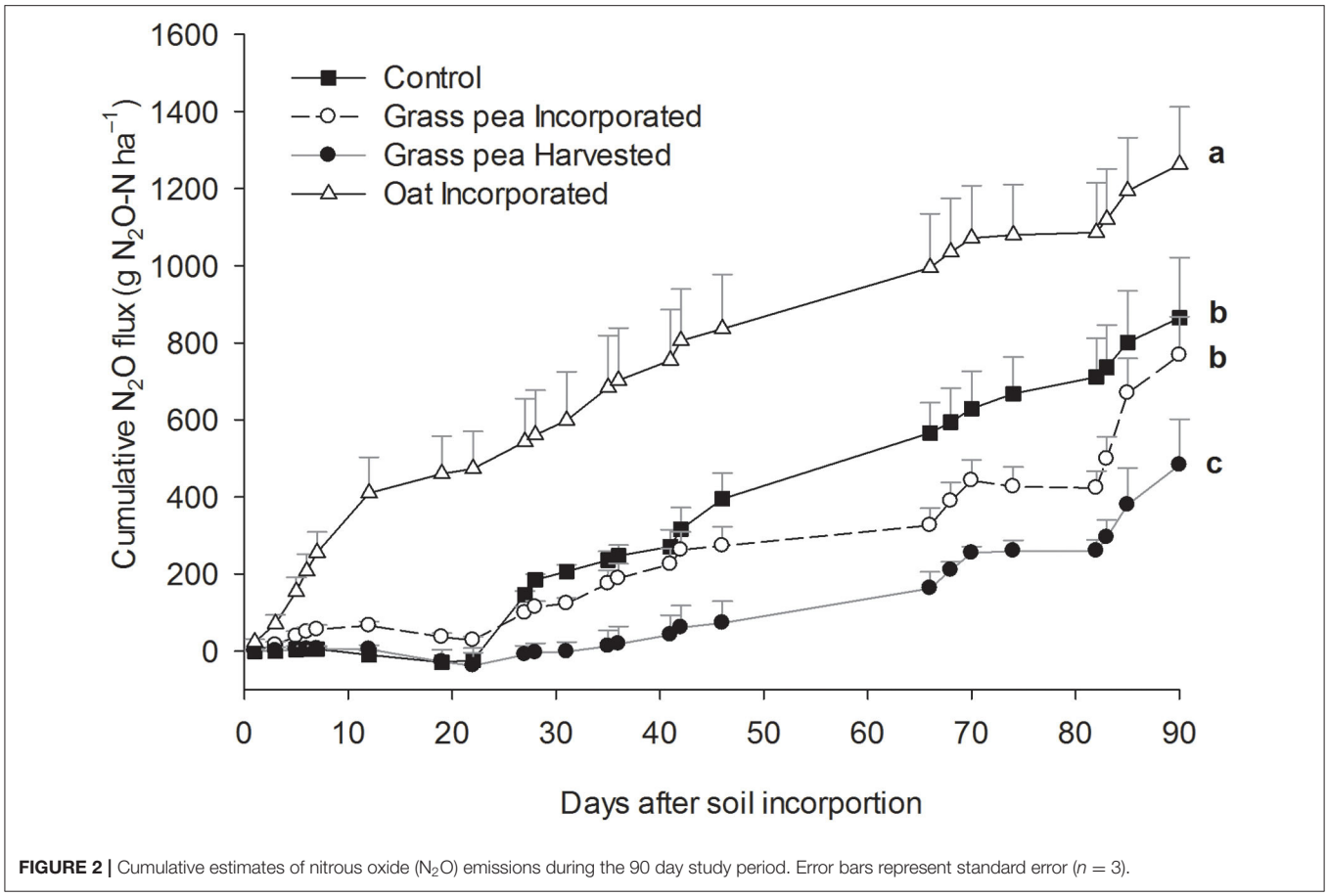

FIGURE 2 | Cumulative estimates of nitrous oxide (N_2O) emissions during the 90 day study period. Error bars represent standard error ($n = 3$).

$= 0.97$; $P < 0.0001$) strongly with each other, indicating rapid contribution of decomposing biomass of cover crops to N_2O fluxes (**Figure 3**).

Biomass Growth, Yield, and N Concentrations and Uptake of Crabgrass

Biomass growth of crabgrass measured as RVI was significantly greater ($P < 0.05$) from the control plots on days 41 and 46 after incorporation of cover crops compared to the other treatments and remained nominally greater thereafter (**Figure 4**). Crabgrass germination occurred in all plots 7 days after planting, but growth by crabgrass on plots assigned to spring cover crops was severely affected by drought. Crabgrass expressed classic symptoms of drought stress, including dark bluish-green rolled leaves and small plant size. A rapid increase in RVI was observed on control plots after irrigation on day 23, while such increases were observed in response to the other treatments after a rainfall on day 45.

The biomass yield of crabgrass in response to the control was roughly twice ($P < 0.05$) the yields generated by crabgrass in response to cover crops (**Figure 5A**). The N concentrations of crabgrass biomass produced on grass pea- and oat-incorporated plots were significantly greater than concentrations in crabgrass biomass produced by the control and grass pea-harvest treatments (**Figure 5B**). However, there were no significant differences among treatments for total N accumulated in crabgrass biomass (**Figure 5C**).

DISCUSSION

Our hypothesis of lower N_2O emissions from decomposing oat residue than the grass pea residue was rejected, as results showed the opposite. In particular, emissions from oat-cultivated plots were greater than from grass pea-cultivated plots during the first 2 weeks after soil incorporation. Generally, greater amounts of N_2O emissions are expected from decomposing legume residues, as N in low C/N ratio legume residues mineralizes rapidly after soil incorporation, increasing amounts of NO_3^- in soil pools, which serves as a substrate for denitrification (Baggs et al., 2000; Huang et al., 2004; Gomes et al., 2009; Basche et al., 2014). However, this response was not observed in the current study.

A possible reason for the greater level of N_2O emissions from the oat treatment could be the greater amounts of C provided by oat biomass at termination, which increased the amounts of mineralizable C for denitrification as indicated by greater CO_2 fluxes (Cameron et al., 2013). Additionally, since the amount of N in oat biomass was comparable to that in biomass of grass pea, oats scavenged available soil N that was released by denitrification after termination. Therefore, greater denitrification rates due to greater availability of mineralizable C, combined with the large

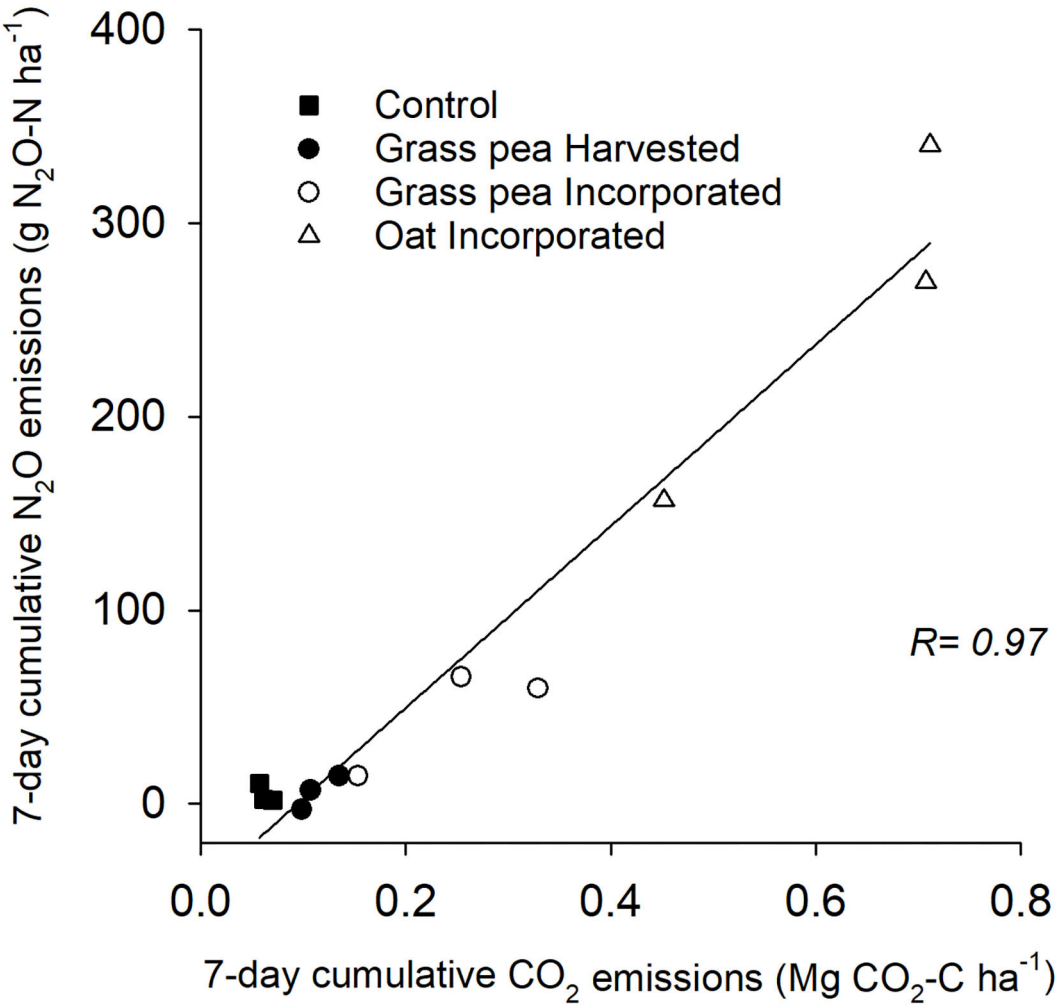

FIGURE 3 | Correlation of cumulative carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions from individual collars across the treatments during the first 7 days of soil incorporation of cover crop biomass. Pearson's correlation coefficient (*R*) between the cumulative emissions is shown.

amounts of N noted in oat biomass, may have contributed to the greater N_2O emissions from oat-incorporated plots.

This study noted that the cumulative N_2O emissions from control plots were not significantly different from the N_2O emissions generated by the grass pea-incorporated plots. A possible explanation for this similarity might be greater accumulation of N by grass pea (through N fixation and scavenging N from soil) during growth than was actively mineralized from biomass after soil incorporation. This assumption was supported by generally greater nitrate availability in control plots during the initial stages of the experiments.

Previous studies have reported that residues of cover crops could enhance N_2O emissions from agricultural soils after incorporation, though mitigation is possible by management of the residues of cover crops (Sanz-Cobena et al., 2014; Kim et al., 2017; Kandel et al., 2019a; Singh et al., 2020). Residues of cover crops that are incorporated into soil generally increase mineralizable C and NO_3^- contents in soils, which are conducive for N_2O emissions (Mitchell et al., 2013). In the current study, the increase in mineralizable C in residues within the incorporation treatments was evidenced by greater CO_2 emissions compared to control and grass pea-harvested treatments during the first 7 days of the study (before germination of crabgrass), though average concentrations of soil NO_3^- remained similar among the treatments. Thereby, it can be deduced in the current study that increased soil-mineralizable C provided by residues of incorporated cover crops had possibly stronger influences on N_2O emissions than N mineralized from soil-incorporated residues.

Poor responses of growth and yield of crabgrass in response to biomass removal of grass pea might be due to low fertilizer values for the remaining residues, since soil in this study is considered highly fertile. In a nearby site with less fertile soil, biomass removal of hairy vetch (*Vicia villosa*) grown as a green N crop resulted in poor growth and yield of crabgrass in the same season as the current study (Kandel et al., 2019a).

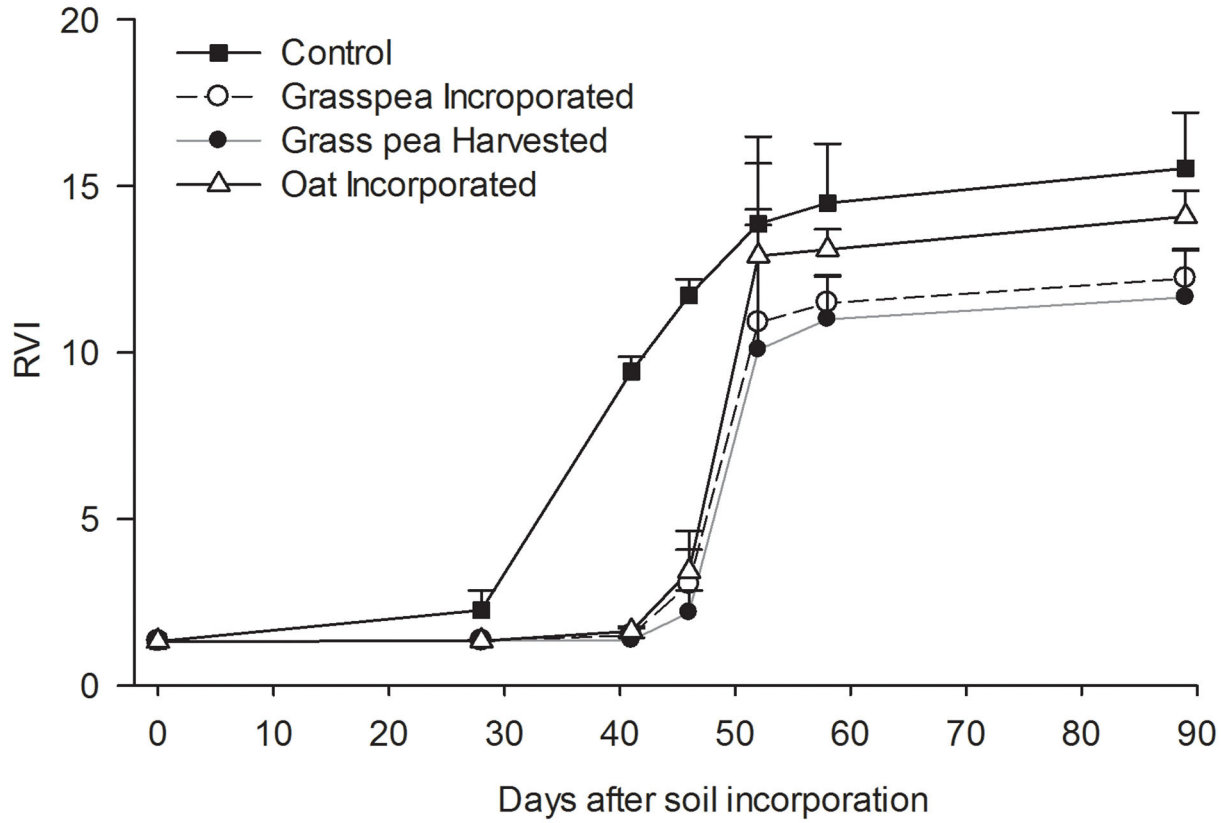

FIGURE 4 | Dynamics of ratio vegetation index (RVI) measured as a proxy for green biomass of crabgrass. Error bars represent standard error ($n = 3$). Unidirectional error bars are shown for clarity.

These results from two contrasting sites within the same year indicate management applied to green N crops, such as biomass removal, should be based on soil types and their fertility status.

Yields generated by crabgrass in response to the control treatment were significantly greater than yields generated by treatments that included cover crops. This response was likely due to depletion of available soil moisture by the cover crops. The total precipitation received during the growing period of the cover crops, and the following summer crop, was lower than the long-term average precipitation, although supplemental irrigation was applied to help alleviate some of the deficit. This response indicated that replacing a period of spring fallow with a spring-grown cover crop can negatively affect yields by the following summer crop, particularly during dry years. Such depletion of the limited pools of available soil moisture is a common phenomenon in double-cropped systems throughout the drought-prone US Great Plains (Nielsen et al., 2002; Rao and Northup, 2009; Aiken et al., 2013).

Crabgrass biomass produced on grass pea-incorporated plots contained ∼40 kg ha^{-1} more N than crabgrass produced on grass pea-harvested plots, though these responses were not significantly different. This response indicated that ∼39% of N in aboveground biomass of grass pea was transferred to crabgrass. These transfers indicate some degree of function for soil-incorporated residues of cover crops to serve as organic forms of N fertilizer. However, these levels of transfer are well below levels reported for applied inorganic N. Northup and Rao (2016) reported wheat biomass grown in rotation with summer legume-based green N crops contained 32% (±6%) of the amount of N in hay crops than was recorded in response to the recommended amount of N fertilizer (80 kg N ha^{-1}). Similar differences were recorded in N accumulated in wheat grain in response to green and inorganic N sources (Kandel et al., 2019b).

The effectiveness of the cover crops as source of N to following crops mainly depends on the amount of N in their biomass (Kaye et al., 2019; Singh et al., 2020). Additionally, the chemical properties of biomass, particularly cell wall fractions that govern decomposition and mineralization, are also important for the transfer of nutrients from residues of cover crops to recipient crops since the amounts of such properties in biomass govern the speed of decomposition by soil microbes (White et al., 2014; Singh et al., 2020). Although we expected better N fertilizer value from grass pea due to biological N fixation and higher N content in biomass, crabgrass performed at similar levels under both grass pea and oat cover crops. This might be due to a greater amount of uptake of soil N and recycling of N to soil by oat than grass pea

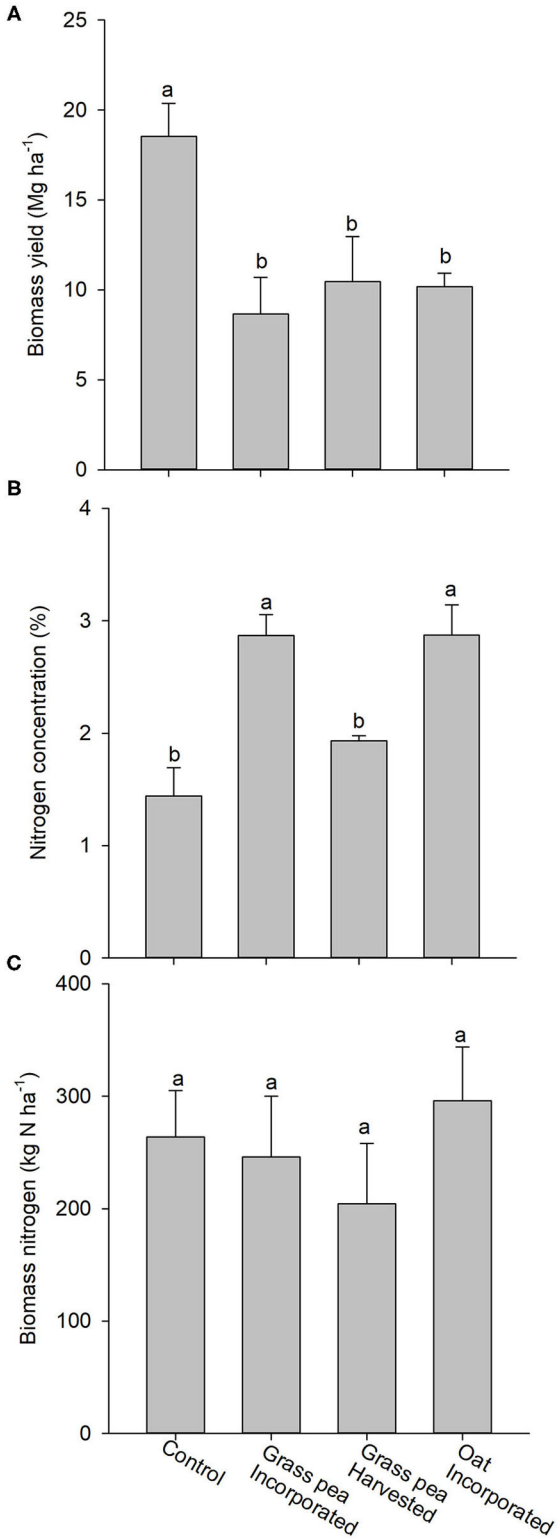

FIGURE 5 | (A) Mean aboveground biomass produced by crabgrass after 90 days of growth. **(B)** Nitrogen (N) concentrations of the harvested biomass of crabgrass. **(C)** Total amount of N in the harvested crabgrass biomass. Error bars represent standard error ($n = 3$). Different letters on the top of bars indicate statistical difference ($P < 0.05$).

since the total amount of N in both crops was similar. Also, oat biomass was terminated at an earlier growth stage (∼60 days after emergence) than full maturity, so the less-mature biomass would decompose rapidly, as noted in the larger CO_2 fluxes compared to responses to grass pea.

CONCLUSIONS

This study showed that the use of short-duration spring crops that are grown as sources of green N is not a straightforward process, with responses driven by interactions among the chosen crop and its productivity, growing conditions, and availability of soil moisture. We observed that post-incorporation N_2O emissions were greater from oat crops that were incorporated compared to an incorporated legume-based crop grown for green N. Although greater amounts of N_2O emissions were expected from grass pea, the higher amounts of oat biomass, which provided similar amounts of N per unit area, resulted in greater amounts of mineralizable C that contributed to greater levels of N_2O production.

We also noted that the form of management applied to the biomass produced by crops has the potential to mitigate the production of greenhouse gases for decomposing residues, though some forms will limit the availability of N for following crops. The 90 day cumulative emissions of N_2O from the grass pea plots that were harvested generated half the emissions noted from plots where grass pea biomass was incorporated. This response showed that incorporated aboveground biomass of green N crops have the capacity to act as major sources of N_2O emissions and the potential to mitigate these emissions by harvesting biomass for forage.

Results also recorded that yields of crabgrass in response to spring-grown green N crops or as hay, can be negatively affected during years where drought periods occur during spring through summer. Plots receiving spring-planted crops generated half the production of the control plots, which included spring fallowing. This response indicated that replacing spring periods of fallow with short-duration crops grown for green N or hay can severely affect the yield of following summer forages during dry years.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

HS and TK contributed to the conceptualization, methodology, and investigation. HS contributed to the formal analysis and writing of the original draft preparation. PG and BN contributed to the resources. TK, PG, BN, and VK contributed to the writing, review, and editing. TK contributed to the supervision. BN and PG contributed to the project administration. PG contributed to the funding acquisition. All authors have read and agreed to the published version of 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/fsufs.2020.604934/full#supplementary-material

Supplementary Figure 1 | (a) Average long-term and study year air temperature for the months of May, June, July, and August. **(b)** Average long-term and study year precipitation for the months of May, June, July, and August.

Supplementary Figure 2 | Correlation matrices with Pearson's correlation coefficients (R) of nitrous oxide (N_2O) emissions and soil variables on 25 measurement dates during the study period. EC, electrical conductivity.

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frontiers in Sustainable Food Systems

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Effects of Three Types of Organic Fertilizers on Greenhouse Gas Emissions in a Grassland on Andosol in Southern Hokkaido, Japan

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Reduction of chemical fertilizers and effective use of livestock excrement are required for the realization of sustainable agriculture and reduction of greenhouse gas (GHG) emissions. The purpose of this study was to estimate the reduction rate of GHG emissions represented by comparing global warming potential (GWP) using organic fertilizers instead of chemical fertilizers. The study was conducted in a managed grassland on Andosol in southern Hokkaido for 3 years from May 2017 to April 2020. There were five treatment plots: no fertilizer, chemical fertilizer, manure, slurry, and digestive fluid. Organic fertilizers were applied such that the amount of NPK did not exceed the recommended application rate, and the shortage was supplemented with chemical fertilizers. Fluxes in CO_2 caused by heterotrophic respiration (RH), CH_4, and N_2O were measured using the closed chamber method. Net ecosystem carbon balance (NECB) was obtained as net primary production + organic fertilizer application—RH—harvest. The GWP was estimated by CO_2 equivalent NECB and CH_4 and N_2O emissions in each treatment. Chemical fertilizer nitrogen application rates in the organic fertilizer treatments were reduced by 10% for manure, 19.7% for slurry and 29.7% for digestive fluid compared to chemical fertilizer only, but the grass yields were not significantly different among the fertilizer treatments. The 3-year NECB showed significantly smallest carbon loss in manure treatment, and smaller carbon loss in the organic fertilizer treatments than in the chemical fertilizer only. The reduction rate in the GWP with use of organic fertilizers relative to that of chemical fertilizer was 16.5% for slurry, 27.0% for digestive fluid, and 36.2% for manure. The NECB accounted for more than 90% of the GWP in all treatments. CH_4 emissions were $< 0.1\%$ of the GWP. On the other hand, N_2O emissions accounted for more than 5% of the GWP, and was larger in the order of slurry > chemical fertilizer only > digestive fluid > manure. As a conclusion, these organic fertilizers can be used without no reduction of crop yield instead of chemical fertilizer, however, manure is the best way to increase soil carbon and to decrease GWP, followed by digestive fluid.

Keywords: CH_4, global warming potential, manure, methane fermentation digestive fluid, slurry, N_2O, soil carbon sequestration

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INTRODUCTION

The anthropogenic impact on the climatic system has increased annually, and greenhouse gas (GHG) emissions in 2018 reached a record high of 55.3 Gt CO_2 eq yr^{-1} (UNEP, 2019). Approximately 24% of the GHG emissions come from agriculture, forestry and other land use (AFOLU) (IPCC, 2014). Mitigation in the AFOLU sector is urgently needed.

Soil is the largest carbon storage pool, approximately twice the amount of carbon in the atmosphere and three times the amount in terrestrial biomass (Schlesinger and Jeffrey, 2000). However, agricultural soil looses soil carbon because of organic matter decomposition and erosion, and its recovery is required (Lal, 2020). Furthermore, agriculture is the largest source of CH_4 and N_2O (Blandford and Hassapoyannes, 2018). Therefore, improvement of carbon storage in farmland and reduction of CH_4 and N_2O emissions from farmland are important as climate change mitigation measures in agriculture.

Grasslands are a very important ecosystem for the production of herbivorous livestock (Soussana et al., 2007). Because grasslands are not tilled for several years to several decades, the organic matter content in the surface soil increases because of plant residues, livestock excreta, or organic matter derived from applied manure (Ciais et al., 2013). A 3-year study in valley inland and coastal grasslands in California showed that manure application increased soil carbon by 26 and 37%, respectively (Ryals et al., 2014). These show the soil carbon sequestration reducing the concentration of CO_2 in the atmosphere (Paustian et al., 1997). Therefore, when organic matter application and no-tillage are adapted continuously, grasslands are expected to exhibit climate change mitigation effects as a carbon storage in agricultural soil.

Evaluation of carbon storage in agricultural land includes carbon output from the harvest system and carbon input into the system by the application of organic matter in addition to carbon cycling in the ecosystem through the atmosphere, plants, and soil (Shimizu et al., 2009). Studies on the net ecosystem carbon balance (NECB) and GHG balance caused by manure application in southern Hokkaido, Japan showed that although CO_2 emissions increased because of manure application, there were no differences in CH_4 and N_2O emissions, and the carbon input from manure application reduced the global warming potential (GWP) (Mukumbuta et al., 2017a). However, N_2O emissions in the manure and chemical fertilizer combinedly applied grasslands tended to be higher than the chemical fertilizer only applied grasslands (Shimizu et al., 2013). A study comparing the difference in CH_4 and N_2O emissions from soil with manure or slurry application in grasslands in northern Tochigi Prefecture, Japan, showed no significant difference between the two organic fertilizers (Mori and Hojito, 2015). Research on the environmental factors controlling CO_2, N_2O, and CH_4 fluxes has shown that CO_2 flux has a significant relationship with soil temperature (Shimizu et al., 2009), N_2O flux increased from 60% water-filled pore space (WFPS) peaking at 80% WFPS (Katayanagi et al., 2008), and CH_4 was normally absorbed by soil but the CH_4 uptake decreased with nitrogen application (Hu et al., 2002), and CH_4 emitted from poorly drained soil (Shimizu et al., 2013).

In recent years, the use of livestock manure for methane fermentation has increased from the perspective of treating livestock excrement (Holm-Nielsen et al., 2009). Biogas energy can be obtained by fermenting livestock manure. Utilizing the methane fermentation digestive fluid, which is the fermentation residue, as liquid fertilizer not only prevents the outflow of pollutants into rivers, but also provides a supply of nutrients to farmlands and resource-recycling for livestock farming. Despite being fermented, the methane fermentation digestive fluid can be used as a liquid fertilizer with the same components as the raw slurry material (Matsunaka et al., 2003) and has no foul odor as compared with the slurry (Immovilli et al., 2008).

Different organic fertilizers show different physicochemical properties (Harada et al., 1993; Mori and Hojito, 2015). In particular, methane fermentation digestive fluid tends to have a higher pH and a higher ammonium nitrogen concentration than slurries (Yuyama et al., 2007). Therefore, application of digestive fluid makes soil nutrient status and can reduces the application of chemical fertilizer. Furthermore, increase of soil pH decreased N_2O emissions (Mukumbuta et al., 2018). However, digestive fluid had lower C/N ratio than raw slurry (Holly et al., 2017), which can increase N_2O emission (Toma and Hatano, 2007). On the other hand, a study in Wisconsin showed there was no significant difference of N_2O production between digestive fluid and raw slurry applications (Holly et al., 2017).

Effect of organic fertilizers on soil moisture is also an important factor. Digestive fluid has a high water content and increases soil moisture just after the application. Soil moisture is a significant factor influencing nitrogen mineralization, nitrification and denitrification, which strongly influence N_2O production in soil (Linn and Doran, 1984). Also increase of soil moisture may increase CH_4 in upland fields, and the CH_4 emission remaining in the digestive fluid during the fermentation reaction can occur after application to upland fields (Nakamura et al., 2008).

Since organic fertilizers do not always contain NPK in the best balance for crop growth, it is necessary for farmers to properly manage nutrients for the application of organic fertilizer. For this, for example in Hokkaido, the local government suggests an upper limit of the application rate of organic fertilizer to prevent excessive nutrients being applied and recommends that insufficient nutrients induced by this is supplemented using chemical fertilizers (Hokkaido Government Agricultural Department, 2015). Therefore, NPK composition of organic fertilizers influence the application rate of organic fertilizer and reduction rate of chemical fertilizer, which influence NECB and GHG balance.

Therefore, in this study, influences of three organic fertilizers (manure, slurry and digestive fluid) treatments on NECB and GHG balance in grassland are compared with chemical fertilizer only and control (no fertilizer) treatments. The GHG emissions from grassland soil, crop growth and harvest and organic matter application with the five treatments for 3 years in a grassland southern Hokkaido, Japan were measured. The emission factor of N_2O in managed upland soil which is used in the IPCC

guideline for the National GHG Inventory Report (IPCC, 2006) was also calculated.

In this study, following results were expected: (1) The three types of organic fertilizers have the similar effect of fertilization and can reduce the amount of chemical fertilizer application rate; (2) N_2O emissions are lower in organic fertilizer treatments than in chemical fertilizer only treatment due to the reduction in chemical fertilizer nitrogen application rate; (3) The contribution of CH_4 emissions to total GHG emissions is small; (4) NECB becomes manure > slurry > digestive fluid treatments, which is significantly larger than that in chemical fertilizer only treatment.

MATERIALS AND METHODS

Study Site

This study was conducted in a grassland cultivating reed canary grass in the Shizunai Experimental Livestock Farm, Field Science Center for the Northern Biosphere of Hokkaido University in Southern Hokkaido, Japan (Shizunai) (42°26′05.4″N, 142°28′52.1″ E) from May 2017 to April 2020. The study site has a humid continental climate, with cold winters and cool summers. The average temperature over the past 10 years (2007–2016) was 8.4°C, annual rainfall was 1,273 mm, deepest monthly snow was 1 to 22 cm, and snowfall of 10 cm or more was observed from December to March.

The soil was derived from Tarumae (b) volcanic ash, and the mottled upper end of the layer appears within the 0–50 cm soil horizon, and consequently was classified as Wet Andosols (The Fifth Committee for Soil Classification and Nomenclature of the Japanese Society of Pedology, 2017). The soil properties of the 0–7 cm surface layer (Ap1) were pH (H_2O) 5.64 ± 0.04, total carbon 36.7± 1.74 g kg^{-1}, total nitrogen 2.7± 0.05 g kg^{-1}, and C/N ratio 13.4. Before 2017, when this research began, the study site had been used as a grassland since 2009, and fertilization with chemical fertilizer and harvest were conducted twice a year. From 2009 to 2016, nitrogen, phosphorus, and potassium were applied as chemical fertilizer at an average of 86 kg T-N ha^{-1} yr^{-1}, 71 kg P_2O_5 ha^{-1} yr^{-1}, and 104 K_2O ha^{-1} yr^{-1}, respectively. Except for 2010, 10 Mg FM ha^{-1} yr^{-1} of manure was applied every year after September when the second grass was harvested, and liquid urine fertilizer was also applied in 2012.

Fertilization Treatments and Field Management

The study period consisted of 3 years from May 14, 2017 to April 26, 2020, including May 14, 2017 to April 26, 2018 (348 d), April 27, 2018 to April 26, 2019 (365 d), April 27, 2019, to April 26, 2020 (366 d). Fertilization was conducted twice a year with a base fertilizer (spring) and supplement fertilizer (summer). Harvest was performed twice a year for the first and second grasses. In this study, five treatments of fertilization were tested: no fertilizer (N), chemical fertilizer (F), manure (M), slurry (S), and digestive fluid (D). Fifteen subplots of 5 × 10 m were set up in five treatments × three replicates in a random block design. In each subplot, a 5 × 8 m vegetation survey area, a 5 × 2 m gas sampling area, and a 50 × 50 cm bare area, excluding roots, was set up in the gas sampling area. In the bare area, a root permeable sheet (BKS9812, TOYOBO CO. Ltd., OSAKA, Japan) was inserted at a depth of ∼30 cm at the boundary with the planting area to prevent the entry of roots. Plants growing in the bare area during the survey period were regularly removed by hand.

Organic Fertilizer Used

Manure, slurry, and digestive fluid were used as organic fertilizers. Every year, the manure used was from Shizunai, and the digestive fluid was from Sapporo Experimental Farm, Field Science Center for the Northern Biosphere of Hokkaido University (Sapporo) (43°04′41.1″N, 141°20′03.6″E). The manure was made from a mixture of cow excreta, horse excreta, and bedding litter and turned over once every 10 days during winter. The slurry used was from Shizunai in 2017. However, the Shizunai slurry had a high water content and a low nitrogen content because it was mixed with rainwater. Due to this, in 2018 and 2019, Sapporo slurry was used. The slurry in Shizunai was from cow excreta, horse excreta, and rainwater and was stored in a slurry reservoir in the barn until use. The slurry in Sapporo was made from cattle, pig, and chicken excreta, and water was added as appropriate to increase fluidity. The digestive fluid used was from Sapporo, which was made from methane fermentation of the slurry in Sapporo. Each organic fertilizer was collected 1 month before application, and water content, pH, TN, NH_4^+-N, P, K, and TC were analyzed. The components of each organic fertilizer are shown in **Table 1**.

Design of Fertilization

Table 2 shows the application rates of organic and chemical fertilizers for each year. The nitrogen application rate depended on the legume rate (Hokkaido Government Agricultural Department, 2015). In 2017 and 2019, because the legume rate was 5–15%, nitrogen application rate was 100 kg ha^{-1}. On the other hand, in 2018, the nitrogen application rate increased to 160 kg ha^{-1} because the legume rate decreased to < 5%. The organic fertilizer application rate was determined such that the organic fertilizer N, P, or K application rates did not exceed the recommended application rate of N, P, or K, and any shortage in N, P, or K was made up by chemical fertilizers. Chemical fertilizer was applied at the ratio of application at the base: supplement of 2:1, whereas organic fertilizer was applied only used as a base application. Both chemical and organic fertilizers were applied by top dressing.

Measurements

Environmental Factors

Daily air temperature and precipitation were obtained from the close Automated Meteorological Data Acquisition System (AMeDAS) station of the Japan Meteorological Agency, which are located about 14 km from the study site for air temperature and about 100 m from the study site for precipitation. Soil temperature at a 5 cm depth was measured at the same time as the gas flux measurements using a thermistor thermometer (CT-414WR, CUSTOM, Tokyo, Japan), and volumetric soil moisture content at 0–6 cm depth was measured using the frequency domain reflectometry (FDR) method (DIK-311A; Daiki, Saitama,

TABLE 1 | Chemical components of organic fertilizer used.

		Water content	pH	TN	NH_4^+-N	P_2O_5	K_2O	TC	C/N
		%		%FM	%FM	%FM	%FM	%FM	
2017	M	70.0 ± 0.49	8.39 ± 0.06	0.34 ± 0.17	0.02 ± 0.00	1.27 ± 0.38	3.81 ± 0.47	9.49 ± 0.50	28.07
	S	96.8 ± 0.05	7.93 ± 0.01	0.11 ± 0.02	0.07 ± 0.00	0.07 ± 0.02	1.53 ± 0.06	0.79 ± 0.01	7.15
	D	95.7 ± 0.05	7.85 ± 0.01	0.19 ± 0.03	0.13 ± 0.00	0.27 ± 0.10	0.43 ± 0.04	1.56 ± 0.06	8.36
2018	M	78.4 ± 0.88	8.52 ± 0.32	0.62 ± 0.02	0.09 ± 0.03	3.43 ± 0.22	2.45 ± 0.31	8.95 ± 0.50	14.33
	S	92.9 ± 0.10	7.22 ± 0.03	0.21 ± 0.01	0.19 ± 0.00	0.53 ± 0.00	0.58 ± 0.01	2.93 ± 0.09	13.77
	D	94.9 ± 0.14	7.79 ± 0.03	0.19 ± 0.00	0.18 ± 0.01	0.49 ± 0.02	0.53 ± 0.09	1.95 ± 0.04	9.88
2019	M	73.6 ± 0.88	7.91 ± 0.07	0.58 ± 0.04	0.10 ± 0.00	1.73 ± 0.39	3.08 ± 0.39	11.1 ± 0.23	19.17
	S	93.1 ± 0.09	6.05 ± 0.00	0.24 ± 0.01	0.12 ± 0.00	0.37 ± 0.12	0.36 ± 0.04	2.98 ± 0.17	12.22
	D	95.1 ± 0.08	7.56 ± 0.05	0.24 ± 0.00	0.13 ± 0.00	0.24 ± 0.12	0.36 ± 0.12	2.08 ± 0.04	8.81

Values represent mean ± standard deviation. FM is the fresh weight, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot. n = 3.

TABLE 2 | Annual organic fertilizer application rate each year, chemical fertilizer application to chemical fertilizer plots, and chemical fertilizer supply to organic fertilizer plots.

		Organic fertilizer						Chemical fertilizer		
		Application rate	TC	TN	P_2O_5	K_2O	C/N	TN	P_2O_5	K_2O
		Mg FM ha^{-1}	kg ha^{-1}					kg ha^{-1}		
2017	N	0	0	0	0	0	–	0	0	0
	F	0	0	0	0	0	–	100	80	180
	M	8.4	797	28	106	320	28.1	95	59	0
	S	19	152	21	14	293	7.2	93	64	0
	D	58	914	109	154	247	8.4	65	38	0
2018	N	0	0	0	0	0	–	0	0	0
	F	0	0	0	0	0	–	160	80	180
	M	9.0	806	56	309	221	14.3	139	5.2	0
	S	38	1,115	81	204	221	13.8	127	0	0
	D	26	508	51	130	140	9.9	123	27	60
2019	N	0	0	0	0	0	–	0	0	0
	F	0	0	0	0	0	–	100	80	180
	M	7.4	828	43	128	228	19.2	90	23	0
	S	62	1,849	151	232	227	12.2	69	0	0
	D	42	874	99	101	153	8.8	66	40	58

FM is the fresh weight, N is the no fertilizer plot, F is the chemical fertilizer plot, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot.

Japan). The water-filled pore space (WFPS,%) was calculated as:

$$WFPS = \left(\frac{\theta}{p}\right) \times 100 \quad (1)$$

where θ is volumetric soil water content ($m^3\ m^{-3}$) and p is soil porosity ($m^3\ m^{-3}$); p was measured by a three-phase meter (DIK-1150; Daiki, Saitama, Japan).

Soil sampling was conducted at the same time as the gas flux measurement during from April to November when the soil was not frozen. The collected soil was sieved at 2 mm. Soil NO_3^--N content was determined by water extraction with the ratio of soil: deionized water = 1:5, and the NO_3^--N concentration in the water extraction was measured by ion chromatography (DIONEX ICS-1100; Thermo Fisher Scientific, MA, USA). Soil NH_4^+-N content was determined by KCl extraction with the ratio of soil:KCl (2 mol L^{-1}) = 1:10, and the NH_4^+-N concentration in the KCl extraction was measured by the indophenol blue method.

Gas Fluxes

Soil CO_2, CH_4, and N_2O fluxes were measured by the static closed chamber method (Toma and Hatano, 2007). In the gas sampling area of each treatment, chambers made of stainless steel were installed. Chambers with a diameter of 40 cm and a height of 30 cm were used to measure CH_4 and N_2O fluxes in the planting area, and those with a diameter of 20 cm and a height of 25 cm were used to measure the CO_2 flux in the bare plot, which was assumed to correspond to microbial heterotrophic respiration (RH). The chambers were placed onto bases, which

were permanently installed during the measurement period. The chamber bases were inserted into the soil to a depth of 5 cm for at least 12 h before the first gas sampling. During the snowfall period, chamber bases were set up directly onto the snow (Katayanagi and Hatano, 2012). Gas flux measurements were performed between 8:00 and 13:00 for seven consecutive days after the application of the fertilizer, once a week during the plant growing season and once a month during winter. Changes in the concentrations of CO_2, CH_4, and N_2O in the headspace of the chambers with time was measured according to a previously reported procedure (Nakano et al., 2004; Toma and Hatano, 2007; Shimizu et al., 2013), that is, gas samples in the chamber headspace were taken at 0 and 6 min for CO_2 and 0, 15, and 30 min for CH_4 and N_2O after closing chambers by using a 25 mL gas tight syringe. A gas sample of 250 mL was injected into a 500 mL Tedlar bag for CO_2. For CH_4 and N_2O, each gas sample (20 mL) was placed into an evacuated glass vial (10 mL). The CO_2 concentration was determined using an infrared CO_2 analyzer (Model ZEP9GC11; Fuji Electric, Tokyo, Japan), and the CH_4 and N_2O concentrations were determined using gas chromatography equipped with a flame ionization detector (GC-8A; Shimadzu, Kyoto, Japan) and an electron capture detector (GC-14B; Shimadzu, Kyoto, Japan), respectively.

The gas flux from the soil was calculated using the following linear regression equation (Toma et al., 2011):

$$F = \rho \times \left(\frac{V}{A}\right) \times \left(\frac{\Delta c}{\Delta t}\right) \times \left(\frac{273}{T}\right) \times \alpha \quad (2)$$

where F is the gas flux (mg C m^{-2} h^{-1} for CO_2 and CH_4, mg N m^{-2} h^{-1} for N_2O); ρ is the density of each gas under standard conditions ($CO_2 = 1.997 \times 10^6$ mg m^{-3}, $CH_4 = 0.717 \times 10^6$ mg m^{-3}, $N_2O = 1.978 \times 10^6$ mg m^{-3}), V is the volume of the chamber (m^3), A is the surface area of the chamber (m^2), Δc/Δt is the rate of change in gas concentration in the head space of the chamber during the sampling time (10^{-6} m^3 m^{-3} h^{-1}); T is the air temperature inside the chamber (°C); and α is the ratio of molar mass of carbon to the molecular weight of CO_2 and CH_4, or of nitrogen to N_2O. For N_2O and CH_4, theΔc/Δt of R more than 0.95 was used for the flux calculation. The flux of CO_2 was calculated at two points of 0 and 6 min based on the theoretical consideration that the increase in the CO_2 concentration in the chamber loses linearity after about 8 min (Nakano et al., 2004). However, the relationship between the multiple-times sampling and the two-points sampling is linear of 1: 1 (Mukumbuta et al., 2017b).

Cumulative gas emissions were calculated by linear interpolation between sampling events and numerical integration of the underlying area using the trapezoid rule as follows (Jin et al., 2010):

$$Cumulative\ gas\ emission = \sum_{i=1}^{n} (Ri \times 24 \times Di) \quad (3)$$

where Ri is the mean gas flux (mg m^{-2} h^{-1}) of the two successive sampling dates, Di is the number of days in the sampling interval and n is the number of sampling times.

Plant Production and Harvest

Aboveground biomass, belowground biomass were measured four times a year including two times of harvest, mid-April (beginning of crop growing season), late June (the first crop harvest), early September (the second crop harvest), and early November (end of crop growing season), that is, total 13 times from April 2017 to April 2020. Grass samples were taken from the vegetation survey areas of each treatment plot. All the aboveground biomass, including green and dead biomass were collected from the 0.5 × 0.5 m quadrate in April and November. Aboveground biomass at the time of harvest was obtained as the sum of harvest and residue. The harvest was measured by clipping at 5 cm above the ground in the 1 × 1 m quadrate. The residue was measured by collecting the stubbles and dead biomass in a 0.5 × 0.5 m quadrate. Regarding the belowground biomass, the root samples were collected from the 0.5 × 0.5 m area × 0.3 m deep by collecting soil and passing through an 8 mm sieve in the field. Roots were washed in a 2 mm sieve in the laboratory. All the samples were oven-dried at 70°C for 72 h and weighed. Each dried sample was analyzed for total carbon content.

Net primary production (NPP) was estimated as the increments of aboveground and belowground biomass (Mu et al., 2006), that is, annual aboveground NPP (ANPP) and belowground NPP (BNPP) were estimated as follows:

$$\begin{aligned} ANPP = {} & H(1) + R(1) - ABb + H(2) + R(2) - R(1) \\ & + ABe - R(2) + ABb' - ABe \end{aligned} \quad (4)$$

where H and R are the harvest and the residue at crop harvest, respectively (1 and 2 in the parentheses mean the first and second crop harvest, respectively); ABb, Abe, and ABb' are the aboveground biomass at the beginning and the end of crop growing season and the beginning of crop growing season in next year, respectively.

Equation 4 can be shortened as follows:

$$ANPP = ABb' - ABb + H(2) + H(1) \quad (5)$$

As belowground biomass is not harvested, BNPP can be obtained as follows:

$$BNPP = BBb' - BBb \quad (6)$$

where BBb and BBb' are the belowground biomass at the beginning of crop growing season and in the next year, respectively.

Calculations

N_2O Emission Factor

The N_2O emission factor indicates the cumulative N_2O emissions per unit applied nitrogen. According to the calculation method proposed by Shimizu et al. (2013), the N_2O emission factors derived from chemical fertilizers and organic fertilizers were calculated as follows:

$$EF_{CF} = \frac{E_{CF} - E_{NF}}{N_{CF} in\ CF\ plot} \times 100 \quad (7)$$

$$EF_{OF} = \frac{\{E_{OF} - (N_{CF}in\ OF\ plot \times EF_{CF}) - E_{NF}\}}{N_{OF}in\ OF\ plot} \times 100 \quad (8)$$

where EF_{CF} is the N_2O emission factor for chemical fertilizer (%), EF_{OF} is the N_2O emission factor for organic fertilizer (%); E_{CF}, E_{NF}, and E_{OF} are the N_2O emissions in the chemical fertilizer plot, no fertilizer plot, and organic fertilizer plot, respectively (kg N ha^{-1}); N_{CF} in CF plot and N_{CF} in OF plot were the chemical fertilizer N application rates in the chemical fertilizer plot and organic fertilizer plot (kg N ha^{-1}), respectively, and N_{OF} in the OF plot was the organic fertilizer N application rate (kg N ha^{-1}).

Net Ecosystem Carbon Balance

Net ecosystem carbon balance (NECB) was obtained as net biome production (Schulze et al., 2000). The NECB in agricultural land was obtained by adding carbon input from the application of organic fertilizer (C_{input}) and carbon export via harvest (C_{output}) for net ecosystem production (NEP). The NEP is estimated as the difference between net primary production (NPP) by photosynthesis of plants and heterotrophic respiration (RH) by decomposition of soil organic matter. Concerning carbon emission with CH_4 flux, in uplands, CH_4 flux is known to be very small compared to CO_2 (Toma et al., 2011), therefore it was not included in the NECB calculation. Therefore, NECB (Mg C ha^{-1} yr^{-1}) is calculated as follows:

$$NECB = C_{input} + ANPP + BNPP - C_{output} - RH \quad (9)$$

GWP and GHG Balance

The NECB and cumulative emissions of CH_4 and N_2O were converted to GWP_{CO2}, GWP_{CH4}, and GWP_{N2O}, respectively, using the CO_2 conversion coefficient [CO_2:1, CH_4:28, N_2O: 265 (IPCC, 2014)]. The GHG balance (Mg CO_2 eq ha^{-1} yr^{-1}) was obtained as GWP, which are the sum of GWP_{CO2}, GWP_{CH4}, and GWP_{N2O} as follows:

$$GWP_{CO2} = -NEBC \times \frac{44}{12} \quad (10)$$

$$GWP_{CH4} = CH_4 \times \frac{16}{12} \times 28 \quad (11)$$

$$GWP_{N2O} = N_2O \times \frac{44}{28} \times 265 \quad (12)$$

$$GWP = GWP_{CO2} + GWP_{CH4} + GWP_{N2O} \quad (13)$$

Statistical Analysis

The Shapiro-Wilk test was performed on each GHG flux, environmental factors, cumulative GHG emissions, carbon balance, and GHG balance to confirm normality. If normality was not found, logarithmic conversion was performed and the test was performed again to confirm normality. Differences in GHG emissions among years and among treatments were tested using a two-way analysis of variance (ANOVA). Differences in 3-year total GHG emissions, net ecosystem carbon balance and GHG balance among treatments were tested using a one-way ANOVA. If a significant difference ($p < 0.05$) occurred in the test, multiple comparisons were performed using the Tukey HSD method. In order to explain the relationship between the C/N and N_2O emission factors of organic fertilizers, the normality of each was confirmed by the Shapiro-Wilk test, and a simple regression analysis was performed. The analysis was performed using R (R Development Core Team, 2018; version 3.5.1).

RESULTS

Environmental Factors

Air temperature was highest in August and lowest in February (**Figure 1A**). The average annual temperatures during the study period in each year were 8.0, 8.4, and 8.9°C in 2017, 2018, and 2019, respectively, which were lower, similar, and higher than the average values for the last 10 years (8.4°C), respectively. Annual precipitation during the study period of each year was 1,227, 1,254, and 1,227 mm in 2017, 2018, and 2019, respectively, and the average annual precipitation for the past 10 years was 1,273 mm (**Figure 1A**).

Soil temperature tended to be similar to air temperature during the no-freeze period and ranged from 1.6 to 25.6°C, which was significantly lower in 2018 than in 2017 and 2019, although there was no significant difference among the treatments (**Figure 1B**).

WFPS ranged from 38 to 100%, tended to increase after heavy rainfall, and to decline when there was high temperature and no rainfall (**Figure 1C**). The WFPS was significantly lower in the no-fertilizer plot and the slurry plot in 2019, and there was no significant difference among the other treatment plots.

Soil NO_3^- -N content showed almost no peak in 2017, but several peaks after fertilization in 2018 and 2019 (**Figure 1D**). The highest mean NO_3^- -N was 82.6 mg kg^{-1} in the slurry plot. Conversely, the lowest average NO_3^- -N was 75.3 mg kg^{-1} in the non-fertilized plot.

Soil NH_4^+ -N content showed high peak in the slurry plot (39.7 mg kg^{-1}) after topdressing in 2017 (**Figure 1E**). In 2018, peaks were observed in the chemical fertilizer plot (36.1 mg kg^{-1}), manure plot (54.8 mg kg^{-1}), and slurry plot (52.3 mg kg^{-1}) immediately after the first fertilizer application. In 2019, no significant peak was observed in any treatment plot. The highest mean NH_4^+ -N content was in the slurry plot at 10.8 mg kg^{-1}. Conversely, the lowest mean of NH_4^+ -N content was 9.0 mg kg^{-1} in the non-fertilized plot.

GHG Fluxes

The CO_2 (RH) flux ranged from −49 (3 March, 2020) to 262 mg C m^{-2} h^{-1} (22 August, 2017) and increased with increasing temperature (**Figure 2A**). Additionally, a decrease in CO_2 (RH) flux was observed when WFPS was 100%.

The CH_4 flux ranged from −401 to 357 μg C m^{-2} h^{-1} and fluctuated highly (**Figure 2B**). The peaks of CH_4 flux were observed after the application of organic fertilizers in 2017 and 2018, especially in the slurry plot in the winter of 2018. In 2019, there was a large daily fluctuation with high CH_4 uptake by the soil.

The N_2O flux ranged from −115 to 839 μg N m^{-2} h^{-1} just after the application of organic fertilizer (**Figure 2C**). A peak of N_2O flux occurred in the digestive fluid plot in 2017 (347.82 μg N m^{-2} h^{-1}). On the other hand, in 2018 and 2019, no peak in N_2O

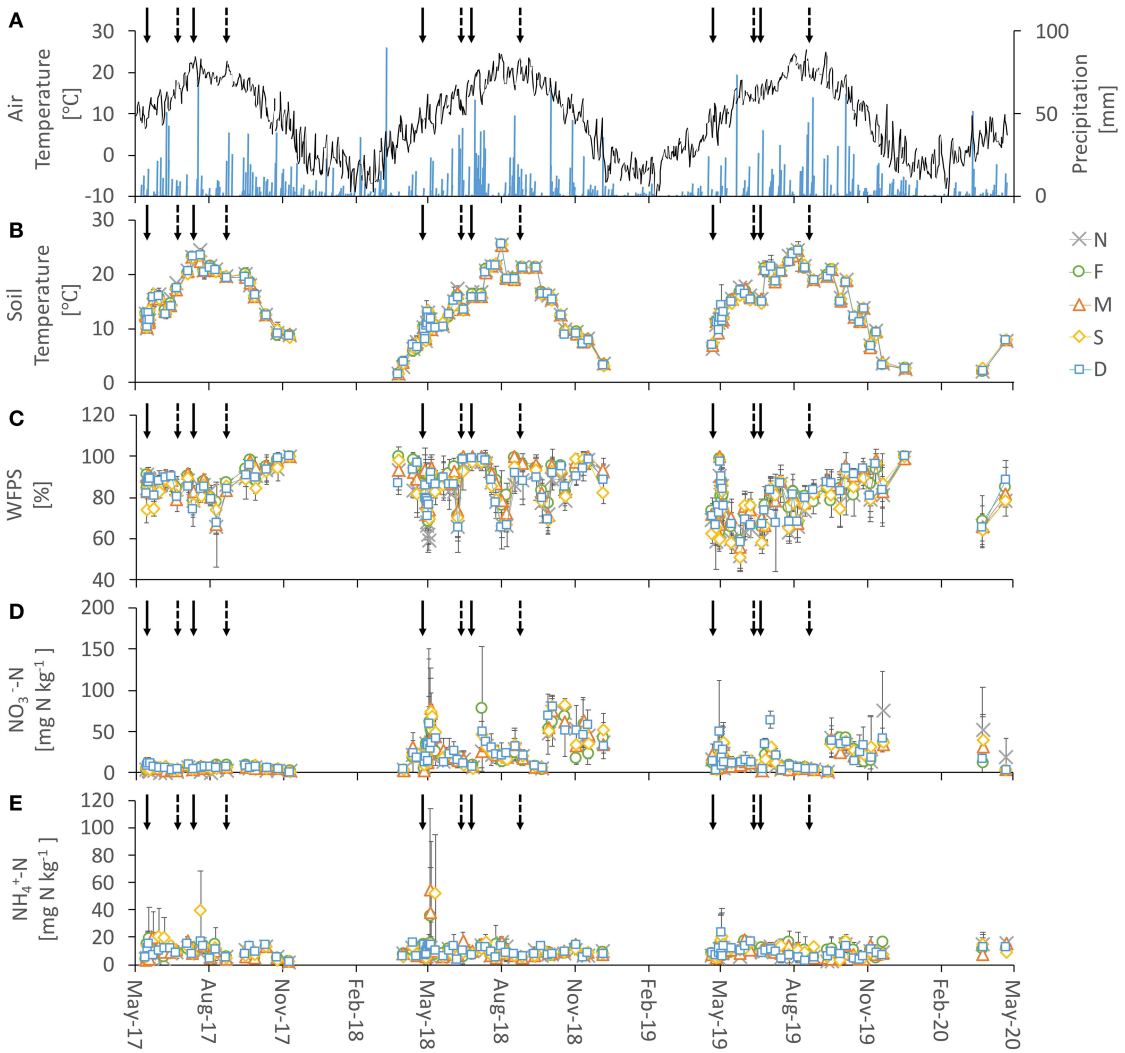

FIGURE 1 | Change in air temperature and precipitation **(A)**, soil temperature at a depth of 5 cm **(B)**, water filled pore space (WFPS) at the 0–6 cm soil depth **(C)**, soil NO_3^--N content **(D)**, soil NH_4^+-N content **(E)**. Error bars represent standard deviations, solid and broken arrows represent fertilization and harvest, respectively. $n = 3$.

flux was observed just after the application of organic fertilizer. The peak of N_2O flux was smaller throughout the year in 2018 than in 2017 and 2019.

There was no significant correlation between N_2O flux and soil NO_3^--N and NH_4^+-N contents (**Figures 3A,B**). However, the emission peaks of N_2O flux larger than 191 μg N m^{-2} h^{-1} of top 5% were clearly observed and tended to increase when the soil NO_3^- -N content was 2–12 mg N kg^{-1} (except for one at the time of just after supplement fertilizer application for second crop in digestive fluid treatment in 2019). Concerning soil NH_4^+-N content, almost all N_2O fluxes including the high peaks were found in 5–18 mg N kg^{-1}.

There was no difference in the relationship between gas flux and environmental factors caused by fertilization treatment (**Figure 4**). The CO_2 flux increased with increasing soil temperature and decreasing WFPS (**Figures 4A,B**). There was no significant correlation between CH_4 flux and soil temperature and WFPS (**Figures 4C,D**). The emission peak of N_2O flux larger than 191 μg N m^{-2} h^{-1} of top 5% was observed when the soil temperature was 12–23°C and the WFPS was 80–100%. However, at just after the harvest of first crop, all treatments showed high peaks in the range of 60–70% WFPS (**Figures 4E,F**).

GHG Emissions

The result of two-way ANOVA shows that CO_2(RH) emissions exhibited significant difference among years but no significant difference among the treatments (**Table 3**). It was maximum in the chemical fertilizer treatment in 2017, in no fertilizer treatment in 2018, and in the slurry treatment in 2019.

The result of ANOVA showed that CH_4 emissions exhibited no significant differences among years and treatments (**Table 3**). However, CH_4 uptake was observed with the no fertilizer or chemical fertilizer treatments in 2017, manure treatment in 2018, and all treatments in 2019 with lower precipitation.

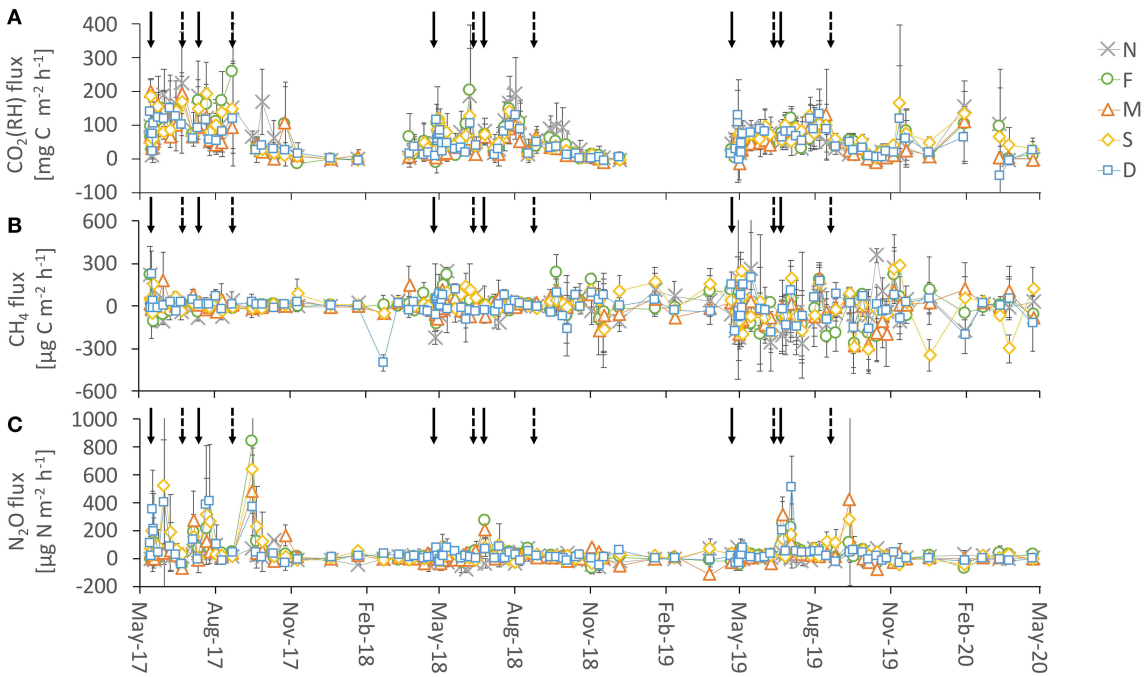

FIGURE 2 | Change in CO_2(RH) flux **(A)**, CH_4 flux **(B)**, and N_2O flux **(C)**. Error bars represent standard deviations, solid and broken arrows represent fertilization and harvest, respectively. $n = 3$.

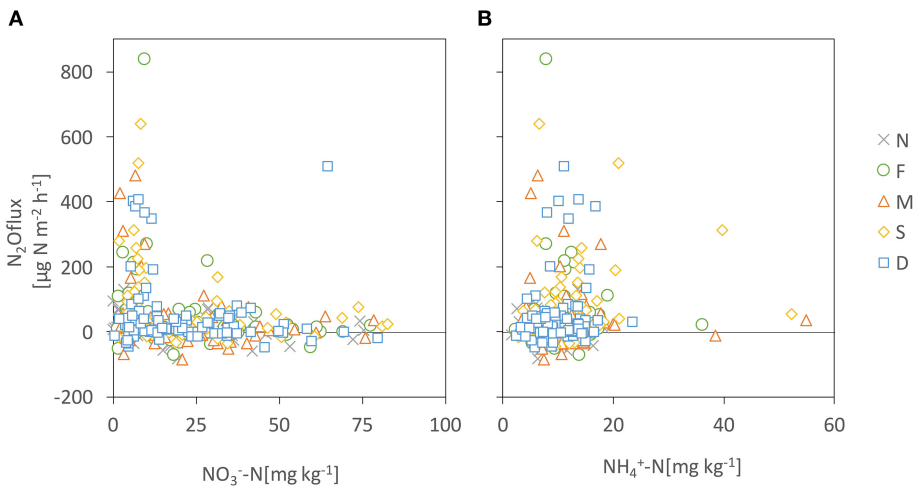

FIGURE 3 | Relationship between N_2O flux and soil NO_3^--N content **(A)** and NH_4^+-N content **(B)**. N is the no fertilizer plot, F is the chemical fertilizer plot, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot.

The result of ANOVA showed that N_2O emissions exhibited significant differences among years and treatments (**Table 3**). N_2O emission was significantly higher in fertilizer treatments than in no fertilizer treatment, but there was no significant difference among the fertilizer treatments. However, the slurry treatment tended to be the highest N_2O emission for all years.

The result of ANOVA showed no significant difference in the 3-year total CO_2(RH) among the treatments (**Table 4**). However, it tended to be the highest in the chemical fertilizer treatment (13.79 Mg C ha^{-1}), and among the organic fertilizer treatments, slurry > digestive fluid > manure.

There was no significant difference in the 3-year total CH_4 emission among the treatments (**Table 4**). However, 3-year total CH_4 emission tended to be highest in the slurry treatment (0.90 kg C ha^{-1}) and lowest in the manure treatment (−1.19 kg C ha^{-1}) among the organic fertilizer.

There was significant difference in 3-year total N_2O emission among the treatments (**Table 4**). Three-year total N_2O emission

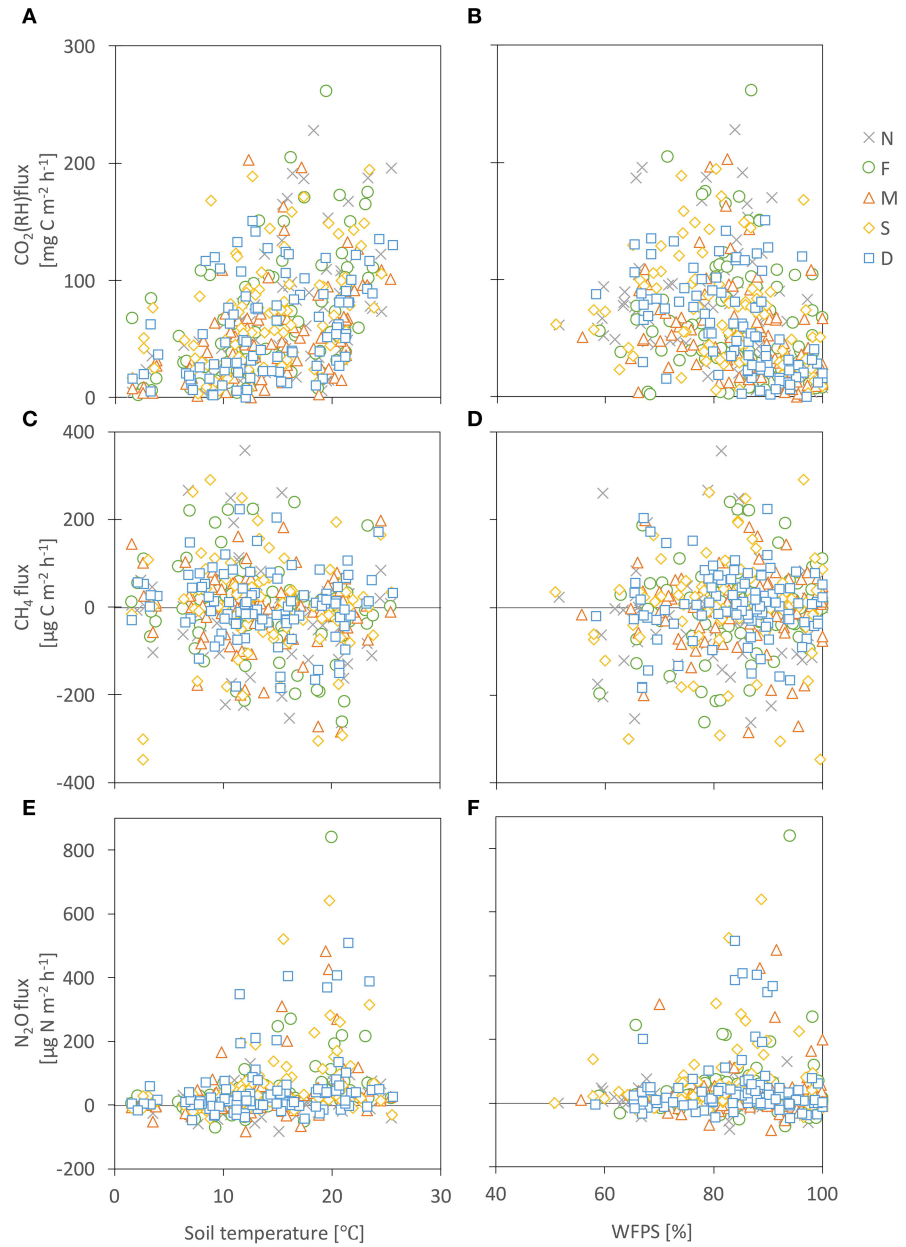

FIGURE 4 | Relationship between CO_2(RH) flux and soil temperature **(A)** and WFPS **(B)**, CH_4 flux and soil temperature **(C)**, and WFPS **(D)**, and N_2O flux and soil temperature **(E)** and WFPS **(F)**. N is the no fertilizer plot, F is the chemical fertilizer plot, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot.

was highest in the slurry treatments (10.8 kg N ha^{-1}) and lowest in the manure treatment (6.21 kg N ha^{-1}), although there was no significant difference among the fertilizer treatments.

N_2O Emission Factor

There was not a substantial variability on the N_2O emission factor among years and treatments (**Table 3**). The 3-year average of the N_2O emission factor was in the order chemical fertilizer > slurry > digestive fluid > manure (**Table 4**). However, among the organic fertilizer treatments, there was a significant negative relationship between the C/N ratio and N_2O emission factor (**Figure 5**).

Grass Yield

The 3-year cumulative grass yield was not significantly different among fertilizer treatments and was significantly higher than that of the no fertilizer treatment (**Figure 6**). This was achieved despite of the reduction of chemical fertilizer for nitrogen by 10.0–29.4%, phosphorus by 56.3–73.3%, and potassium by 78.2–100% in 3 years as the concentrations of phosphorus

TABLE 3 | Cumulative CO_2(RH), CH_4, and N_2O emissions and the N_2O emission factor (EF_{N2O}) for each year.

		CO_2(RH)		CH_4		N_2O		EF_{N2O}	
		Mg C ha^{-1}		kg C ha^{-1}		kg N ha^{-1}		%	
2017	N	4.91 ± 0.52	A	−0.16 ± 0.99	AB	1.10 ± 0.99	DE	– –	–
(348 days)	F	6.05 ± 2.44	A	−0.06 ± 1.12	AB	5.06 ± 1.12	AB	3.96 ± 1.57	A
	M	4.08 ± 1.14	A	1.22 ± 1.06	AB	3.70 ± 1.06	ABCD	−4.09 ± 11.3	A
	S	4.24 ± 0.70	A	1.23 ± 2.26	AB	6.29 ± 2.26	A	7.07 ± 13.8	A
	D	3.61 ± 0.75	A	0.60 ± 1.21	AB	4.79 ± 1.21	ABC	1.02 ± 1.76	A
2018	N	3.72 ± 1.73	A	1.10 ± 0.99	AB	0.13 ± 0.99	E	– –	–
(365 days)	F	3.18 ± 0.54	A	0.89 ± 0.35	AB	1.03 ± 0.35	DE	0.56 ± 0.51	A
	M	2.30 ± 0.20	A	−1.36 ± 0.64	AB	−0.02 ± 0.64	E	−1.65 ± 0.61	A
	S	2.99 ± 0.55	A	4.66 ± 0.87	A	1.82 ± 0.87	CDE	1.21 ± 0.57	A
	D	2.30 ± 0.56	A	1.90 ± 0.57	AB	1.25 ± 0.57	DE	0.84 ± 0.56	A
2019	N	4.40 ± 2.74	A	−2.03 ± 0.64	AB	1.27 ± 0.64	E	– –	–
(366 days)	F	4.56 ± 1.28	A	−2.78 ± 1.27	B	2.09 ± 1.27	BCDE	0.82 ± 1.67	A
	M	3.25 ± 2.06	A	−1.05 ± 2.38	AB	2.53 ± 2.38	BCDE	1.22 ± 3.09	A
	S	6.05 ± 1.10	A	−4.99 ± 0.34	B	2.70 ± 0.34	BCDE	0.57 ± 0.31	A
	D	4.19 ± 1.47	A	−1.71 ± 0.94	AB	1.96 ± 0.94	CDE	0.16 ± 1.53	A

ANOVA	*d.f.*	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
year	2	6.85	0.004	12.3	<0.001	30.3	<0.001	0.49	0.62
Treatment	4	1.93	0.13	0.31	0.87	6.66	0.001	1.60	0.22
Treatment × year	8	0.78	0.62	1.97	0.09	1.70	0.09	1.01	0.44

Values represent mean ± standard deviation. Values with the same letters are not significantly different ($P < 0.05$). N is the no fertilizer plot, F is the chemical fertilizer plot, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot. ANOVA is analysis of variance, and d.f. is degrees of freedom. n = 3.

TABLE 4 | Cumulative CO_2(RH), CH_4, and N_2O emissions and the N_2O emission factor (EF_{N2O}) for 3-year total.

		CO_2(RH)		CH_4		N_2O		EF_{N2O}	
		Mg C ha^{-1}		kg C ha^{-1}		kg N ha^{-1}		%	
3 years	N	13.0 ± 0.50	A	−1.09 ± 2.33	A	2.50 ± 1.67	B	– –	–
(1,079 days)	F	13.8 ± 3.27	A	−1.94 ± 4.48	A	8.17 ± 1.79	AB	1.58 ± 0.16	A
	M	9.63 ± 1.18	A	−1.19 ± 4.00	A	6.21 ± 1.82	AB	−1.09 ± 1.40	A
	S	13.3 ± 0.61	A	0.90 ± 3.32	A	10.8 ± 3.32	A	1.48 ± 1.08	A
	D	10.1 ± 2.63	A	0.79 ± 2.09	A	8.00 ± 2.27	AB	0.58 ± 1.46	A

ANOVA	*d.f.*	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Treatment	4	2.89	0.08	0.43	0.78	5.55	0.01	3.45	0.07

Values represent mean ± standard deviation. Values with the same letters are not significantly different ($P < 0.05$). N is the no fertilizer plot, F is the chemical fertilizer plot, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot. ANOVA is analysis of variance, and d.f. is degrees of freedom. n = 3.

and potassium in organic fertilizers were high (**Table 2**). Thus, the fertilizer application design for each fertilizer area was appropriate.

Net Ecosystem Carbon Balance

NECB in all treatments was negative, that is, the ecosystem lost carbon (**Table 5**). NECB was significantly lower in no fertilizer and chemical fertilizer treatments than the manure treatment. Although there was no significant difference among the fertilizer treatments, NECB tended to be larger in organic fertilizer treatments than in the chemical fertilizer treatment. ANPP was significantly lower in the no fertilizer treatment than the fertilizer treatment, and there was no significant difference among the chemical and organic fertilizer treatments. On the other hand, BNPP was negative, although there was no significant difference. Although ANPP + BNPP was positive, NEP was negative because of a larger RH than ANPP+BNPP. Therefore, a larger NECB in the organic fertilizer plot was caused by the contribution of C_{input} with organic fertilizer application, that is, carbon input by organic fertilizer enhances soil carbon sequestration. NECB in organic fertilizer plots tended to be in the order manure > digested fluid > slurry.

GHG Balance

The contribution of CH_4 emission to the GWP for 3 years was very small, which was < 0.1%. On the other hand, the

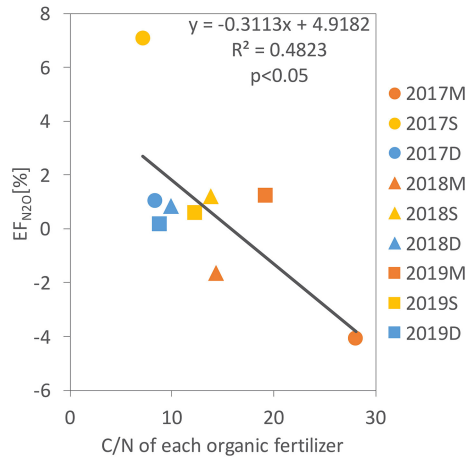

FIGURE 5 | Relationship between N_2O emission factor and C/N ratio of organic fertilizers. M is the manure plot, S is the slurry plot, and D is the digestive fluid plot. Solid line reveals the result of simple regression analysis.

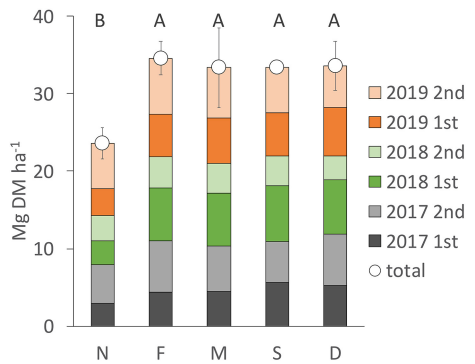

FIGURE 6 | Cumulative dry matter yield of grass. N is the no fertilizer plot, F is the chemical fertilizer plot, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot. Error bars represent standard deviations.

contribution of N_2O emissions to the GWP was larger than 5% (**Table 6**). The 3-year GWP was significantly smaller in the manure plot (43.1 ± 2.8 Mg CO_2 eq ha^{-1} yr^{-1}) than that in the no fertilizer (65.4 ± 3.7 Mg CO_2 eq ha^{-1} yr^{-1}) and chemical fertilizer plots (67.4 ± 13.6 Mg CO_2 eq ha^{-1} yr^{-1}), but there was no significant difference among the organic fertilizer treatments. The cumulative values for organic fertilizer treatments were slurry > digestive fluid > manure, and the GWP was 16.5, 27.0, and 36.2% smaller than those in the chemical fertilizer treatment, respectively.

DISCUSSION

Effect of Different Fertilizers on the Net Ecosystem Carbon Balance

In this study, the no-fertilizer treatment exhibited a significantly smaller NECB than did the fertilizer treatments. All organic fertilizers from manure, slurry, and digestive fluid tended to have a larger NECB, although there was no significant difference compared to that of the chemical fertilizer treatment. This indicates that organic fertilizers have larger carbon storage than do chemical fertilizers. Previous studies conducted on grasslands also showed higher NECB in manure treatment than in chemical fertilizer treatment (Matsuura et al., 2014; Shimizu et al., 2015; Mukumbuta et al., 2017a). In this study, NECB was negative for 3 years and all treatment plots became carbon sources. On the other hand, in previous studies by Matsuura et al. (2014) and Shimizu et al. (2015), NECB was positive in the manure treatment. The manure application rate in the previous study was 2.1–7.7 Mg C ha^{-1} $year^{-1}$, whereas that in this study was 0.15–1.9 Mg C ha^{-1} $year^{-1}$. This depended on the raw material of the manure. The manure used in the previous study was a bark manure with a lower C/N ratio and lower potassium content.

Therefore, to increase soil organic carbon using organic fertilizer, the quality of organic fertilizer, especially the ratio of carbon to nutrients, should be taken into consideration.

Effect of Different Fertilizer on the CH_4 and N_2O Emissions

In this study, the slurry treatment showed the highest CH_4 emission in 3-year total, although CH_4 emissions were not significantly different (**Table 4**). However, CH_4 emission showed a large variation among the years. Additionally, a previous study showed that direct CH_4 emission from organic fertilizer often occurred just after the application of organic fertilizer to the soil (Mori and Hojito, 2015). It was also expected that the anaerobic conditions produced by the liquid fertilizer in the slurry and digestive fluid would promote CH_4 emission immediately after fertilization (da Silva Cardoso et al., 2020). However, in this study, no peak of CH_4 flux was observed immediately after fertilization. This was probably because the temperature was relatively low immediately after fertilization and no microbial degradation occurred (Ryals and Silver, 2013).

The relationship between N_2O flux and environmental factors was not significantly different among fertilizer treatments. That is, N_2O flux peaks were observed when the soil NO_3^--N content was 2 to 12 mg N kg^{-1} (except for one plot of just after supplement fertilizer application for second crop in digestive fluid treatment in 2019) (**Figure 3A**), and the WFPS was 80–100% (**Figure 4F**). These suggest that the N_2O emission occurred through denitrification in all fertilizer treatments (Takakai et al., 2006). However, at just after the first crop harvest in 2019, the large N_2O peaks in the lower WFPS than 80%. This was probably because the stronger effect of the disturbance by harvest on N_2O emission than the effect of WFPS, which was shown by Li et al. (2015).

The application of organic fertilizers increases nitrogen mineralization in the soil and, from a physical point of view, increased the water retention of the soil, which increase N_2O emissions (Ryals and Silver, 2013). In particular, the application of slurry tends to promote denitrification to increase N_2O production (Rochette et al., 2004). On the other hand, it has also been reported that there was no significant difference in annual N_2O emission in a grassland in Hokkaido between chemical

TABLE 5 | Net ecosystem carbon balance.

	C_{input}	ANPP		BNPP		C_{output}		RH		NEP		NECB	
						Mg C ha^{-1}							
N	0	8.65 ± 0.82	B	−4.17 ± 0.66	A	9.00 ± 0.92	B	13.0 ± 0.50	A	−8.55 ± 1.13	B	−17.6 ± 1.13	B
F	0	12.9 ± 0.79	A	−3.31 ± 1.87	A	13.3 ± 0.92	A	13.8 ± 3.27	A	−4.17 ± 3.73	B	−17.5 ± 3.73	B
M	2.43	12.5 ± 1.93	A	−3.51 ± 0.42	A	12.9 ± 2.01	A	9.63 ± 1.18	A	−0.63 ± 0.98	A	−11.1 ± 0.98	A
S	3.12	12.6 ± 0.27	A	−3.60 ± 0.33	A	12.9 ± 0.35	A	13.3 ± 0.61	A	−4.30 ± 0.81	AB	−14.1 ± 0.81	AB
D	2.30	13.0 ± 1.32	A	−4.46 ± 0.45	A	13.2 ± 1.25	A	10.1 ± 2.63	A	−1.57 ± 2.68	AB	−12.5 ± 2.68	AB
ANOVA	*d.f.*	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Treatment	4	7.53	0.005	0.79	0.56	6.80	0.007	2.89	0.08	5.66	0.01	5.34	0.01

Values represent mean ± standard deviation. Values with the same letters are not significantly different ($P < 0.05$). N is the no fertilizer plot, F is the chemical fertilizer plot, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot. C_{input} is the carbon input from the application of organic fertilizer, ANPP is the aboveground net primary production, BNPP is the belowground net primary production, C_{output} is the carbon output from the harvest, RH is organic matter decomposition, NEP is net ecosystem production, NECB is the net ecosystem carbon balance. ANOVA is analysis of variance, and d.f. is degrees of freedom. n = 3.

TABLE 6 | GHG balance (CO_2 equivalent).

	CO_2		CH_4		N_2O		GWP	
					Mg CO_2 eq ha^{-1} year^{-1}			
N	64.4 ± 4.1	A	−0.04 ± 0.1	A	1.04 ± 0.7	B	65.4 ± 3.7	A
F	64.1 ± 13.7	A	−0.07 ± 0.2	A	3.40 ± 0.7	AB	67.4 ± 13.6	A
M	40.5 ± 3.6	B	−0.04 ± 0.1	A	2.59 ± 0.8	AB	43.1 ± 2.8	B
S	51.8 ± 3.0	AB	0.03 ± 0.1	A	4.50 ± 1.4	A	56.3 ± 3.6	AB
D	45.9 ± 9.8	AB	0.03 ± 0.1	A	3.33 ± 0.9	AB	49.2 ± 10.1	AB

ANOVA	*d.f.*	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Treatment	4	5.34	0.01	0.43	0.78	5.55	0.01	5.01	0.02

Values represent mean ± standard deviation. Values with the same letters are not significantly different ($P < 0.05$). N is the no fertilizer plot, F is the chemical fertilizer plot, M is the manure plot, S is the slurry plot, and D is the digestive fluid plot. GWP_{CO2} is the CO_2 equivalent NECB (the net ecosystem carbon balance), GWP_{CH4} is the CO_2 equivalent CH_4 emission, GWP_{N2O} is the CO_2 equivalent N_2O emission, GWP is the CO_2 equivalent GHG balance. ANOVA is analysis of variance, and d.f. is degrees of freedom. n = 3.

fertilizer treatment (0.6 kg N ha^{-1} yr^{-1}) and digestive fluid treatment (0.7 kg ha^{-1} yr^{-1}) (Sawamoto et al., 2010). In this study, N_2O emissions from organic fertilizer treatments were higher than that of no fertilizer treatment (**Tables 3**, **4**). The slurry treatment exhibited the highest N_2O emission, and the manure treatment had the lowest N_2O emissions among the fertilizer treatments. However, there was no significant difference among the organic fertilizer treatments and between organic and chemical fertilizer treatments. Similarly, in a previous study conducted in the same Andosols, no significant difference was found between the manure treatment and the chemical fertilizer treatment, although it tended to be higher in the manure treatment (Shimizu et al., 2010; Mukumbuta et al., 2017a). These results suggest that the high soil organic matter content of the Andosols reduce the effect of organic matter application.

Importance of Fertilization Design on Crop Yield

The fact that there was no significant difference in the grass yield between the chemical fertilizer treatment and the organic fertilizer treatments (**Figure 6**) was that the fertilizer application design was correctly performed using a combination of chemical and organic fertilizers without significantly increasing nitrogen loss of the organic fertilizer compared to that of the chemical fertilizer (Sawamoto et al., 2010; Mori and Hojito, 2015). It has been reported that field surplus nitrogen, which is calculated as the difference between nitrogen input by fertilizer application and nitrogen output by plant uptake is a good indicator of N_2O emissions (Shimizu et al., 2010). Field surplus nitrogen did not correlate with plant nitrogen uptake but correlated with N_2O emission and NO_3^--N leaching (Nagatake et al., 2018).

Comparison of the N_2O Emission Factors of Organic Fertilizer

The N_2O emission factors in this study were 0.6–4.0%, −4.1–1.2%, 0.6–7.1%, and 0.2–1.0% for chemical fertilizer, manure, slurry, and digestive fluid, respectively. There was no significant difference among years or fertilizer treatments (**Table 3**), although chemical fertilizer treatment tended to have higher emission factor than organic fertilizer treatments. However, on average, there was a significant negative correlation with the C/N ratio of organic fertilizers (**Figure 5**). This result was consistent with the results on farmland where N_2O emissions were measured using organic matter containing manure and plant residues (Akiyama and Tsuruta, 2003; Huang et al., 2004; Toma and Hatano, 2007; He et al., 2019). The C/N ratio of the microbes in soil is 5–10; that is, the synthesis of the cell requires nitrogen in an amount of 1/5–1/10 that of the carbon,

and some of the nitrogen mineralized through organic matter decomposition will be taken up by the microbes. The lower the C/N ratio of organic matter applied to the soil, the greater the amount of mineralized nitrogen released into the soil because there is more mineralized nitrogen than the microbes can uptake. On the contrary, the higher the C/N ratio of organic matter into the soil, the lower the release of mineral nitrogen into the soil because more mineralized nitrogen is taken up by the cells (Ruser et al., 2001). Soil mineral nitrogen (NO_3^--N and NH_4^+-N) and easily decomposable organic carbon are substrates for nitrification and denitrification that cause soil N_2O emissions. In this study, soil NO_3^--N and NH_4^+-N content tended to be larger in slurry with a low C/N ratio than in manure with a high C/N ratio (**Figures 1E,F**). Therefore, it is considered that the slurry releases more mineralized nitrogen into the soil, which enhances nitrification and denitrification, and promotes N_2O emission.

In this study, N_2O emission factor is obtained by subtracting N_2O emissions from chemical fertilizers and soil-derived emissions (Equations 7 and 8). Negative N_2O emission factor of organic fertilizer shown in **Table 3** suggests that the application of organic fertilizer denitrifies N_2O derived from chemical fertilizer. Several reports show the lower N_2O emission factor of organic matter than that of chemical fertilizer only (Toma and Hatano, 2007; Toma et al., 2007; Jin et al., 2010; Mori and Hojito, 2012; Shimizu et al., 2013; De Rosa et al., 2018).

Comparison of Global Warming Potential Among Fertilizers

The cumulative GWP for 1,079 days tended to be smaller for the organic fertilizer treatments than in the no fertilizer and chemical fertilizer treatments, especially the manure treatment, which had a significantly smaller GWP (**Table 6**). As suggested by Mukumbuta and Hatano (2020), estimates of the NECB showed that organic fertilizers tended to have a higher soil carbon sequestration effect than chemical fertilizers (**Table 5**), which is thought to reduce the GWP of organic fertilizers. Comparing organic fertilizer treatments, the cumulative GWP was the largest in the slurry, which showed the highest N_2O emission factor because of the lowest C/N ratio (**Figure 5**). Although manure application requires relatively higher chemical fertilizer nitrogen application rate among the organic fertilizer treatments (**Table 2**), manure application increased soil carbon sequestration and reduced N_2O emissions, resulted in the highest reduction of GWP. Digestive fluid application reduced chemical fertilizer nitrogen application most, and reduced N_2O emission next of manure.

CONCLUSION

The effects of application of manure, slurry, and digestive fluid on GHG emissions from a grassland on Andosol in a cold temperate climate were evaluated under fertilization management in accordance with regional recommendations. In the plots where organic fertilizer was applied, the amount of chemical fertilizer input could be reduced while maintaining yield. The relationships between CO_2, CH_4, and N_2O emissions and the soil environmental factors (soil NO_3^--N and NH_4^+-N contents, temperature, and WFPS) were not influenced by the type of fertilizer. The N_2O emission factor was highest in the slurry treatment and lowest in the manure treatment, showing a negative correlation with the C/N ratio of organic fertilizers. Additionally, the application of these organic fertilizers has been shown to improve ecosystem carbon balance and reduce the GHG balance. When the GWP for each fertilizer was evaluated based on the results of this 3-year study, it was suggested that manure is the best way to increase soil carbon and to decrease GHG emissions, followed by digestive fluid.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

RH planned this study, compiled all the data, and edited the manuscript. RK, CS, and KY measured greenhouse gas emissions and environmental factors and wrote the results and discussion. AN designed the experimental field, measurement procedures, and sampling schedule. YY and JD measured greenhouse gases and soil environmental factors. NY and KT produced slurries and methane fermentation digestive fluid at the Sapporo Farm. MK produced manure used at the Shizunai livestock farm and managed the field work. All authors contributed to the article and approved the submitted version.

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Effect of Nutritional Variation and LCA Methodology on the Carbon Footprint of Milk Production From Holstein Friesian Dairy Cows

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The UK livestock industry urgently needs to reduce greenhouse gas (GHG) emissions to contribute to ambitious climate change policy commitments. Achieving this requires an improved understanding of emission sources across a range of production systems to lower the burden associated with livestock products. Life cycle assessment (LCA) methods are used in this study to model milk production from two genetic merits of Holstein Friesian cows managed in two novel and two conventional UK dairy systems. Select merit cows sired by bulls with high predicted transmission for fat plus protein yield are compared with Control merit animals sired from UK average merit bulls. Cows were managed in conventional housed and grazed dairy systems with novel Byproduct and Homegrown feeding regimes. A LCA was used to quantify the effect of allocation and management of feed components on the carbon footprint of milk production. Natural variation in nutritional quality of dairy system rations was investigated to quantify uncertainty in the carbon footprint results. Novel production system data are used to assess the effect of introducing home grown legumes and co-product feeds. Control merit footprints across each of the management regimes were significantly higher ($p<0.001$) in comparison with a high production Select merit, on average by 15%. Livestock emissions (enteric, manure management and deposition) and embedded emissions (purchased feeds, fertiliser, and pesticides) were also significantly higher from control merit cows ($p<0.01$). Mass and economic allocation methods, and land use functional units, resulted in differences in performance ranking of the dairy systems, with larger footprints resulting from mass allocation. Pairwise comparisons showed GHG's from the systems to be significantly different in total and source category emissions, with significant differences in mean embedded emissions found between most management systems ($p<0.05$). Monte Carlo simulated system footprints considering the effect of variation in feed digestibility and crude protein also differed significantly from system footprints using standard methods ($p < 0.001$). Dairy system carbon footprint results should be expressed using multiple units and where possible calculations should incorporate variation in diet digestibility and crude protein content.

Keywords: carbon footprint, allocation, nutrition, dairy cattle, functional unit, LCA

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INTRODUCTION

The UK government is committed to reducing GHG emissions to net zero by 2050 with an even more ambitious target date of 2045 in Scotland (Committe on Climate Change, 2019). Agriculture is estimated to be responsible for 10–12% of global anthropogenic greenhouse gas (GHG) emissions (Smith et al., 2014). Emissions stemming from milk production in developed dairy regions such as the UK are estimated at between 1.2 and 1.4 kg CO_2e/kg, respectively, which is lower than the global average of 2.5 kg CO_2e/kg of fat and protein corrected milk (FPCM) (FAO, 2019). Dairy products have been processed and consumed in Britain since the Neolithic era and grassland, including rough grazing, covers ∼80% of the land area in Scotland (Charlton et al., 2019). However, agriculture is now the second largest source of GHG emissions in Scotland and there is an urgent need for this sector to contribute to national GHG emission reductions (SG, 2018). GHG emissions from livestock need to be reduced at a time when global demand for these commodities is increasing (Opio et al., 2013; FAO, 2019). UK GHG emissions from agriculture have declined by ∼14% since 1990, and reductions have largely arisen from a change to the Common Agriculture Policy (CAP), which ended a link between subsidy amounts and animal numbers (DBEIS, 2019, AHDB, 2014). Fewer total livestock numbers have led to lower stocking densities, less manure, and thus lower emissions (Rotz, 2004; del Prado et al., 2010; DBEIS, 2015). Formulating policies to enable further emission reductions on dairy farms will require an understanding of mitigation measures appropriate for specific production systems.

Models used to quantify GHG's are important tools to aid the understanding of mitigation pathways that lie within the intricate footprints of livestock systems (Opio et al., 2013). Estimates of GHG emissions from livestock systems contain uncertainties from model boundaries and allocation, variation in input values, or epistemic uncertainty arising from modelled biological processes, all of which present challenges for researchers and decision makers (IPCC, 2006; Flysjö et al., 2011; Opio et al., 2013; Röös and Nylinder, 2013). Epistemic uncertainty analyses of modelled dairy and beef livestock systems have shown that, overall, nitrous oxide (N_2O) and methane (CH_4) emissions from manure, fertiliser N input, and enteric CH_4 contribute most to variability and can inflate GHG emissions in livestock footprints (Ross et al., 2014; Zehetmeier et al., 2014; Sykes et al., 2019). Variation of N_2O emissions in dairy system footprints was found to mainly stem from the IPCC emission factor for volatilisation and atmospheric deposition of N (Ross et al., 2014). Sensitivity analysis provides a deeper technical understanding of complex systems and is recommended to clarify potential impacts, however Baldini et al. (2017) show that only 20% of 44 reviewed LCA studies of milk production published between 2009 and 2015 carried out sensitivity analysis.

Gradual improvements in methodology have allowed more precise estimates of emissions arising from agricultural systems. However, simultaneously annual global GHG emissions have continued to increase and the interval available to implement reduction strategies narrows (Boden et al., 2017). Numerous examples can be found in the literature comparing carbon footprints arising from various dairy production methods in Scotland (Ross et al., 2014) and across the world (Cederberg and Mattsson, 2000; Basset-mens et al., 2005; Flysjö et al., 2011; O'Brien et al., 2014) including suggestions for establishing a system emitting less CO_2 per unit product or management type (O'Brien et al., 2012). However, differences in LCA methodology, allocation methods (for milk and meat) or functional unit hinder comparability (Baldini et al., 2017) and a meta-analysis of 30 published LCA's with 87 carbon footprints from pasture, mixed, and confinement dairy systems found no average footprint differences per kg of FPCM (Lorenz et al., 2019). Choice of functional unit is also important when expressing results from LCA studies assessing environmental impacts arising from differing dairy systems and when considering effects of intensification (Ross et al., 2017, Salou et al., 2017). As far as the authors are aware, allocation methods assessing feed components and their effect on dairy system carbon footprints are not available in literature.

Studies quantifying uncertainty and assessing sensitivity of milk production LCA's have investigated management changes, C sequestration (O'Brien et al., 2012), manure storage (O'Brien et al., 2012; Battini et al., 2014) and changes in energy consumption (Roer et al., 2013). Methodologies to model CH_4 and N_2O emissions from manure, and enteric CH_4, require measurements of diet digestibility and crude protein (CP) (Dong et al., 2006). The consequences of dietary and other variabilities should be considered, and uncertainties communicated when quantifying dairy farm carbon footprints (Zehetmeier et al., 2014; Milne et al., 2015). Of the nine LCA studies reviewed by Baldini et al. (2017) that incorporated sensitivity analysis an assessment of variability of crude protein and digestibility was not reported, this information is not available in literature for multiple dairy systems as far as the authors are aware.

The digestibility of a dairy cow diet relates to the chemical composition of feed components and also the ration as a whole. Digestibility can be described as the fraction of a food that is absorbed, and this is affected by fibre content of feeds, of which the forage components tend to exhibit wider variation (McDonald et al., 2011). Predictions of enteric CH_4 emissions are lower from diets with high digestibility (Röös and Nylinder, 2013) and diet digestibility has been shown to influence uncertainty in beef production footprints (Sykes et al., 2019). Rations containing optimum digestibility and balanced CP can lead to lower GHG emissions because a cow would require less feed to meet nutritional requirements. Conversely, too much CP leads to higher N excretions, which can cause nutrient surpluses that contribute to air and water pollution, as well as climate change. Edouard et al. (2019) showed that IPCC GHG estimates were not as accurate for high levels of dietary N because of increased NH_3 emissions. When compared with soybean meal, the addition of legumes such as faba beans and peas in a ruminant ration were shown to have higher digestibility, CP and energy, which can be beneficial for dairy cow nutrition (Volpelli et al.,

2012), as long as rations are balanced to ensure higher levels of CP do not reduce nitrogen use efficiency (NUE).

Legumes are found in a wide range of ecosystems and the majority are genetically distinct form other plant species due to a symbiotic relationship with rhizobia. These are soil bacteria located within root nodules with the ability to fix nitrogen from the atmosphere (Kenicer, 2005). Within crop rotations, legumes can displace the need for imported nitrogen fertilisers, as well as nurture and condition the soil, which can have positive environmental and resource security consequences along with disease suppressing qualities (Lüscher et al., 2014; Stagnari et al., 2017). Home-grown protein feeds for animal production are increasingly being encouraged in the EU to reduce the protein deficit that relies upon soya imports which can fluctuate in price and can be associated with rainforest loss (European Parliament, 2018; Taherzadeh and Caro, 2019). Introducing legumes such as spring beans into crop rotations has the potential to reduce emissions through displacement of fertilisers, which in Scotland would translate to 100 to 180 kg/ha less *N* per year for spring and winter cereals, respectively (Iannetta et al., 2019). Legumes are estimated to generate <20% of the emissions associated with synthetic fertilisers, however N_2O emissions can still occur from leguminous crop residues (Senbayram et al., 2015; Stagnari et al., 2017). Increasing the ratio of corn to alfalfa silage on large and small dairy farms in northern US has been shown to raise farm gate GHG emissions per kg of FPCM (Kim et al., 2019). An increase in the use of forage legumes within dairy systems should therefore be considered to improve outcomes for livestock and the wider environment.

This study adds to literature on Scottish dairy farm carbon footprinting carried out by Ross et al. (2014) by presenting novel Homegrown and By-product feeding systems in comparison with more conventional management techniques. This paper seeks to clarify the impact of dairy systems on the environment using a modelling approach to address specific questions; (i) what effect does the method of allocation of emissions from animal feeds in dairy systems have on the carbon footprint of milk produced, (ii) what effect does alternative system inputs, such as legumes and co-products have on the composition of carbon footprints and (iii) what is the effect of variation in feed digestibility and CP content on the global warming potential of milk produced in dairy systems under a range of management scenarios. Carbon footprints from Holstein Friesian cows managed in novel and conventional UK dairy feeding regimes are presented using life cycle assessment (LCA) methods. Monte Carlo simulations were applied to describe uncertainty brought about by variation in nutritional quality of the diets. Carbon footprint results using mass and economic allocation of feed components, land use functional units and considering variation in CP and digestibility were compared by ranking performance. Knowledge surrounding effects of UK farm grown forage legumes on dairy carbon footprints and insight into the influence of variation of feed digestibility and CP content in LCA uncertainty are novel aspects of this study. Additional impact categories are not presented in this manuscript, in order to focus on GHG emissions and climate change.

MATERIALS AND METHODS

Dairy Systems Description

Data in this study originates from the Langhill herd of Holstein Friesian cows which form one of the worlds' longest running genetic line × feeding systems experiments (Pollott and Coffey, 2008). Data were used from all cows belonging to the herds based at the SRUC's Crichton Royal Farm, Dumfries, Scotland between 2006 to 2010 and 2012 to 2015. A Select (S) group of cows sired by bulls with high predicted transmitting abilities (PTA) for fat plus protein yield are compared with a Control (C) group sired from UK average merit bulls (Pryce et al., 2001). System experiments were managed according to the same rules and each regime was designed to allow animals to express their potential for milk production, within the limitations based on the feed rations offered. All experimental cows were milked three times per day and if not grazing were housed in the same building, with cubicles and concrete passageways that were cleaned with automatic scrapers. A complete diet was offered as a total mixed ration (TMR), irrespective of milk yield and stage of lactation. The four dietary treatments compared in this analysis were,

i. a high forage (HF) composite system which can be defined as a conventional regime; grazing cows when availability of grass is adequate and housing during inclement winter months when animals are fed conserved forage and concentrate through a total mixed ration (TMR),
ii. a novel home grown (HG) partially housed system, defined here as a regime where all feed is grown on farm using legume-based protein sources with no purchased feeds except minerals, and where animals are housed for one period each day and fed a conserved forage TMR,
iii. a low forage (LF) conventional housed system with animals confined all year round and being fed a diet of conserved forage and concentrate through a TMR,
iv. a novel by-product (BP) fully housed system that required no on-farm land by feeding mainly non-human edible co-products from the food industry with no forages except straw.

Data were collected over 5 years from January 2006 to December 2010 for LF and HF systems and over 4 years for HG and BP systems from January 2012 to December 2015 providing eight systems in total defined as Low Forage Control (LFC), Low Forage Select (LFS), High Forage Control (HFC), High Forage Select (HFS), Byproduct Control (BPC), Byproduct Select (BPS), Homegrown Control (HGC) and Homegrown Select (HGS). During 2011, cows consuming an LF diet transitioned to BP diet and HF to a HG diet. Herd size for each sub-group was maintained at ∼50 cows during the experiments. Herds were managed in feed groups which contained cows of both genetic merits and animals remained in the same system for three lactations or until there was a suitable replacement. Cows within each herd calved all year round (AYR) and were dried off 8 weeks prior to estimated calving date. Calving intervals for the LF and HF herds are presented in March et al. (2017). Youngstock were managed as one group, with rations attributed by age, for heifer calves 0–12 months, and from 12 to 24 months.

Data Collection

Each sub-group was treated as a whole farm in the life cycle inventory. Four dietary treatments and two genetic merits of cow allowed a comparison of eight diverse dairy production systems summarised annually. Milking and dry cow populations were evaluated for each sub-group and replacement animal populations were categorised by age for calves and heifers as 0–12, 12–24, >24 months. Milk yield was measured for individual animals at each milking with a sample taken once per week and analysed for fat and protein content by infra-red spectroscopy. Samples were analysed using a Milkoscan minor spectrophotometer (Foss Ltd., Denmark) and calibrated by methods of AOAC (2000). Outputs of milk were summed for the systems annually and weekly fat and protein constituents were averaged. Liveweights were recorded three times per day after milking and weekly liveweights were recorded for dry cows and replacements.

In each dietary regime Select and Control merits were housed together on one side of the building in two management groups which rotated every 3 days either being fed using automatic gates or behind a strap. A formulated TMR was offered daily, and average rations for each diet are presented in **Table 1**. Individual feed intakes for milking cows were measured using HOKO gates (Insentec BV, Marknesse, The Netherlands). LF and HF cows were fed 0.75 kg/day fresh weight of a standard 16% CP complementary parlour concentrate whilst milking. Dairy system inputs and outputs were determined annually using data extracted directly from SRUC's Langhill herd database and averages of production indicators are presented in **Table 2** for Select and **Table 3** for Control systems. Intakes of grass were not measured, however, periods of time spent grazing were recorded and samples of fresh grass were taken and analysed. Dry cows consumed a straw based diet and were then fed a transition diet 3 weeks before calving which consisted of 33% of the average daily dry matter intake of the appropriate milking cow ration plus 5 kg straw. Housed youngstock were managed as a group and offered a ration that included straw, distillers grains, and molasses.

Forage components of the LF, HF and HG system diets were grown on the farm. Maize, wheat, and spring beans were sown annually, lucerne every 2 years and grassland for pasture and silage every 5 years. Up to three cuts of ryegrass silage were harvested each year and any instances of double cropping of fields were noted with the lengths of time attributed to each crop allocated accordingly. For example, a field to be sown for maize may have been cut for silage before ploughing and there were instances where a grass silage cut was taken from a field sown for red clover bales. Harvested crops were stored on farm in covered clamps. Land required annually for those systems consuming crops grown on the farm was determined from amounts and DM's of each crop component fed to the herds and the crop DM and yield at harvest. Dry matter losses occurred at harvest, during ensiling or baling with estimated losses from grass silage, wheat alkalage, red clover bales and maize silage applied when considering land requirements for sub-groups because crops were not grown or ensiled separately for each of the dairy systems. An estimate of surface and effluent DM losses in the ensiling clamp were added to an estimate of wilting, leaching, respiration and mechanical field losses of crop to determine total land requirement by crop (Bastiman and Altman, 1985; Xiccato et al., 1994). Total land for each system year was calculated by

TABLE 1 | Ration constituent proportions in fresh weight and dry weight[a].

Diet	Component	Fresh weight (kg/day)	Dry weight (kg/day)	Fresh weight proportion
Low forage	Wheat grain	4.3	3.83	0.096
	Sugar beet pulp molasses	3.5	3.14	0.078
	Soya bean meal	3.1	2.81	0.068
	Distillers grains (wheat)	1.5	1.34	0.033
	Soya hulls	0.6	0.57	0.014
	Rumen-protected fat	0.3	0.33	0.008
	Bicarbonate & vegetable protein	0.4	0.3	0.009
	Grass silage	19.8	6.6	0.436
	Maize silage	8.2	2.2	0.181
	Alkalage	3.3	2.2	0.073
	Minerals/vitamins	0.25	0.25	0.006
	Total	45.4	23.6	
By-product	Barley straw	6.5	5.30	0.200
	Molassed sugar beet pulp	5.5	4.90	0.169
	Breakfast cereal (maize gluten)	3.3	3.00	0.102
	Distillers grains (wheat)	8.0	2.20	0.246
	Biscuit meal	2.2	2.00	0.068
	Wheat distillers dark grains	2.2	2.00	0.068
	Soya bean meal	2.2	2.00	0.068
	Cane molasses	2.0	1.30	0.062
	Minerals (high P)	0.2	0.20	0.006
	Rumen-protected fat	0.4	0.38	0.012
	Total	32.5	23.3	
High Forage	Grass silage	17.5	9.6	0.426
	Maize silage	12.9	3.2	0.314
	Alkalage	4.9	3.2	0.119
	Rapeseed meal	1.7	1.5	0.041
	Barley distillers grains	2.5	2.3	0.061
	Wheat distillers grains	1.4	1.2	0.034
	Minerals/vitamins	0.2	0.2	0.005
	Total	41.1	21.2	
Homegrown (Winter ration)	Grass silage	35.1	9.0	0.587
	Spring beans	5.5	4.7	0.092
	Wheat grain	4.0	3.4	0.067
	Red clover silage	10.0	2.0	0.167
	Maize silage	4.0	1.0	0.067
	Lucerne silage	2.0	0.6	0.017
	Minerals/vitamins	0.2	0.2	0.003
	Total	60.8	20.9	

[a] *Ration formulation for predicted intakes to satisfy a dairy cow of 650 kg and yielding 30 kg/day.*

TABLE 2 | Average annual production characteristics for high production select merit cows.

	Low forage		By-product		High forage		Home grown	
	Mean	sd	Mean	s.d.	Mean	s.d.	Mean	s.d.
Herd size	45	3.5	50	2.1	51	3.8	55	2.2
Body weight, kg/cow	647	11.4	663	21.7	626	8.6	651	33.3
Daily DMI, kg/cow	19.0	3.93	22.1	4.42	17.3	3.72	17.2	3.31
Daily yield, kg/cow	35.6	1.36	34.6	1.41	26.8	0.82	24.6	0.67
Fat, %	3.9	0.74	3.5	0.25	4.0	0.06	3.9	0.31
Protein, %	3.3	0.40	3.2	0.07	3.3	0.05	3.4	0.07

DMI, Dry matter intake; sd, standard deviation.

TABLE 3 | Average annual dairy system production characteristics for control merit cows.

	Low forage		By-product		High forage		Home grown	
	Mean	sd	Mean	s.d.	Mean	s.d.	Mean	s.d.
Herd size	50	0.6	52	1.8	54	1.0	55	2.4
Body weight, kg/cow	625	11.3	632	5.8	599	12.0	611	18.5
Daily DMI, kg/cow	18.1	4.02	20.3	5.53	16.35	1.16	15.52	2.28
Daily yield, kg/cow	31.1	1.54	28.8	1.55	24.1	0.83	22.8	0.61
Butterfat, %	3.6	0.13	3.3	0.17	3.8	0.07	3.6	0.25
Protein, %	3.1	0.06	3.0	0.04	3.2	0.03	3.2	0.1

DMI, Dry matter intake; sd, standard deviation.

TABLE 4 | GHG emissions and land use factors applied to dairy system purchased feed components.

Diet	Component	Economic allocation g CO_2 e/kg	Mass allocation g CO_2 e/kg	Land use m^2/kg
Low forage	Wheat grain	434	349	1.43[a]
	Sugar beet pulp molasses	120	245	0.22
	Soya bean meal	575	750	2.42
	Distillers grains (wheat)	285	5,428	0.98[a]
	Soya hulls	333	754	0.33
	Rumen-protected fat	501	2,941	0.33
By-product	Barley straw	196	306	0.61
	Sugar beet pulp molasses	120	245	0.22
	Breakfast cereal (maize gluten)	296	1,015	1.11
	Distillers grains (wheat)	285	5,428	0.98[a]
	Biscuit meal	118	126	1.25
	Wheat distillers dark grains	285	5,795	0.98[a]
	Soya bean meal	575	750	2.42
	Molasses cane	262	681	0.15
	Minerals (high P)	180	180	0.33
	Rumen-protected fat	501	2,941	0.33
High forage	Rapeseed meal	529	1,221	1.50
	Barley distillers grains	285	5,428	1.15[a]
	Wheat distillers dark grains	285	5,795	0.98[a]

Source unless otherwise stated: Feedprint (Vellinga et al., 2013), [a]SAC Consulting (2016).

adding on-farm land required to an estimate of off-farm land. Off-farm land was approximated using economic allocation of feed components within each of the diets, national data for crops SAC Consulting (2016) and Feedprint (Vellinga et al., 2013) for processed feeds. **Table 4** presents estimated feed component land use requirements and GHG emissions associated with mass and economic allocation methods for purchased feed inputs. TMR's were sampled monthly and wet chemistry analysis provided measurements of metabolisable energy (ME) content, dry matter (DM), digestibility and crude protein (CP) content of the ration (AOAC, 2000). Within the BP system only non-human edible purchased concentrates and straw were consumed (**Table 1**). Leguminous by-products, soya bean meal and soya hulls were included in the LF and BP housed system TMR's at proportions of 14 and 9%, respectively. Legumes grown on the farm for the HG system represented 25% of the winter TMR and there were no legumes or leguminous by-product components fed within the HF regime (**Table 1**).

Applications of nitrogen (N), phosphorus (P), and potassium (K) fertilisers and organic fertiliser to crops grown on the farm were determined by the farm manager using a long-term nutrient management plan. Fertiliser use data recorded for each crop area with application rate and fertiliser type was compiled annually and apportioned to each system by crop land requirement for that system. Organic fertiliser was applied as solid manure or as liquid slurry using a splash plate, trailing shoe, or by shallow injection. Manure was managed as solid or liquid storage or deposited at pasture. Liquid manure was pumped from the building and stored in uncovered slurry tanks and solid manure was stored uncovered outdoors. All youngstock and dry cow manure was managed as solid storage. All manure from housed milking cows was stored in liquid storage. Types, amounts and application rates of insecticide, fungicide and herbicide sprays applied to each crop were obtained from the Langhill herd database and directly from the supplier (pers. comm. Richard Bray). Forestry and other land managed within the farm boundary such as broadleaf woodland and biodiversity strips were apportioned using annual data prepared by the farm manager for farm subsidy applications (Pers. Comm. H. McClymont, SRUC) depending on the age and type of woodland.

Use of petrol and diesel, including the fuel needs of contractors, for each required activity was recorded in the Langhill database and then attributed to a feeding system by task, such as fertiliser application, and then by genetic group. Activities on the farm that required fuel related to crop management included fertiliser applications and herd management, such as feeding. Electricity use (kWh) was estimated from average milk yield per cow in each system using the method of Sheane et al. (2010) which applies 0.051 kWh/kg milk for yields of

6,500–8,500 litres per cow and 0.045 kWh/kg milk for yield >8,500 litres. Electricity was estimated from milk yield because power consumed was not recorded separately for each of the systems. Average annual energy use data is provided in the **Supplementary Material**. Annual diet digestibility and CP for each of the systems were determined from proportional intakes from monthly TMR and feed component sample analysis.

Life Cycle Assessment

Goal and Scope

Life Cycle Assessment (LCA) is a key approach to determine environmental or other impacts along a product chain and is carried out according to ISO 10440 and ISO 10444 standards. This research applies an attributional "cradle to farm gate" LCA method as defined by the British Standards Institute (BSI) PAS2050 (BSI, 2011) for the assessment of the life cycle GHG emissions of goods and services. Boundaries applied in this study were "cradle to farm gate" which included all stages from production of farm inputs and raw materials until the milk or animals left the farm. This boundary included supply chain input resources and emissions that are generated off farm, such as those associated with growing and manufacturing purchased feeds, transport, fertiliser manufacture as well as fuel and electricity production. On farm system components included applications of fertilisers, sprays, fuel, crops and field activities, animal feed, livestock of all ages and the management of their manure. Minor emission sources excluded on the basis of materiality PAS2050 (BSI, 2011) in this study were indirect emissions such as staff travel, maintenance of farm buildings, disposal of dead animals and ancillary purchases such as medicine and disinfectants used to clean infrastructure.

A standard functional unit (FU) related to dairy LCA's of fat and protein corrected milk was applied using the following equation (IDF, 2015),

$$\text{FPCM} = \text{Production (kg/year)} \times [0.1226 \times \text{Fat (\%)} + 0.0776 \times \text{Protein (\%)} + 0.2534]$$

FU's applied in this study are one kilogramme of FPCM milk leaving the farm gate, total hectares of land required and FPCM production per hectare of total land required. When considering intensification land use is important, because globally land availability is a limiting factor for agriculture (Salou et al., 2017). Area based emissions are used in policy setting and results presented in this research allow comparisons with other studies that consider land such as O'Brien et al. (2012), Ross et al. (2017), Salou et al. (2017).

Allocation describes how GHG's are attributed to the products, and possible co-products that leave the farm and the methods applied affect the estimation of emissions. In this case, as no crops were sold, co-products included animals culled and manure exported from the system. Methods of allocation available in LCA studies include biological causality, system expansion, economic allocation, mass allocation and no allocation (Audsley et al., 1997). In this study a whole farm approach is taken and emissions are allocated between milk and meat using no allocation to meat and 100% to milk. Emissions from co-product feeds were allocated proportionally by component as described by Vellinga et al. (2013). Greenhouse gas emissions attributed to feeds purchased for the LF, HF and BP systems followed an economic allocation method by feed component in the first instance and a mass allocation method as a comparison, shown in **Table 4**. This comparison is reported for Select merit herds in the BP, LF, HF and HG feed systems. Additional impact categories are not presented in this study to focus on types and sources of GHG emissions from a range of dairy systems.

Inventory Analysis

A life cycle inventory of annual data from five system years 2006–2010 for HF and LF diets and four system years 2012–2015 for HG and BP treatments was compiled for both genetic merits. Emissions from livestock were calculated using monthly herd dynamic data that was prepared for each of the systems for all years to determine livestock within each of the age categories, those culled, died, or sold, as well as dry and transition cows. Manure management emissions for each of the systems were allocated by the length of time cattle spent at either liquid storage, solid storage or depositing at pasture. Data showing proportions of time that cattle spent in each manure management category are provided in the **Supplementary Material**. Liquid slurry stems from the housed milking cows and the proportions of time spent grazing were determined and used to allocate deposition directly at pasture. Dry, transition cows and young stock generated solid manure. Manure generated by the dairy systems that was not applied to the crops was exported.

Livestock emissions from dairy cattle included those stemming from manure and enteric CH_4, direct and indirect N_2O from manure management, and additionally leaching of N from the soil, and ammonia (NH_3) volatilisation arising from the application and deposition of manure. Amounts of N excreted were estimated from N consumed minus N utilised for production, growth and maintenance, which were derived from dry matter intake and CP content of the diets (Dong et al., 2006). Tier II emission factors (EF's) were applied for livestock and manure management and Tier I for fertiliser and crop residue N_2O (de Klein et al., 2006; Dong et al., 2006). GHG emissions from production, processing, and distribution are embedded in purchased feeds brought onto the farm. Embedded emissions from fertiliser included those associated with manufacture and distribution, which, in the case of the Haber process for N, can be energy intensive. The global warming potentials associated with each feed component within the TMR's of the four diets are presented in **Table 4**. The LF and HF diets included a proportion of distillery products and the BP system comprised of purchased co-products from the bakery, distillery, brewing and confectionary industries. Estimated GHG emissions per kg for crops in the HG diet are presented in the **Supplementary Material** and the GWP of minerals in the LF, HF and HG diets was 261 kg CO_2e/kg using mass and economic allocation (Vellinga et al., 2013). The CT (2010) database was used to source emission factors applied to the production of fertilisers and pesticides (**Table 5**). Land category emissions arising from fertiliser application include N_2O from

TABLE 5 | Selected emission factors and calculations applied within the model.

Category	Description	Units	Factor	Source
Land	Direct from application of N to soils (EF_1)	kg N_2O-N kg N^{-1}	0.01	IPCC, 2006 Ch11 11.11
	Indirect volatilisation, atmospheric deposition of N (EF_4)	kg N2O-N kg N^{-1}	0.01	IPCC, 2006 Ch11 11.24
	Leaching and runoff of N (EF_5)	kg N2O-N kg N^{-1}	0.0075	IPCC, 2006 Ch11 11.24
	% *N* losses from leaching/runoff ($Frac_{LEACH}$)	kg N	30%	IPCC, 2006 Ch11 11.24
Livestock	Direct urine and dung N deposition at pasture (EF_{3PRP})	kg N2O-N kg N^{-1}	0.02	IPCC, 2006 Ch11 11.11
	Indirect N volatilisation from urine and dung at pasture ($Frac_{GASM}$)	kg N	0.2	IPCC, 2006 Ch11 11.24
	Direct volatilisation of solid storage manure (EF_{3SS})	kg N2O-N kg N^{-1}	0.005	IPCC, 2006 Ch10 10.62
	Feed system and age specific enteric CH_4 equation 10.21	kg CH_4 $head^{-1}$ $year^{-1}$		IPCC, 2006 Ch10 10.31
	Enteric (CH_4 conversion factor)	% of gross feed energy	6.5%	IPCC, 2006 Ch10 10.30
Embedded	Production of fertiliser N	kg CO_2e/kg	7.11	CT Footprint Expert 3.1
	Production of fertiliser P	kg CO_2e/kg	1.85	CT Footprint Expert 3.1
	Production of fertiliser K	kg CO_2e/kg	1.76	CT Footprint Expert 3.1
	Herbicides	kg CO_2e/kg ai	29.5	Audsley et al., 2009
	Insecticides	kg CO_2e/kg ai	28.5	Audsley et al., 2009
	Fungicides	kg CO_2e/kg ai	37.6	Audsley et al., 2009
Energy	Diesel	kg CO_2e/l	3.17	DEFRA/DECC, 2015
	Petrol	kg CO_2e/l	2.66	DEFRA/DECC, 2015
	Electricity	kg CO_2e/kWh	0.48	DEFRA/DECC, 2015
Sequestration	Broadleaf woodland > 20 yrs	C fraction DM growth	0.48	IPCC, 2006 Ch4

VS, volatile solids; DM, dry matter.

soil, volatilisation, leaching and run-off as well as N_2O emissions from crop residues. Carbon sequestration of farm woodland (by age and type) is modelled using Tier 1 IPCC (2006) methodology and reported separately as a reduction of net emissions. Selected emission factors and equations applied within this study are shown in **Table 5**.

LCA - Impact Assessment

The impact category of interest in this study is climate change, which was assessed by estimating total GHG emissions expressed in kg CO_2e stemming from the annual inventories of the dairy systems. The inventory prepared for each of the eight systems provided annual farm inputs and outputs from 36 distinct annual inventories which refer to nine calendar years in total for subsequent analysis using SRUC's Agricultural Resource Efficiency Calculator (Agrecalc) v1.4 (SRUC, 2014). Agrecalc (SRUC, 2014) is a carbon foot-printing and resource efficiency tool designed to model emissions at farm level using IPCC methodology (Dong et al., 2006). A PAS2050 (BSI, 2011) accredited model is available online and the tool is used by consultants, farmers, and livestock researchers (Toma et al., 2013; Sykes et al., 2017). Factors applied in Agrecalc (SRUC, 2014) to convert GHG emission flows to CO_2e were 25 and 298, for emissions of CH_4 and N_2O, respectively.

Statistical Analysis

Statistical analyses were carried out in R version 3.5.2 (R Core Team, 2013) using lme4, car, and lattice packages (Bates et al., 2015), to determine the effect of dairy production system upon product GHG emissions. Footprints applied in the statistical analysis were estimated using economic allocation of feeds and a FPCM FU for comparability with other studies. A linear mixed model was fitted and included fixed effects of feeding regime, genetic merit, and a random effect of year. An ANOVA, and a Tukey pairwise comparison test was carried out to determine significance of the production systems using the following model,

$$Y_{ijk} = \mu + F_i + M_j + T_k + F_i M_j + \varepsilon_{ijk}$$

Where,

y_{ijk} GHG emissions using economic allocation and expressed per kg FPCM

μ = *grand mean*

F_i = *feed type* $(i = 1\ to\ 4)$ *fixed effect*

M_j = *genetic merit* $(j = 1\ to\ 2)$ *fixed effect*

T_k = *year* $(k = 1\ to\ 9)$ *random effect*

ε_{ijk} = *residual error*

Sensitivity and Uncertainty Analysis

Stochastic simulations were carried out using ModelRisk5 (Vose Software) to assess the effect of annual variation in neutral cellulase gammanase digestibility (NCGD) and CP content of the rations on dairy system GHG emissions. Agrecalc (SRUC, 2014) was used to estimate baseline carbon footprints from all feed systems and both genetic merits using economic allocation of feeds and average annual values for NCGD and CP content. A FPCM FU is used for consistency and ease of comparability. Variation applying mass allocation of purchased feed emissions is not assessed in this study. Monte Carlo simulation using

repeated random sampling was used to generate distributions of footprints for the dairy systems that accounted for uncertainty stemming from variability in NCGD and CP content for each treatment group. Descriptive statistics for the NCGD and CP distributions are shown in **Table 6**. Exponential and Log Laplace distributions were fitted to NCGD and CP analysis results, respectively, and Monte Carlo simulations with 10,000 iterations (seed = 2,605) were carried out. To determine if there was a significant difference in sensitivity the footprint outputs from the linear mixed model detailed in the previous section were refitted and modelled and an Anova was carried out to test for a significant difference between the models.

RESULTS

Statistical Analysis

An ANOVA was conducted to compare GHG's and test for significant differences between the four feeding regimes and two genetic merits using an economic allocation of feed emissions and results (**Table 7**). Normality checks and Levene's test were carried out and the assumptions were met. The effect of the interaction was significant ($p < 0.01$). There was a significant difference in mean GHG's per kg FPCM between the feed groups [$F_{(3, 28)} = 15.6$, $p < 0.001$] and the genetic merits [$F_{(1, 28)} = 46.5$, $p < 0.001$]. *Post hoc* comparisons using the Tukey test showed mean Select and Control merit GHG totals to be significantly different ($p < 0.05$) in LF, BP and HF feed types but no significant difference was found between the HG and HF diet. Tukey test results showed that the LF diet was significantly different from BP ($p < 0.05$), the HF and the HG ($p < 0.001$) diets for GHG totals. Significant differences in mean embedded emissions were found between all management systems ($p < 0.05$) and livestock emissions were all significantly different ($p < 0.05$) apart from HFS and HGS regimes (**Table 7**).

TABLE 6 | Diet digestibility and crude protein content, mean sd and range.

	NCGD (g kg DM^{-1})			Crude protein (g kg DM^{-1})		
	Mean	sd	Range	Mean	sd	Range
Low forage	83.9	4.32	12.8	18.0	0.97	2.5
By-product	74.9	2.83	6.0	20.3	0.27	0.8
High forage	72.8	2.64	8.5	17.1	0.44	1.1
Home grown	75.0	3.04	6.7	18.1	1.62	3.9

sd, standard deviation; NCGD, neutral cellulase gammanase digestibility.

Effect of Allocation Method

For Select merit herds the average annual carbon footprint for milk produced in each of the dairy systems was calculated using both economic and mass allocation of feed components which resulted in large differences in the ranking of the different systems (**Figure 1**). In the housed systems, with economic allocation, the BP diet is associated with higher emissions per kg product at 1.07 kg CO_2e/kg FPCM compared with the LF system, which averaged 0.95 kg CO_2e (**Table 7**). Economic allocation of feed components in the HF and HG grazed system footprints led to similar product emissions per kg of FPCM at 1.15 and 1.16 kg CO_2e, respectively, as a result of lower embedded emissions in the HG system (**Table 7**) that were outweighed by higher emissions from energy and fuels. The HG system is connected with higher fuel use associated with crop production on farm. Economic allocation of emissions for the HF and HG TMR's were

TABLE 7 | Least square means (lsm) of dairy system GHG's by emission type.

		Land	Livestock	Embedded	Energy	Sequestration	Total
Variable	**Level**	**CO_2e/kg FPCM**					
Management system	LFS	0.06^{a}	0.49^{a}	0.34^{a}	0.08	-0.01^{a}	0.96^{a}
	BPS	0.00	0.61^{b}	0.39^{b}	0.07	0.00	1.07^{b}
	HFS	0.09^{b}	0.73	0.28^{c}	0.08	-0.03^{b}	1.15^{c}
	HGS	0.11^{c}	0.73	0.22^{d}	0.15^{a}	-0.05^{c}	1.16^{c}
	LFC	0.07^{d}	0.59^{c}	0.40^{e}	0.09	-0.02^{d}	1.13^{d}
	BPC	0.00	0.71^{d}	0.46^{f}	0.07^{b}	0.00	1.24^{e}
	HFC	0.10^{e}	0.84^{e}	0.33^{g}	0.08	-0.03^{e}	1.33^{f}
	HGC	0.12^{f}	0.81^{f}	0.25^{h}	0.16^{c}	-0.06^{f}	1.28^{e}
Diet	LF	0.06^{a}	0.54^{a}	0.37^{a}	0.08^{a}	-0.02^{a}	1.04^{a}
	BP	0.00	0.66^{b}	0.43^{b}	0.07^{a}	0.00	1.16^{b}
	HF	0.09^{b}	0.79^{c}	0.31^{c}	0.08^{a}	-0.03^{b}	1.24^{c}
	HG	0.11^{c}	0.77^{d}	0.23^{d}	0.16^{b}	-0.05^{c}	1.22^{c}
Genetic Line	Control	0.07	0.74^{a}	0.36^{a}	0.10	−0.03	1.24^{a}
	Select	0.06	0.64^{b}	0.31^{b}	0.09	−0.02	1.08^{b}
R^2		0.97	0.71	0.79	0.92	0.97	0.87

Different superscripts within a column denote significant differences between levels of the same variables ($p < 0.05$). LF, low forage; BP, by-product; HF, high forage; HG, home grown; S, select; C, control.

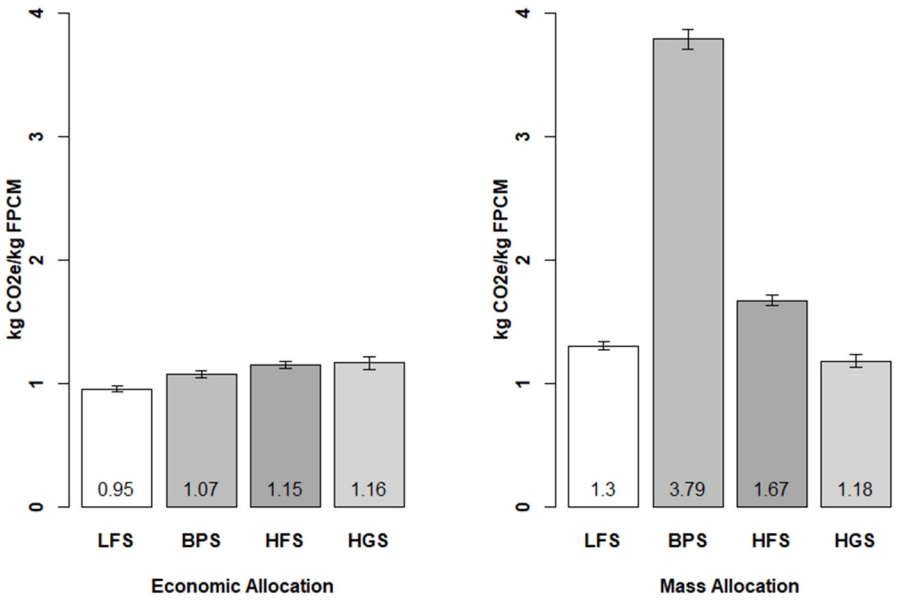

FIGURE 1 | Select merit dairy system average annual footprints per kg of FPCM using Economic and Mass allocation of feed components.

206 and 252 kg of CO_2e, respectively. Mass allocation of feed components led to increases in system footprints per kg FPCM because emissions were higher for all diet TMR's, except HG. TMR emissions were 473, 2,072, 757 and 252 kg CO_2e/tonne, for the LF, BP, HF and HG systems, respectively. On average, in the housed systems, BP diet emissions increased per kg product from 1.07 to 3.79 kg CO_2e/kg FPCM using economic and mass allocation, respectively. LF system product emissions averaged 0.95 kg CO_2e/kg FPCM using economic allocation and 1.3 kg CO_2e/kg FPCM using mass allocation (**Figure 1**). Control merit results followed the same rank order and are reported in the **Supplementary Material**.

Sensitivity and Uncertainty Analysis

Simulated footprints were generated using economic allocation of feeds to obtain distributions of dairy management system results if variation in NCGD and CP levels were considered. Mean dairy system footprint simulations considering the effect of NCGD and CP variation differed significantly from mean system footprints estimated using average annual NCGD and CP values ($p < 0.001$). Mean milk footprints were increased in the BP and HF systems and decreased in the LF and HG systems, in comparison with applying an average annual figure for digestibility and CP. Accounting for nutritional variation of the rations throughout the year had widened footprint ranges and altered comparative dairy system performance ranking. Select merit cows in the BP regime incurred greater average emissions per kg FPCM, at 1.21 kg CO_2e, when compared to the LFS, HGS and HFS systems which averaged 0.92, 1.15, and 1.17 kg CO_2e/kg FPCM, respectively (**Figure 2**). Average Control merit footprints followed the same rank and were higher than the Select merit in the same system at 1.09, 1.26, 1.35, and 1.42 kg CO_2e/kg FPCM for LFC, HGC, HFC and BPC systems, respectively (**Figure 2**). Higher average diet digestibility combined with a lower average CP content in the LF system (**Table 6**) led to lower mean emissions, in comparison with BP, HF and HG diets in both genetic merits (**Table 7**) and the standard method applying an average annual figure for digestibility and CP. When compared to the LF, BP and HF regimes Select merit cows managed in the HG system attracted a wider range of potential carbon footprints (**Figure 2**).

Sources of GHG's within the carbon footprints varied by dairy management regime, therefore farm mitigation strategies may prove more effective if applied by system type. Land and crop GHG emissions stem from crop residues, manure and fertiliser application and these ranged from zero in the BPS system to 0.11 kg CO_2e in the HGS system (**Table 8**). Embedded emissions are generated by energy consumed in the manufacture of feeds, fertilisers and pesticides and also in the use of bedding. Embedded emissions were greatest in the BPS housed system, at 0.46 kg CO_2e /kg FPCM, because all feed and bedding were imported. The HGS grazed system attracted higher embedded emissions than the HFS system, as a larger area of on farm crop land replaced purchased feeds (**Table 8**).

Livestock emissions that arise from enteric fermentation and manure management were greater in the HF and BP systems, at 0.79 and 0.68 kg CO_2e/kg FPCM, compared with 0.57 and 0.66 kg CO_2e/kg FPCM in the LF and HG systems, respectively (**Figure 3**). Higher emissions arose from greater amounts of manure stored in the BP system and from depositions while at pasture in the HF system. Emissions related to energy use were greater in the HG system, as this stemmed from the fuel used for crop related activities. Sequestered carbon estimated to occur within the woodland in the LF, HF and HG systems, lowered

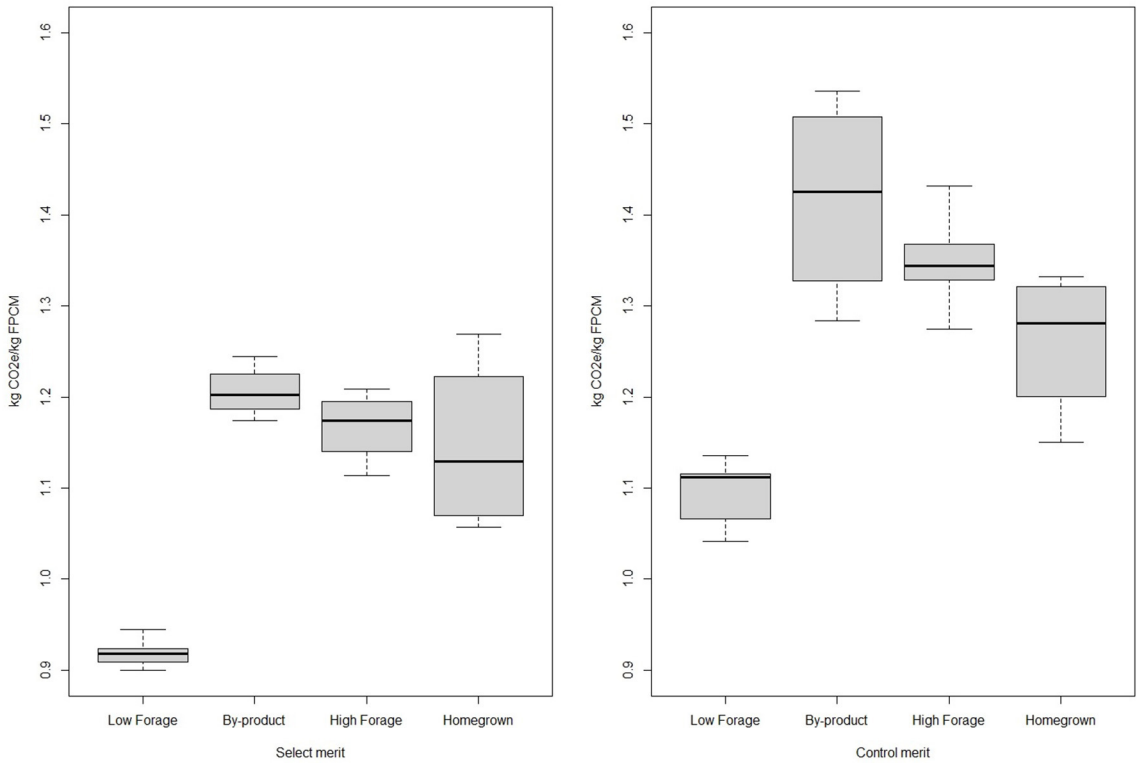

FIGURE 2 | Box plots for Select and Control merits showing economic allocation dairy system emissions with effect of NCGD and CP variation in the ration.

TABLE 8 | Dairy system GHG emissions (kg CO_2e/kg FPCM) by category using economic allocation of feeds and considering nutritional variation of both CP and NCGD.

		Land		Livestock		Embedded		Energy		Sequestration		Total	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Low Forage	S	0.05	0.003	0.57	0.013	0.24	0.014	0.08	0.007	−0.01	0.002	0.92	0.04
By-product	S	0.00	0.000	0.68	0.014	0.46	0.016	0.07	0.004	0.00	0.000	1.21	0.03
High Forage	S	0.08	0.003	0.79	0.036	0.24	0.013	0.08	0.007	−0.03	0.002	1.17	0.06
Home Grown	S	0.11	0.011	0.66	0.036	0.28	0.055	0.15	0.010	−0.05	0.003	1.15	0.11
Low Forage	C	0.06	0.006	0.68	0.020	0.29	0.009	0.09	0.012	−0.02	0.001	1.09	0.05
By-product	C	0.00	0.000	0.80	0.051	0.55	0.045	0.07	0.005	0.00	0.000	1.42	0.10
High Forage	C	0.09	0.008	0.91	0.028	0.30	0.020	0.08	0.013	−0.03	0.002	1.35	0.07
Home Grown	C	0.11	0.012	0.73	0.051	0.32	0.028	0.16	0.008	−0.06	0.003	1.26	0.10

S, select merit; C, control merit; sd, standard deviation; CP, crude protein; NCGD, neutral cellulase gammanase digestibility.

Select merit footprints by 0.01, 0.03, and 0.05 kg CO_2e/kg FPCM, respectively (**Table 8**). Control merit footprints were reduced by 0.02, 0.03, and 0.06 kg CO_2e/kg FPCM, for the LF, HF and HG, systems, respectively (**Table 8**).

Effect of Increased Legume Forages

Economic allocation of feed components generated similar average product emissions for HF and HG systems at 1.15 and 1.16 kg, respectively (**Table 7**). Mass allocation increased the HF milk footprint to 1.67 kg CO_2e/kg due to the proportion of distillers' grains in the ration. Accounting for nutritional variation slightly reduced the HG average to 1.15 kg CO_2e per kg FPCM and increased the HF to 1.17 kg CO_2e/kg FPCM (**Table 8**). If C sequestration was not included, the footprints were, on average, equivalent at 1.19 kg CO_2e/kg FPCM (**Table 8**). Trade-offs between livestock manure emissions and energy use to grow crops has led to similar milk total emissions being returned from the HG and HF systems (**Table 7**). Total on-farm land use per milking cow increased from an average of 0.86 ha to 1.23 ha when comparing the HF and HG systems. The HG system attracted greater embedded emissions than the HF system, these stemmed from the use of fungicide and herbicide applications to the wheat and spring beans.

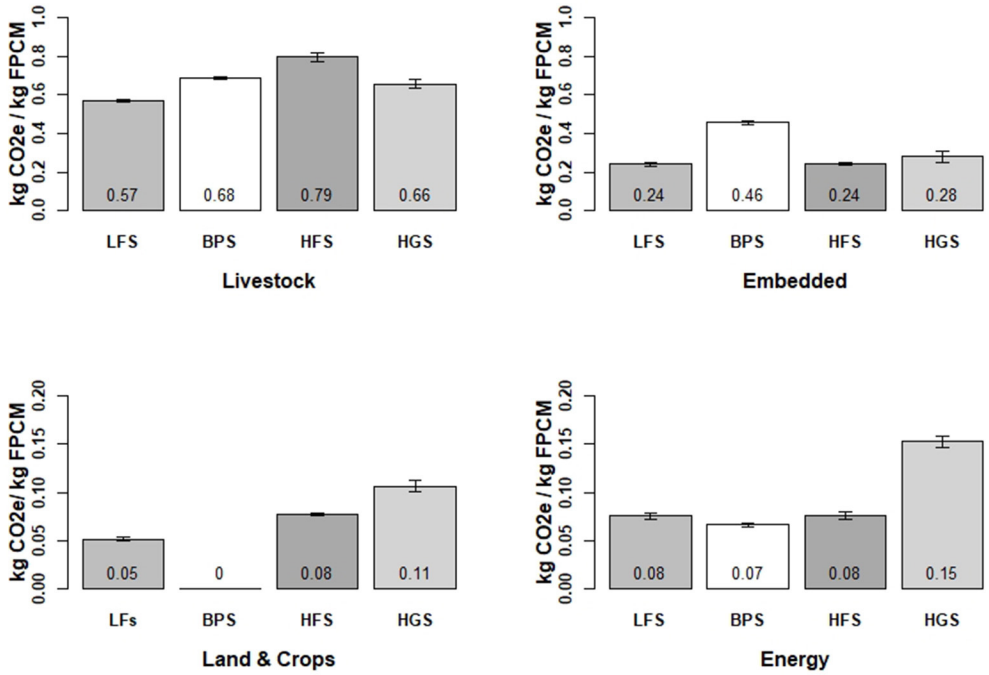

FIGURE 3 | Dairy system GHG's by emission source type with standard error considering NGCD not including C sequestration.

Effect of Genetic Merit

Control merit total product footprints across each of the management regimes were significantly higher ($p < 0.001$) in comparison with high production Select merit cows, on average by 15% (**Table 7**). Livestock and embedded emissions were also significantly higher from control merit cows ($p < 0.01$). On average, comparing raw milk quality across each of the management regimes, Control merit cows yielded less milk volume and produced lower percentage fat and protein content (**Table 3**) than Select merit animals consuming the same diet (**Table 2**). When encompassing nutritional variation lowest to highest mean system ranking for Control merit was LF, HG, HF, BP and this rank order was preserved for Select mean footprints (**Figure 2**). Control merit carbon footprints were higher than the Select merit animals apart from in the LF system, where the Control merit resulted in slightly lower emissions than Select merits in the BP, HG and HF management. The housed LF regime incurred less GHG's / kg FPCM than the BP system irrespective of merit or allocation method mainly because of emissions embedded in the production of feeds. Raw milk at the farm gate produced by Control merit cows, within the BP system, were associated with greater product emissions at 1.42 kg CO_2e/kg FPCM, than other systems and merits.

Effect of Land Use as a Functional Unit

On average, considering both on and off-farm land requirements for Select merit cows (**Table 9**), the BP system required the least amount of land in total, due to the high proportion of human inedible crop products and industry co-products. Land as a functional unit showed the HG system as least GHG intensive. When output of product is included with total land, it was the BP dairy system that emitted fewer GHG per hectare (**Table 10**).

TABLE 9 | Select merit dairy system land use (ha) on and off farm (mean and standard deviation).

Dairy system	On-farm land		Off-farm land		Total land
	Mean	sd	Mean	sd	
Low forage	29.4	4.99	45.1	2.23	74.5
By-product	0.6	0.0	58.9	4.07	59.5
High forage	41.6	8.23	21.8	2.53	63.4
Home grown	67.6	2.82	11.2	2.18	78.8

DISCUSSION

Using an LCA approach, this study demonstrates the importance of allocation method used to attribute GHG emissions of animal feeds and, in addition, the effect of nutritional variation on the carbon footprint of novel and conventional UK dairy systems. The ranked performance of dairy management types depends on the approach used to calculate impact and whether or not uncertainty is included (**Table 11**). Economic allocation resulted in mean dairy system emissions that ranged from 0.95 to 1.16 kg CO_2e/kg FPCM and 1.13 to 1.28 kg CO_2e/kg FPCM for S and C merit, respectively (**Table 7**). Mean system emissions were lower than the UK average of 1.25 kg CO_2e (AHDB, 2014), except for C

TABLE 10 | Dairy system mean GHG emissions (kg CO_2e) per functional unit.

Unit	Method	Merit	Low forage	By-product	High forage	Home grown
kg FPCM	Economic allocation	Select	0.95	1.07	1.15	1.16
FPCM	Mass allocation	Select	1.30	3.79	1.67	1.18
Ha	Economic allocation	Select	6,939	9,512	8,164	6,309
FPCM/ha	Economic allocation	Select	71.1	63.9	72.8	91.7
FPCM	NCGD & CP sensitivity	Select	0.92	1.21	1.17	1.15
FPCM	NCGD & CP sensitivity	Control	1.09	1.42	1.35	1.26

TABLE 11 | Ranked dairy system mean total GHG emissions (kg CO_2e).

Method	Unit	Merit	LF	BP	HF	HG
Economic allocation	FPCM	Select	1	2	3	4
Mass allocation	FPCM	Select	2	4	3	1
Economic allocation	Ha	Select	2	4	3	1
Economic allocation	FPCM/ha	Select	2	1	3	4
NCGD & CP sensitivity	FPCM	Select	1	3	4	2
NCGD & CP sensitivity	FPCM	Control	1	3	4	2

merit cows in HG and HF regimes (**Table 7**). Results expressing emissions totals per kg FPCM and per hectare produced in this study are in line with similar research such as Salou et al. (2017) and O'Brien et al. (2014). Carbon footprint results presented for the housed and composite systems are also in line with confinement and mixed systems reported in a review of 30 LCA studies from 15 different countries (Lorenz et al., 2019).

Effect of Allocation Method and Uncertainty Analysis

Carbon footprints were, on average, higher using mass allocation EF's, and accounting for uncertainty, stemming from changes in diet CP and digestibility, altered the dairy system ranking. Mass allocation of feed component emissions raised product emissions on average by 41%, for the LF and HF rations, which comprised of a mixture of grown crops and purchased concentrates. The BP ration was formulated from mainly non-human edible food and drink industry co-products and the TMR produced only marginally less GHG's than the LF diet. With economic allocation, ration EF's per tonne in the LF diet was 256 kg CO_2e and 249 kg CO_2e in the BP diet. Milk quality in the BP diet was, on average, lower in fat and protein content in comparison with the LF system. In the grazed HF system, mass allocation of feed components increased product footprints because distillers' grains and rapeseed meal elevated emissions. The effect of the elevation in TMR emissions using mass allocation in the HF system is not as pronounced as in the BP system due to the time spent grazing by HF cows. The HG grazed system footprints were least effected by allocation method as purchased feed was limited to minerals and diet component EF's were all equivalent apart from wheat grain. Ration EF's increased using mass allocation because feed components such as industry co-products tend to be associated with higher emissions when additional processing into animal feed is required.

Nutritional quality of animal feed varies, and in this study feed analyses showed a higher mean CP and lower mean digestibility in the BP ration when compared to the LF system. The BP system had the lowest ranges in digestibility and CP content, possibly because there was no effect of local climate on farm grown crops in this ration. Reducing the CP intake of the dairy cow diet would help in reducing GHG emissions (particularly N_2O) and UK research has shown that loss of production can be lower than expected (Reynolds et al., 2016). Other environmental and financial strategies to improve nitrogen use efficiency, such as home-grown legumes, should have positive consequences for GHG emissions through increased protein supply and the reduced need for N application from imported inorganic fertilisers.

Effect of Increased Legume Forages

The HF system ration included feeds requiring crop rotations of grass silage, maize and wheat, which required N fertilisers and were ensiled on farm. Crop products were combined with purchased concentrates and on average the HF diet consisted of ∼75% forage on a DM basis and 1.3 tonnes of concentrate per cow (March et al., 2017). In comparison with the HF diet, the HG ration required less maize crop, as the purchased distillers' grains and rapeseed meal were replaced with farm grown proteins, such as, spring beans and lucerne. The HG herds were grazed for an average of 26% of the year, whereas the HF cows were grazed for an average of 30% annually and attracted greater emissions from dung and urine deposition at pasture. For Select merit cows, however, feed intakes on a DM basis were similar in both the HF and HG systems (**Table 2**). Average milk yield reduced slightly, by 98 kg per cow from 7,575 kg in the HFS system to 7,477 kg in the HGS system, although, milk quality was similar in both the systems (**Table 2**).

The HG system is a comparatively high emitter using economic allocation however, **Table 11** shows this regime outranked all the other systems using mass allocation because no additional emissions are generated by imported products. In this case mass allocation methods and sensitivity analysis of nutritional variability highlight the benefits of a self-sufficient agricultural system, which may contain positive consequences when incorporating mitigation measures or when moving to more circular economic methods of farming. The HG system also had the lowest area-based emissions, a consequence of the replacement of synthetic N fertilisers by N inputs through biological fixation in comparison with the HF system. Legumes altered the composition of the footprints however the long-term effects of soil conditioning or crop disease prevention were not quantified by carbon footprinting and carbon sequestration modelling needs to be improved (Sykes et al., 2017) to reflect these and other desirable consequences. Mitigation of emissions

related to inputs could be achieved in the HG system by reducing pesticide use and using renewable energies on farm.

Effect of Genetic Merit

On average across all diets, product emissions from Control merit cows using an economic allocation were 15% higher, indicating that improving genetic merit offers an immediate emissions reduction strategy, mainly through increased milk yields. In addition, GHG emissions can be reduced by selecting for feed efficiency (Bell et al., 2011). This could be accelerated using techniques such as genomics in the herd to enhance overall feed efficiency and *in vitro* fertilisation (Gifford and Gifford, 2013; Pryce and Bell, 2017; Hailu, 2018). Financial analysis of the LF and HF regimes found a Control merit cow in a housed regime to be least profitable because milk yields were not sufficiently high to justify the feed costs (March et al., 2017). Considering the diets, the total emissions differed significantly ($p < 0.05$), apart from the HF and HG rations, however, livestock, energy and embedded emission types did differ significantly between these systems. This highlights that dairy systems mitigation potentials, and measures implemented, should be quantified and designed by considering the production method and the emission source.

Effect of Land Use as a Functional Unit

Novel rations such as those used in the BP system required less land, however, incorporating high percentages of co-product based animal feeds can lead to greater GHG emissions as a consequence of upstream processing, such as drying or milling, which can be energy intensive (Vellinga et al., 2013). Ruminant diets for high yielding cows can be formulated to achieve lower emissions, and to make more efficient use of human inedible co-products (Wilkinson and Garnsworthy, 2017), however, not all co-product feeds are low carbon, and feeding TMR's all year round usually requires cows to be housed in adequate modern animal housing facilities with slurry storage systems. In Scotland, industry co-products have traditionally been used as animal feeds, however, feeds such as distillers' grains contain added water, which stems from the mashing stage of the whisky making process. This can hugely inflate mass balance emissions, and for some products drying grains requires a substantial input of energy, as the water content has to be reduced from ∼75% to under 10% (Bell et al., 2012). Product quality in the BP system was also reduced (**Tables 2**, **3**), this was reflected through lower milk fat and protein which have financial consequences for farm income.

Comparisons of low input grass based, mixed, and fully housed intensive dairy systems are valuable to explore uncertainty and mitigation pathways, rather than to justify efficacy of a particular method of farming. Between and within countries agricultural practises vary and livestock farming is to some extent governed by history, culture, and tradition (Boogaard et al., 2011). Overall focus should be turned to mitigation of emissions, adaptation to changing climates, improving comparability of LCA's, and communicating uncertainty. GHG emissions from dairy farming can be mitigated through multiple pathways such as increasing the longevity of cows within a herd, improving fertility, lowering initial calving age and by reducing enteric methane and improving digestibility of cow rations (Garnsworthy, 2004; Wilkinson and Garnsworthy, 2017). Reduced enteric CH_4 and increased digestibility could be achieved through the reformulation of the diet or through feeding additives and supplements (Knapp et al., 2011, 2014). In less intensive dairy systems, enteric CH_4 emissions can be reduced by increasing yields (Yan et al., 2010). Renewable energies and technologies such as anaerobic digestion can be effective in reducing emissions from manure storage, with one study reporting reductions of up to 36% (Weiske et al., 2006; Battini et al., 2014).

CONCLUSIONS

Mass and economic allocation methods, and land use functional units, are shown to generate alternatively ranked footprint results. Monte Carlo simulated system footprints considering the effect of variation in feed digestibility and crude protein differed significantly from system footprints using standard methods. Implications are that dairy farm footprint calculations should incorporate the variation in diet digestibility and crude protein content where possible. Using an economic allocation, a localised home-grown feeding regime had the highest C footprint, however, this more self-sufficient system was associated with the lowest footprint using mass allocation and attracted the lowest area-based emissions, when not considering milk output. This result suggests the need for dairy system footprint results to be expressed in multiple units and to be mindful that methods used to allocate inputs can affect carbon footprint results. It is expected that in developing economy-wide reductions in greenhouse gas emissions, mass and area-based assessments of mitigation are most likely to guide the delivery of policy objectives.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because raw data is not publicly available. Requests to access the datasets should be directed to Margaret D. March, maggie.march@sruc.ac.uk.

ETHICS STATEMENT

The animal study was reviewed and approved by SRUC Animal Experiments Committee (AEC).

AUTHOR CONTRIBUTIONS

MM, RR, and AS contributed to the concept and methodology. MM and AS carried out the analysis. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Seasonal Nitrous Oxide Emissions From Hydroponic Tomato and Cucumber Cultivation in a Commercial Greenhouse Company

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Nitrous oxide (N_2O) is considered as the most critical greenhouse gas (GHG) emitted by agricultural and horticultural food production. Hydroponic vegetable cultivation in greenhouse systems has a high potential for N_2O emissions due to the intense application of nitrogen-containing fertilizers. Previous studies on model hydroponic systems indicate that N_2O emissions per unit area can be several times higher than typically found during field cultivation. However, reliable data from production-scale hydroponic systems is missing. Here we report our findings from monitoring the N_2O emissions in a commercial production greenhouse, located in the east of Germany, over a period of 1 year. We used the static chamber method to estimate N_2O fluxes in the root zones of hydroponic tomato and cucumber cultures on rock wool growing bags with drip fertigation. Regular sampling intervals (weekly-biweekly) were used to calculate whole season cumulative N_2O emissions and N_2O emission factors (EFs) based on the amount of nitrogen fertilizer applied. Our results indicate that the seasonal N_2O emissions from hydroponic greenhouse cultivation are considerably smaller than expected from previous studies. In total, we estimated average cumulative N_2O emissions of 2.3 and 1.5 kg N_2O–N ha^{-1} yr^{-1} for tomato and cucumber cultures, respectively. Average EFs were 0.31% for tomato cultivation with drain re-use (closed hydroponic system), and 0.13% for cucumber cultivation without drain re-use (open hydroponic system). These values lie below the general EF for N_2O from agricultural soils, noted with 1% by the intergovernmental panel on climate change (IPCC). In conclusion, considering the high yield of greenhouse cultivation, hydroponic systems provide a way of producing vegetables climate-friendly, in terms of direct GHG emissions. Further attention should be given to reducing energy inputs, e.g., by using regenerative sources or thermal discharge from industrial processes, and to increasing circularity, e.g., by using recycling fertilizers derived from waste streams. Especially in urban and peri-urban areas, the use of hydroponics is promising to increase local and sustainable food production.

Keywords: horticulture, food systems, soilless cultivation, greenhouse gas emissions, *Solanum lycopersicum*, *Cucumis sativus*, N_2O (nitrous oxide)

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INTRODUCTION

The global food sector is responsible for about 26% of total anthropogenic greenhouse gas (GHG) emissions (Poore and Nemecek, 2018), of which roughly 12% can be attributed to the use of manure and synthetic fertilizers on agricultural soils (Smith et al., 2014). The excessive use of fertilizers also leads to eutrophication of aquatic systems, losses in biodiversity and comprised drinking water (Robertson and Vitousek, 2009; Steffen et al., 2015). Additionally, the current industrial agriculture is very resource intensive in terms of land and water consumption (Campbell et al., 2017; Springmann et al., 2018). Therefore, more sustainable ways for providing the growing world population with food are searched for (Jurgilevich et al., 2016; FAO, 2019; Gerten et al., 2020), including the reduction of GHG emissions from plant cultivation and fertilizer losses to the environment. As one recommendation is to shift human nutrition to a more plant-based diet (Poore and Nemecek, 2018; Springmann et al., 2020), minimizing environmental impacts from vegetable cultivation might become crucial in future. Greenhouse cultivation, especially in hydroponic systems, has the potential to grow vegetables in a very resource-efficient way (Gruda, 2009; Savvas et al., 2013), by maximizing yield per area and by minimizing water consumption and nutrient losses. However, there is little knowledge about fertilizer-derived GHG emissions from greenhouse vegetable cultivation (Gruda et al., 2019). Direct GHG emissions from fertilized plant cultivation mainly consist of the release of nitrous oxide (N_2O) and methane (CH_4) from soils or other growing substrates, while carbon dioxide (CO_2) emissions from the root zone are considered to be in balance with photosynthetic CO_2 fixation by the aboveground biomass (Smith et al., 2014). While CH_4 emissions mainly occur in flooded soils under anaerobic conditions, N_2O emissions also occur under well-aerated conditions. N-fertilizers (e.g., ammonium and nitrate) that are not immediately taken up by plants are available to microbial N-transformation processes, such as nitrification and denitrification, which are associated with the release of N_2O (Firestone and Davidson, 1989; Baggs, 2011). N_2O has a global warming potential about 300 times higher than CO_2 on a 100-year scale and, in addition, depletes the vital stratospheric ozone layer (Myhre et al., 2013). Due to the high amounts and dosage rates of N fertilizers as well as favorable climate conditions, N_2O emissions from greenhouse systems might be generally high (Gruda et al., 2019). Indeed, the few existing studies on hydroponically grown cucumbers (Daum and Schenk, 1996b; e.g., Daum and Schenk, 1996a, 1998) and tomatoes (e.g., Hashida et al., 2014; Yoshihara et al., 2014, 2016) revealed substantial N_2O emission rates, with average values of up to 70 g N_2O–N ha^{-1} d^{-1}. These studies from only two teams, however, were selective and conducted under laboratory experimental greenhouse conditions and may not properly reflect N_2O emissions from commercial tomato and cucumber production. A more recent study on cucumbers cultivated in a large phytotron with cabins of 30 m^2, which was specifically constructed to measure gas fluxes in the root zones of multiple plants, found relatively low N_2O emission rates of ∼17 g N_2O–N ha^{-1} d^{-1} during plant cultivation (Nett et al., 2019). Nevertheless, the same study also showed that under certain conditions, like the enhanced degradation of roots after fruit removal and shoot-cutting, N_2O emission rates can rise to high levels in the range of 180–390 g N_2O–N ha^{-1} d^{-1}. In general, the N_2O release from growing media is known to depend on complex interactions of different variables (Butterbach-Bahl et al., 2013) and has been extensively studied for soils (Stehfest and Bouwman, 2006). The redox potential and oxygen status are of major importance for the microbial processes related to N_2O production (Davidson et al., 2000; Baggs, 2011). Sub-oxic hot-spots together with the supply of organic carbon (C), e.g., as root exudates or from decaying roots, typically increase denitrification rates (Morley and Baggs, 2010; Giles et al., 2017), yielding N_2O emissions from the reduction of nitrate or nitrite. In contrast, nitrifying microorganisms, which convert ammonium to nitrate with N_2O as a side product, typically favor aerobic conditions and are mostly independent of organic C supply (Firestone and Davidson, 1989). Further variables known to affect microbial N cycling are temperature and pH in the growing medium (Farquharson and Baldock, 2007). Because the influence of the different variables on N_2O emission rates can strongly vary over time and with plant growth stage (Daum and Schenk, 1996a), it is important to monitor the emissions regularly during the growing season. In this study we report for the first time seasonal data on N_2O and CO_2 emissions from the root zones of tomato and cucumber plants cultivated in a commercial production greenhouse using rock wool hydroponic systems. Our objectives were to (i) estimate whole-season N_2O emissions and N_2O emission factors from the amount of applied fertilizers, (ii) relate the N_2O emission rates to various influencing variables, including climate conditions inside the greenhouse and plant growth stage, (iii) assess how organic growing substrates alter N_2O and CO_2 emission rates from tomato cultivation compared to rock wool substrate, and (iv) determine how the re-use of rock wool substrate affects N_2O and CO_2 emission rates from cucumber cultivation compared to the use of factory-fresh rock wool. We hypothesized that (a) N_2O emissions from hydroponic vegetable cultivation vary widely over the growing season and are higher on a per unit area basis than for comparable soil-based crops, (b) organic growing substrates increase N_2O and CO_2 emission rates from the root zone of tomato plants, and (c) re-used rock wool with root residues from previous cultivation increases N_2O and CO_2 emissions from cucumber cultivation.

MATERIALS AND METHODS

Study Location

The study site at Fontana Gartenbau GmbH, a small to mid-sized enterprise, is located in the east of Germany in the Oderbruch valley (52°33′06.5″N, 14°33′23.0″E). The company mainly produces tomatoes, cucumbers and ornamental plants on a total greenhouse area of 2.15 ha. Tomatoes and cucumbers are gown hydroponically on growing bags, mostly plastic-coated rock wool mats, in heated glasshouses (**Supplementary Figure 1**). Tomato cultivation is done in a modern Venlo type glasshouse with a height of 4.8 m, inclined roof openings and adjustable thermal screens above the plant canopy. Cucumber cultivation is done

in a ridge and furrow type glasshouse with a height of ∼2.4 m and inclined roof openings. Tomatoes are typically cultivated in a year-round culture from January to November. Cucumbers are cultivated in two distinct cultures, an early season culture from March to May/June and a late season culture from June/July to October. In both, tomato and cucumber cultures, the CO_2 concentration is enriched in the greenhouse air in order to enhance plant productivity and fruit yield. For this purpose, the CO_2 concentration is monitored in the center of each greenhouse at a height of 1.7 m using an NDIR CO_2 sensor (EE820, E+E Elektronik Ges.m.b.H, Engerwitzdorf, Austria) connected to a climate computer system with software from Priva Building Intelligence GmbH (Tönisvorst, Germany). When CO_2 concentrations < 800 ppm are measured, technical CO_2 (≥99.7% CO_2, AIR LIQUIDE Deutschland GmbH, Düsseldorf, Germany) is supplied from a storage tank, resulting in average CO_2 concentrations between 400 and 600 ppm during the day. The CO_2 supply is regulated by solenoid valves and distribution in the greenhouse is *via* perforated PE pipes (19 mm inner diameter, opening slots every 15 cm) at the bottom of every second plant row. Climate variables are measured with sensors provided by Priva Building Intelligence GmbH (Tönisvorst, Germany) and processed by the Priva climate software. In each greenhouse, temperature, and relative humidity are measured on top of the plant stand (3/2 m height in the tomato/cucumber greenhouse) at two locations, one in the south block and one in the north block. The average values from the two locations in each greenhouse are used by the climate software to control heating and ventilation. Solar radiation, wind direction, wind speed, outdoor temperature, and precipitation are measured at a weather station outside the greenhouses and used to control ventilation and shading/energy shielding (only in the tomato greenhouse: up to 50% shading at solar radiation values >500 W/m² and energy shielding during the night). The radiation values inside the greenhouse are calculated by correcting the measured values of solar radiation from outside with the light transmittance of the greenhouses and the proportion of shading used. Irrigation amounts and frequencies are automatically adjusted according to greenhouse temperatures and solar radiation.

Tomato Cultivation

GHG emissions from year-round tomato cultivation were monitored mainly in the 2019 culture and partly in the 2020 culture, because the measurements were not possible before 14 March 2019. Tomato (*Solanum lycopersicum*) seedlings, grafted with two shoots on one scion of the cultivar Pureza (Enza Zaden, Enkhuizen, The Netherlands) on a rootstock cv. Maxifort (De Ruiter Seeds, Bleiswijk, The Netherlands) and pre-cultivated on rock wool cubes (10 × 10 × 6.5 cm), were planted on 14 January 2019 in the greenhouse and continuously grown until 21 November 2019. The rock wool cubes with seedlings were put in a distance of 0.5 m on growing bags (filled with nutrient solution on 9 January 2019), yielding a shoot density of 2.4 m^{-2}. For gas flux measurements, growing bags of three different substrates, with four replicates each, were used: (1) rock wool mats (100 × 20 × 7.5 cm; Grotop master, Grodan B.V., Roermond, The Netherlands), (2) coir mats (100 × 15.5 × 8.5 cm; Coir Project GbR, Segnitz, Germany), and (3) perlite granules mixed with wood fibers (henceforth referred to as "perlite/wood fiber;" 100 × 19.5 × 6.5 cm; Kleeschulte Erden GmbH & Co. KG, Rüthen, Germany). The growing bags for sampling were distributed in the middle of the plant stand of a 4,300 m² greenhouse section, always in distance of 6 m to the central gangway (**Supplementary Figure 2**). The growing bags were installed on elevated, hanging panels with gutters at both sides allowing the collection of drain solution. Water and nutrients were supplied *via* drip fertigation in a closed-cycle system, where the collected drain solution is reutilized after bio-filtration (aerated slow filtration through rock wool with a flow rate of 2.4 m^3 h^{-1}). Mineral fertilizers (**Supplementary Table 1**) were added to obtain EC values of 3–4 mS cm^{-1} and the pH of the supplied nutrient solution was adjusted to 5.6 using nitric acid. The volumes and EC values of added nutrient solution were adjusted according to plant demand/seasonal timing. No nutrient solution was added after 2 November 2019 in order to drying out the growing bags by plant water uptake. In total, nitrogen fertilizers corresponding to 612 kg N ha^{-1} were added to the tomato culture from March to November 2019. Tomato shoots were trained on a wire, and were successively lowered and hanged around the gutters when reaching the top of the wire. Old leaves and lateral shoots were regularly pruned, always leaving 12–14 leaves at one shoot. Harvesting of red tomato fruits was done once or twice per week from April to November. Total yield of marketable tomatoes was around 361 t ha^{-1} for the tomato culture of 2019. The tomato cultivation in 2020 followed the above described protocol. Growing bags were filled with nutrient solution on 14 January 2020 and tomato seedlings were planted on 20 January 2020. For gas flux measurements, only rock wool growing bags were used and six sampling points were distributed analogously to 2019 in the greenhouse section (**Supplementary Figure 2**). In total, nitrogen fertilizers corresponding to 127 kg N ha^{-1} were added to the tomato culture from January to February 2020.

Cucumber Cultivation

GHG emissions from cucumber cultivation were monitored in the late season culture of 2019 and in the early season culture of 2020. Cucumber (*Cucumis sativus*) seedlings of the cultivar Sencere (Nunhems B.V., Nunhem, The Netherlands), pre-cultivated on rock wool cubes (10 × 10 × 6.5 cm), were planted on 6 June 2019 in the south block and on 9 July 2019 (due to late delivery of seedlings) in the north block of the greenhouse. The rock wool cubes with seedlings were put in a distance of 0.5 m on growing bags with rock wool mats (100 × 20 × 7.5 cm; Grotop expert, Grodan B.V., Roermond, The Netherlands), yielding a shoot density of 1.4 m^{-2}. For gas flux measurements, four new growing bags and four growing bags, which were already used in the early season culture of 2019 (re-used), were distributed within the cucumber greenhouse (**Supplementary Figure 2**). The growing bags for sampling were located in the middle of the plant stand of the 6,000 m² greenhouse, always in distance of 6 m to the central gangway. Normally, the growing bags were placed on a 5 cm polystyrene layer on the ground. For measurement purposes, the growing

bags were additionally put on panels, as they are used for tomato cultivation. Water and nutrients were supplied *via* drip fertigation in an open system, where the drain solution is discarded. Mineral fertilizers (**Supplementary Table 1**) were added to obtain EC values of 2.5–3 mS cm^{-1} and the pH of the supplied nutrient solution was adjusted to 5.6 using nitric acid. The volumes and EC values of added nutrient solution were adjusted according to plant demand/seasonal timing, and in order to obtain a surplus volume of about 30% as drain solution. No nutrient solution was added after 19 October 2019 in order to drying out the growing bags by plant water uptake. In total, nitrogen fertilizers corresponding to 725 and 516 kg N ha^{-1} (excluding the surplus of 30%) were added to the south and north blocks, respectively, during the late season cucumber culture in 2019. Cucumber shoots were trained on a wire and lateral shoots were removed before the main shoot reached the top of the wire. Thereafter, the main shoot was cut off and the uppermost two-three lateral shoots were allowed to grow downwards. Harvesting of cucumber fruits started on 21 June 2019 in the south block and on 29 July in the north block, and was from then on done six times per week until the end of the culture on 23 October 2019. Total yield of marketable cucumbers was around 359 t ha^{-1} for the late season culture of 2019. The early season culture in 2020 was similar to 2019. Both, the south and north block, were planted on 27 February 2020. For gas flux measurements, only new rock wool growing bags were used and six sampling points were distributed analogously to 2019 in the greenhouse (**Supplementary Figure 2**). Cultivation was done until 19 May for the first half of the greenhouse (problems with powdery mildew) and until 2 June for the second half of the greenhouse.

Measurement of Greenhouse Gas Emissions

To measure the fluxes of greenhouse gases (CO_2, CH_4 and N_2O) in the root zone of tomato and cucumber plants the closed chamber method described for soil by Parkin and Venterea (2010) was used and modified for hydroponics. Acrylic glass chambers, previously described by Halbert-Howard et al. (2020), were installed around the growing bags separating root zone air space from shoot air space (**Supplementary Figure 3**). To make this possible, the chambers consisted of two halves and had two openings on top for the plant stems. Air exchange was prevented by rubber gaskets (foam rubber and silicone) on the bottom of the chambers, between the two chamber halves and around the plant stems. Pressure imbalances and temperature effects inside the chamber were avoided by a vent tube and reflective aluminum foil on the chamber outside. The chamber air space differed depending on the type of studied growing bag and was 15.6, 18.3, and 19.0 dm^3 for rock wool, coir and perlite with wood fiber, respectively. Gas samples were drawn through a butyl septum on the camber top using a 50 ml polypropylene syringe with a stainless steel needle. For each gas flux determination, four gas samples were taken at 20 min intervals over 1 h (0, 20, 40, and 60 min after closing the chamber). For transport, 30 cm^3 of gas sample was deposited into previously vacuumed 20 ml glass vials with magnetic screw caps and silicone/PTFE septa (model N 18, Macherey-Nagel GmbH and Co KG, Düren, Germany), yielding a slight overpressure to avoid contaminations from ambient air. To ensure tightness of glass vials, the vacuum was checked prior to sampling and only vials with a pressure <100 mbar were utilized. Gas analyses were carried out directly on the day of sampling using a gas chromatograph (GC 2,010 Plus, Shimadzu Corporation, Kyoto, Japan) with an electron capture detector (ECD) for N_2O, a thermal conductivity detector (TCD) for CO_2, and a flame ionization detector (FID) for CH_4. External standards (AIR LIQUIDE Deutschland GmbH, Düsseldorf, Germany) were used to calibrate the GC system for each measuring sequence. Standard concentrations were 0.285, 0.380, 0.592, 1.97, 5.12, and 9.4 ppm (±10%) for N_2O; 310, 604, and 1011 ppm (±2%) for CO_2; and 1.04, 5.02, and 10.1 ppm (±2%) for CH_4. Depending on sample N_2O concentrations, a low calibration curve (0.285–0.592 ppm) and a high calibration curve (0.592–5.12/9.4 ppm) were used, as background effects were more pronounced for small N_2O concentrations (<0.592 ppm).

DATA EVALUATION

Gas fluxes were calculated using the R software [version 3.6.3; R core team (2020)] and the R package "gasfluxes" [version 0.4-4; Fuss (2020)], with the latter automatically selecting for the best fit model from either linear, robust linear, and non-linear (HMR model) regressions. For flux calculation, the measured concentrations (in ppm) were transformed to μmol m^{-3} according to the ideal gas law under the assumption of SATP conditions ($T = 25°C$ and $p = 101.3$ kPa). Because each chamber measurement always included two plants, the area (A) to which the fluxes referred was calculated as: $A = 2 \times Dp^{-1}$, with Dp being the plant density (in m^{-2}). Further input variables for flux calculation were the chamber air volume (in m^3) and the time after closing the chamber (in h). The use of non-linear regression was restricted, as suggested by the gasfluxes package authors, by using the margin of uncertainty from the external standards (±10% for N_2O and ±2% for CO_2 and CH_4) as surrogate for the measurement precision of the GC system. The resulting gas fluxes (in μmol m^{-2} h^{-1}) were further converted to g N_2O–N ha^{-1} d^{-1}, g CH_4 ha^{-1} d^{-1} and kg CO_2 ha^{-1} d^{-1} based on molar masses. Cumulative emissions (kg N_2O–N ha^{-1} and Mg CO_2 ha^{-1}) were calculated on the basis of daily N_2O and CO_2 emission rates by linear interpolation between sampling days and summing up daily emission rates over the study periods (trapezoidal method). N_2O emission factors (in %) were calculated through dividing cumulative N_2O emissions by the total amount of N (in kg N ha^{-1}) supplied in the nutrient solution during the study period. Yield-scaled N_2O emissions (in mg N_2O–N kg_{fruit}^{-1}) were calculated through dividing cumulative N_2O emissions by marketable yield (in kg ha^{-1}). CO_2 equivalents (in Mg CO_2 ha^{-1}) were calculated from cumulative N_2O emissions (in kg N_2O ha^{-1}) through multiplying by the 100-yr global warming potential of 298 for N_2O (Myhre et al., 2013).

STATISTICAL ANALYSES

All statistical analyses were performed in the R software (version 3.6.3). Linear mixed-effects models (LMMs) were done using the R package "lme4" [version 1.1-21; Bates et al. (2015)]. LMMs on N_2O and CO_2 emission rates from tomato cultivation in 2019 included sampling date and location inside the greenhouse (north or south block) as fixed factors, and growing bag identity as random intercept. LMMs on N_2O and CO_2 emission rates from cucumber cultivation in the late season of 2019 included sampling date, location inside the greenhouse (north or south block) and substrate (new or re-used rock wool) as fixed factors, and growing bag identity as random intercept. LMMs on N_2O and CO_2 emission rates from tomato cultivation on different substrates in spring 2019 included sampling date and substrate (rock wool, coir or perlite with wood fiber) as fixed factors, and growing bag identity as random intercept. Prior to LMM analyses, data were log(x + 1)- or sqrt-transformed to fulfill the requirements of LMMs (i.e., normality and homogeneity of variances). Regression analyses between mean gas (N_2O and CO_2) emission rates and climate parameters were done for tomato and cucumber cultivation in 2019 using the "lm" function from the R base package. Prior to analysis, N_2O fluxes were log-transformed to fulfill the assumptions of normality and homogeneity of variances, and all flux and climate data were studentized for better comparability between different units and scales. The sampling dates when no nutrient solutions were supplied to plants were excluded from regression analyses to avoid potential bias because gas emissions were strongly reduced, likely due to dry conditions strongly limiting microbial activity. Permutational ANOVAs were done to determine exact *P*-values for block and substrate effects on cumulative emissions from cucumber cultivation in the late season of 2019 (substrate and block effects) and tomato cultivation in spring 2019 (only substrate effects) using the R package "lmPerm" [version 2.1.0; Wheeler and Torchiano (2016)]. If a significant substrate or block effect was found ($P_{exact} < 0.05$), a Tukey HSD *post-hoc* test was done on the results from ordinary ANOVA on (1/x)-or sqrt-transformed data using a level of significance of $\alpha = 0.05$.

RESULTS

Greenhouse Gas Emissions From Hydroponic Tomato Cultivation

The N_2O emission rates showed a strong variation over the growing season in 2019 (**Figure 1A**; **Table 1**), with average values ranging from 1.7 g N_2O–N ha^{-1} d^{-1} in April to 18.7 g N_2O–N ha^{-1} d^{-1} in July. After a first peak (11.3 g N_2O–N ha^{-1} d^{-1}) at the start of tomato harvest on 4 April, the emission rates balanced at a low level (∼4 g N_2O–N ha^{-1} d^{-1}) until 19 June. Consistently high average emission rates (16.3–18.7 g N_2O–N ha^{-1} d^{-1}) were measured during July and August, when temperature and solar radiation were relatively high (**Supplementary Figure 4A**). However, there was no correlation between emission rates and greenhouse climate variables (**Table 2**), as high solar radiation during April and June, together with the highest temperatures in June, did not relate to increased N_2O emissions. In addition, there was a very high variability of N_2O emission rates between the four replicates in July and August. The emission rates then continuously declined from end of August to end of September (7.8 g N_2O–N ha^{-1} d^{-1} on 25 September) but increased again during October (13.7 g N_2O–N ha^{-1} d^{-1} on 22 October), coinciding with an increased occurrence of excessive root growth due to *Agrobacterium rhizogenes* infection. Following the cessation of irrigation and nutrient supply, the average N_2O emission rates dropped below 1.7 g N_2O–N ha^{-1} d^{-1} in November. The measurements in 2020 showed very low N_2O emission rates (<0.4 g N_2O–N ha^{-1} d^{-1}) during the first 7 weeks (15 January to 4 March) of tomato cultivation (**Figure 2A**). These fluxes were in the range of the measurement uncertainty and even a negative flux value was found in one replicate, which was hence excluded from the calculation of cumulative N_2O emissions. After a 2-month sampling break due to the COVID-19 pandemic, missing the onset of harvest, N_2O emission rates in May 2020 (1.1–2.6 g N_2O–N ha^{-1} d^{-1}) were slightly lower compared to May 2019 (2.5–3.5 g N_2O–N ha^{-1} d^{-1}). Cumulative N_2O emissions were calculated from 14 March to 13 November 2019 and 15 January to 04 March 2020 (**Table 3**). Assuming that the N_2O emission rates in the first 2 months of cultivation in 2019 were similar to the ones from 2020, the total N_2O emissions for one tomato cultivation season were on average 2.3 kg N_2O–N ha^{-1}. This corresponds to about 1.100 kg CO_2 ha^{-1} based on the 100-year global warming potential of N_2O. The CO_2 emission rates from tomato cultivation also exhibited a distinct seasonal dynamic during 2019 (**Figure 1B**; **Table 1**), with highest values during the warm summer months. In contrast to N_2O, the CO_2 emission rates strongly correlated with greenhouse temperature and less pronounced with solar radiation (**Table 2**). The highest average emission rate of 88.7 kg CO_2 ha^{-1} d^{-1} was measured on 5 June, when the temperature inside the greenhouse was also highest (**Supplementary Figure 4A**). The lowest emission rates were found on 29 March (10.0 kg CO_2 ha^{-1} d^{-1}), prior to the harvest, and in November (13.0-14.8 kg CO_2 ha^{-1} d^{-1}), after cutting off the nutrient solution supply. Regarding the measurements in 2020, the CO_2 emission rates continuously increased from 1.9 kg CO_2 ha^{-1} d^{-1} on 15 January to 13.5 kg CO_2 ha^{-1} d^{-1} on 4 March (**Figure 2B**). The values measured in May 2020 (33.7–47.2 kg CO_2 ha^{-1} d^{-1}) were comparable to May 2019 (29.8–54.7 kg CO_2 ha^{-1} d^{-1}). In total, the cumulative CO_2 emissions from 14 March 2019 to 3 March 2020 were about 11.8 Mg CO_2 ha^{-1} (**Table 3**). Over the whole study period no significant CH_4 fluxes were detected (calculated fluxes ranged from −0.6 to 0.3 g CH_4 ha^{-1} d^{-1} and were all below the minimum detection limit; **Supplementary Figure 5**).

Greenhouse Gas Emissions From Hydroponic Cucumber Cultivation

In the late season 2019, the N_2O emission rates strongly varied over time and between the north and south block of the greenhouse (**Figure 3A**; **Table 1**). The emission rates were generally low (on average 3.1 g N_2O–N ha^{-1} d^{-1}) until 11

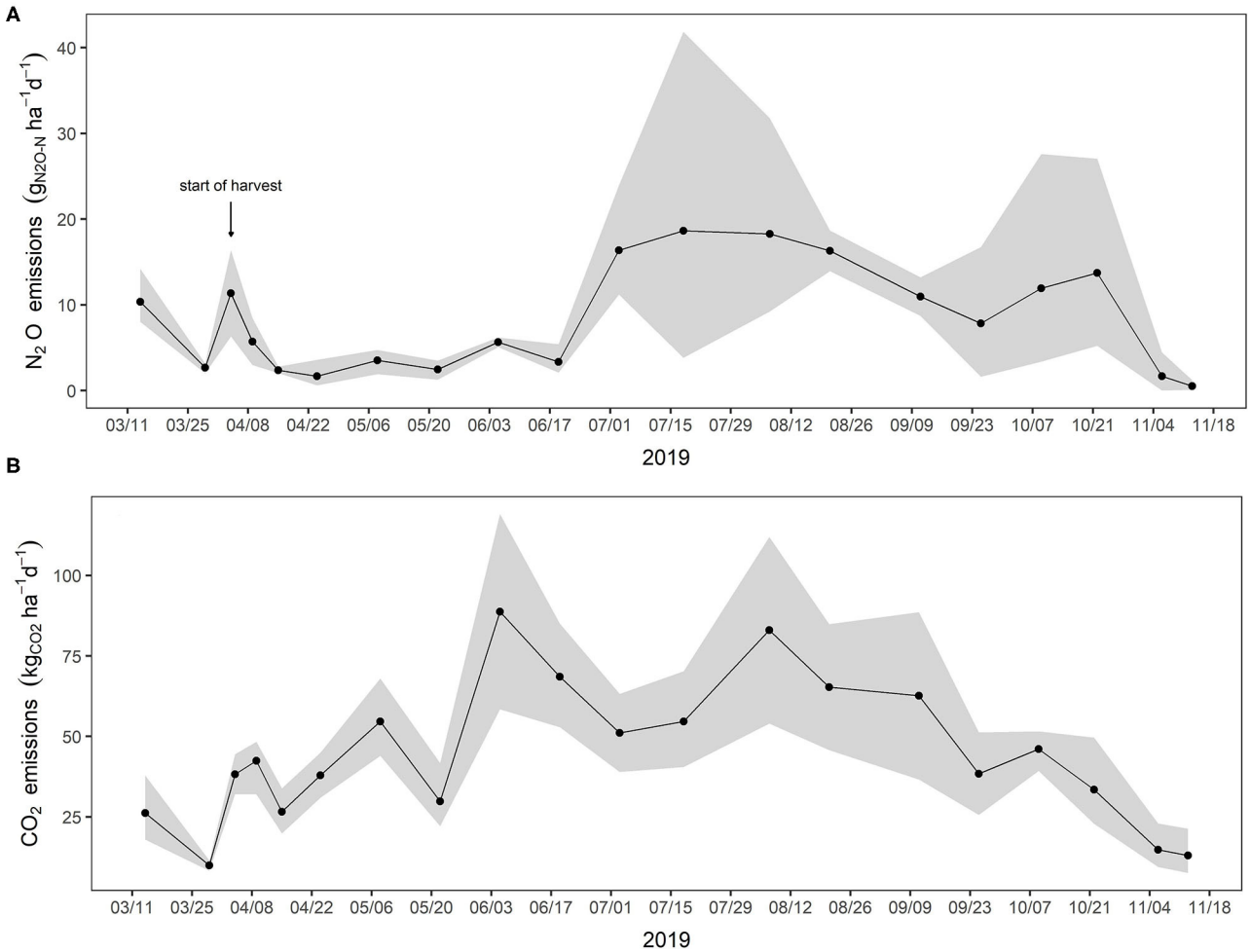

FIGURE 1 | Time series of N_2O **(A)** and CO_2 **(B)** emission rates from the root zone of tomatoes grown on rock wool growing bags in a closed-loop hydroponic system in 2019. Circles show mean values of $n = 4$ replicates and shaded areas the corresponding 95% confidence intervals.

September and increased to high values at 25 September and 9 October in the north block (on average 25.2 g N_2O–N ha^{-1} d^{-1}), while in the south block moderately increased emission rates (on average 14.6 g N_2O–N ha^{-1} d^{-1}) were found at 25 September only. Independent of the block, the N_2O emission rates declined to very low values (on average 0.7 g N_2O–N ha^{-1} d^{-1}) on 22 October, following the cessation of irrigation and nutrient supply. In addition to the temporal and block effects, the emission rates were also affected by the utilized substrate (**Table 1**), with slightly higher values from re-used rock wool than from new rock wool growing bags at most sampling time points (**Figure 3A**). This was also reflected in the linear mixed-effects model by a significant interaction effect of substrate, block and sampling date on N_2O emission rates (**Table 1**). Overall, the N_2O emission rates from late season cucumber cultivation negatively correlated with solar radiation and humidity deficit (**Table 2**). However, the increase of N_2O emission rates in September and October was also coinciding with a substantial spread of mildew, especially in the north block of the greenhouse. Only a few data points could be collected during the early season cucumber cultivation in 2020 (**Supplementary Figure 6A**), because for most of the time sampling was not possible due to the COVID-19 pandemic. The existing data from new rock wool growing bags showed very low N_2O emission rates (on average 0.5 g N_2O–N ha^{-1} d^{-1}) at the beginning of the cultivation period on 4 March, and low emission rates (on average 1.6 g N_2O–N ha^{-1} d^{-1}) at the end of the cultivation period in May (still with fertigation). Cumulative N_2O emissions from the late season cultivation in 2019 were on average 0.74 kg N_2O–N ha^{-1} (**Table 3**). Despite the shorter cultivation period, the N_2O emissions were about 50% higher in the north block (on average 0.89 kg N_2O–N ha^{-1}) compared to the south block (on average 0.60 kg N_2O–N ha^{-1}). Compared to the new rock wool growing bags, the re-used rock wool growing bags had on average 9 and 25% higher N_2O emissions in the north and south block, respectively. Permutational two-way ANOVA showed that the block effect ($P_{exact} = 0.0254$) was significant, while the substrate effect was not significant. The N_2O emissions from cucumber cultivation for the whole year (new

TABLE 1 | Results of linear mixed-effects models analyzing the fixed effects of location inside the greenhouse (Block) and the utilization of new or re-used rock wool growing bags (Substrate, for cucumber cultivation only), and the random effects of individual growing bags on N_2O and CO_2 emission rates from 2019.

Parameter	Factor	Tomato (2019/03/14-11/13)			Cucumber (2019/06/19-10/22)		
		χ^2	df	P_{χ^2}	χ^2	df	P_{χ^2}
N_2O emissions	Date	97.2	19	<0.001	68.7	9	<0.001
(g N_2O–N ha^{-1} d^{-1})	Block	0.03	1	0.857	0.08	1	0.775
	Substrate	–	–	–	6.61	1	0.010
	Block × Date	24.6	19	0.176	30.1	7	<0.001
	Substrate × Date	–	–	–	29.5	9	<0.001
	Block × Substrate	–	–	–	0.01	1	0.944
	Substrate × Block × Date	–	–	–	18.4	7	0.010
CO_2 emissions	Date	147	19	<0.001	117	9	<0.001
(kg CO_2 ha^{-1} d^{-1})	Block	0.09	1	0.770	2.68	1	0.101
	Substrate	–	–	–	0.58	1	0.448
	Block × Date	18.1	19	0.514	67.6	7	<0.001
	Substrate × Date	–	–	–	3.43	9	0.945
	Block × Substrate	–	–	–	2.91	1	0.088
	Substrate × Block × Date	–	–	–	9.48	7	0.220

TABLE 2 | Results of linear regression analyses between average N_2O or CO_2 emission rates (without time points at the end of the growing season when no nutrient solution was applied) and greenhouse climate variables at the corresponding sampling dates in 2019.

Cultivar	Gas flux	Climate variable	r	R^2	$F^a_{1,16/1,7}$	P_F
Tomato	N_2O	Temperature	0.10	0.011	0.17	0.686
		Relative humidity	−0.03	<0.001	0.01	0.908
		Humidity deficit	0.08	0.007	0.12	0.739
		Radiation	−0.02	<0.001	0.01	0.943
	CO_2	Temperature	0.81	0.652	30.0	<0.001
		Relative humidity	−0.12	0.015	0.25	0.624
		Humidity deficit	0.41	0.164	3.14	0.095
		Radiation	0.50	0.254	5.44	0.033
Cucumber	N_2O	Temperature	−0.59	0.344	3.66	0.097
		Relative humidity	0.58	0.332	3.48	0.104
		Humidity deficit	−0.67	0.453	5.81	0.047
		Radiation	−0.75	0.561	8.94	0.020
	CO_2	Temperature	0.91	0.826	33.2	<0.001
		Relative humidity	−0.32	0.104	0.81	0.398
		Humidity deficit	0.53	0.278	2.69	0.145
		Radiation	0.75	0.570	9.28	0.019

For the climate variables temperature (°C), relative humidity (%) and humidity deficit (g m^{-3}), daily averages were used for the analysis. For radiation (J cm^{-2}), the daily sum was used.
[a]*Degrees of freedom depending on the number of sampling time points (18/9 for tomato/cucumber).*
Prior to analysis, N_2O data were log-transformed and all data (gas fluxes and climate variables) were studentized for better comparability.

rock wool growing bags are used in early season and re-used in late season) could only be roughly estimated as the double of the emissions from the late season, i.e., 1.48 kg N_2O–N ha^{-1}, as the data collected from the early cultivation season in 2020 was insufficient. The CO_2 emission rates from the late season cucumber cultivation in 2019 showed a trend to decreasing values from June to October (**Figure 3B**), which strongly correlated with temperature and to a lesser extend with solar radiation inside the greenhouse (**Table 2**). Highest values were found in the south block in June and July (on average 60.4 kg CO_2 ha^{-1} d^{-1}). The CO_2 emission rates were lower in the north block during July (on average 39.8 kg CO_2 ha^{-1} d^{-1}) but increased in August to their maximum (on average 49.6 kg CO_2 ha^{-1} d^{-1}) and were then higher during September than in the south block. The seasonal difference between the two blocks was expressed as a significant interactive effect of block and date in the linear mixed model (**Table 1**). The lowest emission rates (on average 9.8 kg CO_2 ha^{-1} d^{-1}) were measured on 22 October, when the nutrient solution supply was already cut off. The little available data from the early cultivation season in 2020 (**Supplementary Figure 6B**) showed

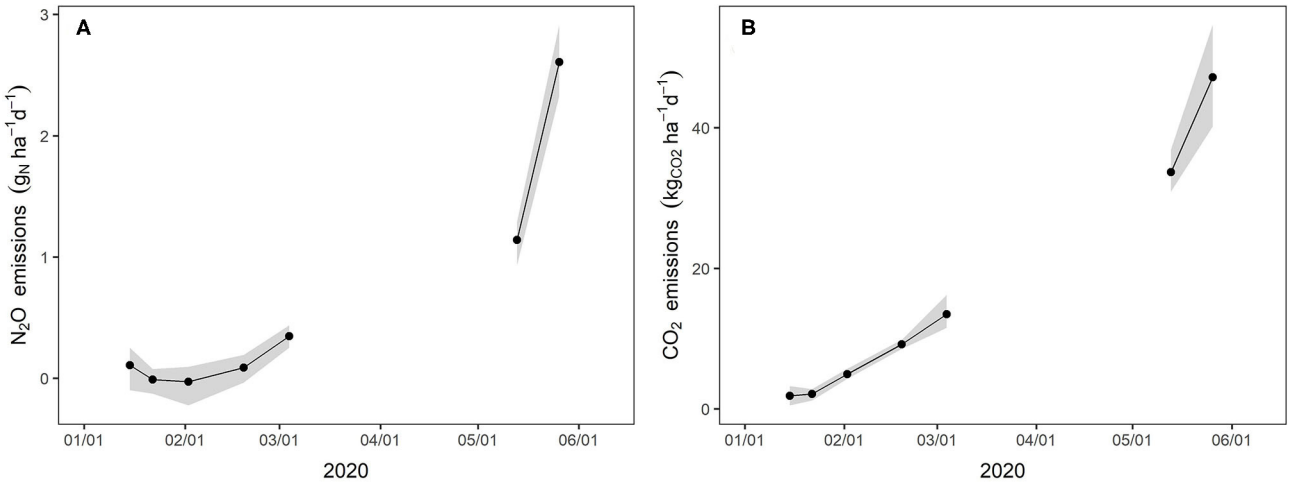

FIGURE 2 | N_2O **(A)** and CO_2 **(B)** emission rates from the root zone of tomatoes grown on rock wool growing bags in a closed-loop hydroponic system in early 2020. Circles show mean values of $n = 6$ replicates and shaded areas the corresponding 95% confidence intervals. Sampling was restricted in March and April due to the COVID-19 pandemic.

TABLE 3 | Cumulative N_2O emissions (cumul. N_2O), N_2O emission factors (EF N_2O) from applied nitrogen fertilizers, yield scaled N_2O emissions, CO_2 equivalents of cumulative N_2O emissions (CO_2 eq.), and cumulative CO_2 emissions (cumul. CO_2) from the root zones of tomato and cucumber plants.

Cultivar	Substrate, block	Time frame	cumul. N_2O (kg N_2O–N ha^{-1})	EF N_2O (%)	Yield-scaled N_2O (mg N_2O–N kg_{fruit}^{-1})	CO_2 eq. (kg CO_2 ha^{-1})	cumul. CO_2 (Mg CO_2 ha^{-1})
Tomato	Rock wool	2019/03/14-2019/11/13	2.29 ± 0.64	0.37	–	1,071	11.5 ± 1.78
		2020/01/15-2020/03/04	0.007 ± 0.001	0.01	–	3.37	0.34 ± 0.01
		2019/03/14-2020/03/04	2.30	0.31	6.36	1,074	11.9
Cucumber	New, South	2019/06/19-2019/10/22	0.53 ± 0.08[a]	0.07	1.49	250	4.90 ± 0.21[a]
	Re-used, South	2019/06/19-2019/10/22	0.66 ± 0.08[a]	0.09	1.85	310	4.89 ± 0.65[a]
	New, North	2019/07/11-2019/10/22	0.85 ± 0.25[a]	0.16	2.36	396	4.41 ± 0.15[a]
	Re-used, North	2019/07/11-2019/10/22	0.93 ± 0.06[a]	0.18	2.60	436	3.79 ± 0.06[a]

[a]*No significant differences were found between groups ($\alpha = 0.05$).*

For cumulative N_2O and CO_2 emissions, mean values of n = 5 (tomato, 2020), n = 4 (tomato, 2019) or n = 2 (cucumber) ± the SEM are shown.

that the CO_2 emission rates were very low at the beginning of cultivation on 4 March (on average 3.4 kg CO_2 ha^{-1} d^{-1}), and then reached values in May 2020 (on average 39.1 kg CO_2 ha^{-1} d^{-1}) comparable to July 2019. The cumulative CO_2 emissions from the late season cucumber cultivation in 2019 were on average 4.5 Mg CO_2 ha^{-1}. The CO_2 emissions were, in contrast to cumulative N_2O emissions, higher in the south block (on average 4.9 Mg CO_2 ha^{-1}) than in the north block (on average 4.1 Mg CO_2 ha^{-1}). The utilization of new or re-used rock wool growing bags had no consistent effect on cumulative CO_2 emissions. This was supported by permutational two-way ANOVA showing a significant block effect ($P_{exact} = 0.0095$), while the substrate effect was insignificant. Assuming similar CO_2 emissions from early and late season, whole year CO_2 emissions from cucumber cultivation were estimated approximately as 9.0 Mg CO_2 ha^{-1}. Over the whole study period no significant CH_4 fluxes were detected (calculated fluxes ranged from −0.1 to 0.2 g CH_4 ha^{-1} d^{-1} and were all below the minimum detection limit; **Supplementary Figure 7**).

Effects of Organic Growing Substrates on N_2O and CO_2 Emissions

The N_2O emission rates from well-drained rock wool, coir and perlite/wood fiber substrates were similar from 14 March to 9 April (**Figure 4A**), with average values ranging from 2.7 to 12.0 g N_2O–N ha^{-1} d^{-1} and varying over time. However, the emission rates from the two waterlogged coir growing bags were substantially higher during this time

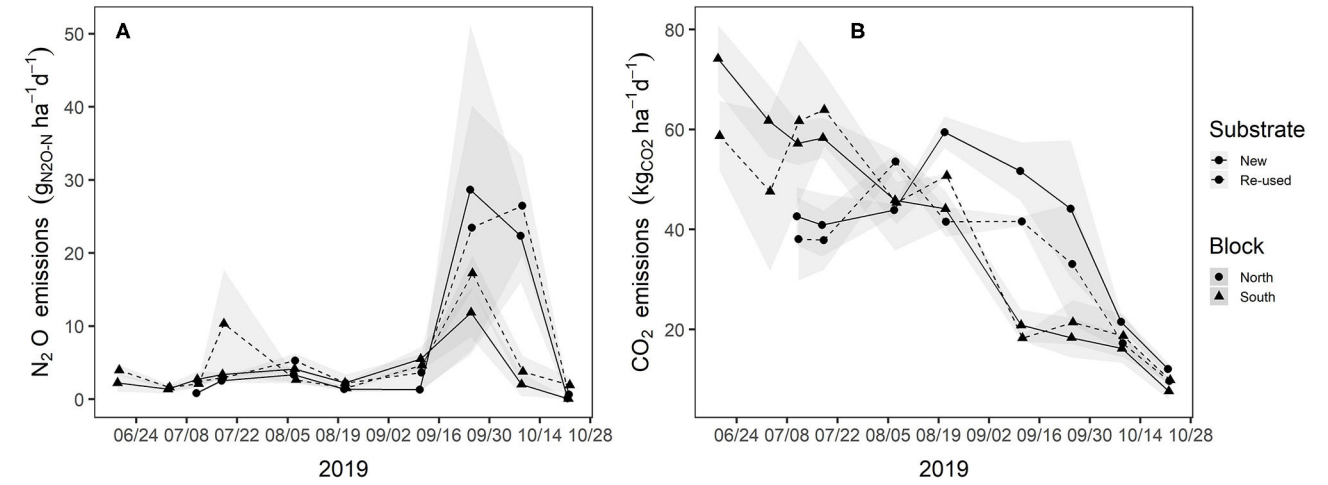

FIGURE 3 | Time series of N_2O **(A)** and CO_2 **(B)** emission rates from the root zone of cucumbers grown on rock wool growing bags in an open-loop hydroponic system in the second half of 2019. Half of the studied rock wool growing bags were new (solid lines), while the other half was previously used in the first half of 2019 (Re-used, dotted lines). Due to a delayed delivery of seedlings, cultivation in the north block (circles) started 3 weeks later than in the south block (triangles) of the greenhouse. Symbols show mean values of $n = 2$ replicates and shaded areas the corresponding 95% confidence intervals.

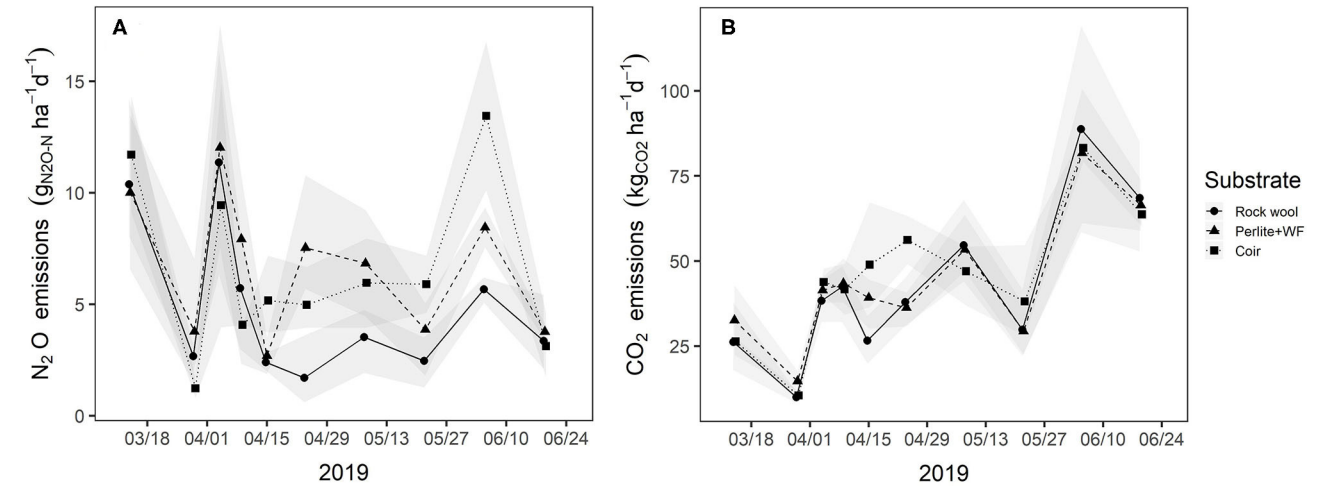

FIGURE 4 | N_2O **(A)** and CO_2 **(B)** emission rates from the root zone of tomatoes grown in perlite/wood fiber growing bags (Perlite+WF, triangles and dashed lines) and coir growing bags (squares and dotted lines) compared to rock wool growing bags (circles and solid lines) in spring 2019. Symbols show mean values of $n = 4$ replicates ($n = 2$ for the first four points of Coir, and for the last point of Coir and Perlite+WF) and shaded areas the corresponding 95% confidence intervals.

(**Supplementary Figure 8A**), yielding average values of 51 to 316 g N_2O–N ha^{-1} d^{-1}. After the waterlogging was eradicated, N_2O emission rates from the previously waterlogged growing bags declined and were similar to the ones from well-drained growing bags on 15 April (**Figure 4A**). From 24 April to 5 June both, coir and perlite/wood fiber growing bags, had about twice as high emission rates (on average 7.6 and 6.7 g N_2O–N ha^{-1} d^{-1} for coir and perlite/wood fiber, respectively) than rock wool growing bags (on average 3.3 g N_2O–N ha^{-1} d^{-1}). On contrast, all substrates had similar emission rates at the last sampling on 19 June. The varying substrate effect over time was reflected by the linear mixed-effects model as a significant interaction effect of substrate and sampling date on N_2O emission rates (**Table 4**). The cumulative N_2O emissions were calculated from 14 March to 5 June, because only two replicates each were measured on 19 June for coir and perlite/wood fiber. The cumulative N_2O emissions from coir and perlite/wood fiber were on average about 50% higher compared to rock wool, while the waterlogged coir growing bags had even 10 times higher emissions (**Table 5**). In both cases, with and without water-logged coir growing bags, a significant substrate effect on cumulative N_2O emissions ($P_{exact} < 0.01$) was found in permutational one-way ANOVA. The CO_2 emission rates from coir and perlite/wood fiber growing bags mostly reflected the

TABLE 4 | Results of linear mixed-effects models analyzing the fixed effects of utilized substrate (rock wool, perlite/wood fiber or coir), and the random effects of individual growing bags on N_2O and CO_2 emission rates from tomato cultivation in spring 2019.

Parameter	Factor	Tomato (2019/03/14-2019/06/05)		
		χ^2	df	P_{χ^2}
N_2O emissions	Date	70.8	8	<0.001
(g N_2O–N ha^{-1} d^{-1})	Substrate	6.68	2	0.035
	Substrate × Date	34.8	16	0.004
CO_2 emissions	Date	137	8	<0.001
(kg CO_2 ha^{-1} d^{-1})	Substrate	3.08	2	0.214
	Substrate × Date	20.9	16	0.183

*Data from two coir replicates with waterlogging during the first four sampling time points were excluded (according to **Figure 4**).*

TABLE 5 | Cumulative N_2O emissions (cumul. N_2O) and cumulative CO_2 emissions (cumul. CO_2) from the root zones of tomato plants grown on rock wool, perlite/wood fiber and coir, with and without waterlogging during the first sampling time points.

Substrate	cumul. N_2O (kg N_2O–N ha^{-1})	cumul. CO_2 (Mg CO_2 ha^{-1})
Rock wool	0.37 ± 0.03^{a}	3.24 ± 0.39
Perlite/wood fiber	$0.55 \pm 0.08^{a,b}$	3.36 ± 0.06
Coir, no waterlogging	$0.55 \pm 0.01^{a,b}$	3.47 ± 0.16
Coir, with waterlogging	3.86 ± 3.04^{b}	4.16 ± 0.28

^{a,b}Letters denote significant differences between groups ($\alpha = 0.05$).
Shown are mean values of n = 4 (rock wool and perlite with wood fiber) or n = 2 (coir with/without waterlogging) ± the SEM.

dynamics found in rock wool (**Figure 4B**), and no significant substrate effects were found in the linear mixed-effects model (**Table 4**). In consequence, also the cumulative CO_2 emissions were comparable between all substrates (**Table 5**). Only slightly higher CO_2 emissions were found from the waterlogged coir growing bags (**Supplementary Figure 8B**). However, there was no significant substrate effect found in permutational one-way ANOVA, regardless of whether the waterlogged coir growing bags were included or excluded.

DISCUSSION

Increasing the sustainability of food production is indispensable regarding current global changes in climate and population. One way of approaching this goal is to increase irrigation and fertilizer efficiency (Gerten et al., 2020). Greenhouse cultivation is known to be very resource-efficient, however, for its expansion potential trade-offs, such as GHG emissions from fertilizer application, need to be assessed (Gruda et al., 2019). In this study, we focused on determining the N_2O emissions from hydroponic tomato and cucumber cultivation under real production conditions. Although the CO_2 emissions from the root zone were about 10 times higher than the N_2O emissions converted to CO_2 equivalents (**Table 3**), the measured CO_2 emissions do not affect the greenhouse gas budget of plant cultivation because of the preceding photosynthetic CO_2 fixation (Smith et al., 2014). In contrast, CH_4 emissions would also affect the GHG budget of plant cultivation but were not traceable in this study. Possibly, CH_4 production was suppressed by rather aerobic conditions in the growing bags and by the high abundance of nitrate fertilizer (Le Mer and Roger, 2001). Despite a few gaps, the data collected during 2019 and 2020 was sufficient for estimating seasonal N_2O emissions from rock wool substrates, since the combined dataset from both years covers all different growth stages of the two vegetable plants. Remarkably, the N_2O emissions reported here are about 10 times smaller compared to findings from previous studies on rock wool-based hydroponic systems. Daum and Schenk (1996a) found that on average 1.2% of the applied N-fertilizer was emitted as N_2O during cucumber cultivation, and Hashida et al. (2014) reported that 4–8% of applied the N-fertilizer was emitted as N_2O during tomato cultivation. In contrast, in our study we found N_2O emission factors of 0.1–0.2% and 0.31% (**Table 3**) for the N supplied during the cultivation of cucumbers and tomatoes, respectively. These values are clearly below the general N_2O emission factor of 1% utilized by the Intergovernmental Panel on Climate Change for estimating N_2O emissions from crop cultivation on soils (IPCC, 2019).

The lower N_2O emissions compared to previous studies could be due to various factors depending on the hydroponic setup. Especially the irrigation rate (Abalos et al., 2014; Yoshihara et al., 2014) and the draining of the substrate could have decreased the emissions by providing more aerobic conditions, thereby decreasing N_2O production from denitrification. The irrigation technique has been found to be a major influencing factor on N_2O emissions from field-based tomato cultivation (Kennedy et al., 2013; Ye et al., 2019). Indeed, we also found strongly increased N_2O emissions from accidentally water-logged growing bags (**Table 5**; **Supplementary Figure 8A**), with up to 40 times higher average N_2O emission rates, underpinning the critical role of precise irrigation and oxygen supply to the root zone in minimizing N_2O production. Another factor limiting N_2O emissions might have been the slightly acidic nutrient solution (pH $\sim$ 5.6) that was supplied to the plants. The activity of nitrifying and denitrifying bacteria is typically highest under neutral and slightly alkaline conditions and decreases with lower pH values (Farquharson and Baldock, 2007). Furthermore, it cannot be excluded that the measurement chambers used here are relatively prone to gas leaking, because the installation and tightening of the chambers in the production greenhouses is very difficult and smaller leaks might have been missed. Nevertheless, mostly linear increases of N_2O and CO_2 concentrations in the chambers over the measurement period of 1 h and extremely high N_2O emission rates found in water-logged substrates (**Supplementary Figure 8**) indicate that the chamber measurements generally worked well. On the other side, it is possible that we rather over-estimated the N_2O emission rates, because the shown gas fluxes were measured during daytime and extrapolated to 24 h. Additional measurements at different daytimes exhibit that the gas emission rates decrease during nighttime (**Supplementary Figure 9**), when no nutrient solution

was supplied and when the temperature inside the greenhouse is lower. Remarkably, comparable N_2O emission rates and N_2O emission factors were reported by Kennedy et al. (2013) for field tomato cultivation, amounting to 0.5 and 0.8% of the applied N-fertilizer from drip-fertigated and conventionally fertilized crops, respectively. Llorach-Massana et al. (2017) found that lettuce crops on perlite bags emitted 0.7-0.9% of the applied N-fertilizer as N_2O. Similarly, low N_2O emission rates were found by (Nett et al., 2019) for cucumber cultivation on substrate-filled pots.

The study of Nett et al. (2019) also showed that N_2O emission rates can strongly increase if sufficient organic C is available in the substrate, as demonstrated by a peak of N_2O emissions following the degradation of roots after cutting shoots. The N_2O emissions from hydroponic systems are probably mainly due to denitrification, as nitrate is typically used as primary N-fertilizer in such systems (de Kreij et al., 2003). Because microbial denitrification is a predominantly heterotrophic process depending on the supply of organic C (Baggs, 2011), the degradation of plant residues can increase the N_2O production by denitrifying microorganisms (Chen et al., 2013), likely also by limiting oxygen availability due to increased C mineralization (Morley and Baggs, 2010). Previous studies (Hashida et al., 2014; Kazuhiro Shoji, 2014) found a strong increase of N_2O emissions from the long-term use of rock wool substrate. However, in this study the N_2O emissions were only slightly increased in re-used rock wool compared to fresh rock wool growing bags used for cucumber cultivation (**Figure 3A**; **Table 1**). Nevertheless, root biomass remained from the previous cultivation in the re-used rock wool growing bags (**Supplementary Table 2**). Possibly, the effect of re-used substrate was overlaid by the strong block effect, which was due to the delayed planting of the north greenhouse block. Because of technical restrictions, the smaller cucumber plants in the north block received the same amount and composition of nutrient solution as the larger plants in the south block. Consequently, the lower water and nutrient demand of cucumber plants in the north block might have resulted in higher moisture and nitrate contents in the growing bags, yielding increased N_2O emission rates compared to the south block (**Figure 3A**). In contrast, we could find a clear effect of the presence of organic material in the growing substrate of tomato plants (**Figure 4A**; **Table 4**), showing that the use of coir and perlite with wood fiber growing bags increased N_2O emissions almost by 50% compared to rock wool growing bags after 5 months of cultivation in June (**Table 5**). This effect might even be higher at the end of the growing season, as emissions from rock wool growing bags increased in July and remained at a relatively high level until the end of October (**Figure 1A**).

In general, we could hardly find a correlation between the greenhouse climatic conditions and the N_2O emission rates (**Table 2**). On the contrary, the CO_2 emissions clearly reflected the changes in temperature over the growing season, as expected for the general microbial activity involved in the decomposition of labile organic C (Davidson and Janssens, 2006). Despite the known temperature sensitivity of N_2O emissions (Grant and Pattey, 2008) the lowest daily mean temperatures found in the greenhouse may have already been at the optimum for denitrification (Farquharson and Baldock, 2007) or a higher share of N_2O was further reduced to molecular nitrogen (N_2) with increasing temperature (Maag and Vinther, 1996). Similarly to temperature, no clear effect of the supplied amount of nutrient solution was visible in our study. The irrigation frequency was adjusted during the cultivation period according to temperature and solar radiation (**Supplementary Figure 10**), whereby strong fluctuations in moisture contents inside the growing bags should have been avoided. In hydroponic systems, the N_2O emission rate was found to strongly depend on plant growth stage (Daum and Schenk, 1996a; Hashida et al., 2014). In line with this, we found that the onset of harvest can temporarily increase N_2O emissions from tomato cultivation, potentially by altering plant C allocation with more C substrates translocated to roots. The increase of N_2O emission rates at the end of the growing season from both, tomato and cucumber cultivation, can be explained by the accumulation of senescent roots delivering C substrates needed for denitrification. In this way, the higher N_2O emission factors from tomato cultivation compared to cucumber cultivation (**Table 3**) might be explained by the higher root biomass of tomato plants (**Supplementary Table 2**), with a potential further increase in organic C due to the re-use of collected drain solution. However, the higher emission rates in September and October might also be related to a lower plant N uptake, considering the negative relation of photosynthetically active radiation and N_2O emission rates found by Yoshihara et al. (2016). In addition, observed plant diseases at the end of the growing season, like the *Agrobacterium rhizogenes* ("Crazy Roots") infection of tomato plants or the mildew on cucumber plants, could also have affected microbial N_2O production by increasing C allocation to roots.

In conclusion, our study demonstrated that hydroponic systems offer a possibility to cultivate vegetable crops with low N_2O emissions if optimal conditions are provided. In particular, high moisture contents together with high C availability in the root zone should be avoided to minimize N_2O production from denitrification. In addition, other reduction possibilities for GHG emissions from greenhouse cultivation should be considered as well. The production of rock wool is associated with high CO_2 emissions, which could be avoided by using alternative (biodegradable) substrates (Dannehl et al., 2015; Kennard et al., 2020). However, there is more research needed on the interaction of different substrates and GHG emissions as well as yield and quality. Considering the moderate increase in N_2O emissions when using organic-based substrates in our study, there might still be a high reduction potential compared to GHG emissions from rock wool production. Similarly, the GHG emissions from fertilizer production might be reduced by utilizing fertilizers recycled from waste streams. First investigations show that recycling fertilizers are suitable for hydroponic tomato production without increasing N_2O emissions (Halbert-Howard et al., 2020). Other measures that can contribute to reducing GHG emissions from greenhouse cultivation include avoiding heat losses by improved greenhouse insolation, using alternative heating and electricity sources, installing energy-efficient lamps, and using renewable sources for CO_2 enrichment in the canopy (Gruda et al., 2019). Taken all these measures

together, hydroponic greenhouse cultivation could help to ensure sustainable vegetable production by reducing the distance between producers and consumers. Because of the high yield to area ratio and the possibility to control most environmental conditions, hydroponic or even aquaponic systems seem to be very promising for food production in urban areas or regions with otherwise adverse climatic conditions.

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

DS and KH initiated the study. MG and KH managed greenhouse technics and plant cultivation during the study period. SK and MG conducted the GHG measurements. SK analyzed the gas samples, evaluated the gas flux data, and prepared the manuscript. All authors were involved in the planning, critically read the manuscript, and provided their feedback.

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REVIEW
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CH_4 and N_2O Emissions From Cattle Excreta: A Review of Main Drivers and Mitigation Strategies in Grazing Systems

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Cattle production systems are an important source of greenhouse gases (GHG) emitted to the atmosphere. Animal manure and managed soils are the most important sources of emissions from livestock after enteric methane. It is estimated that the N_2O and CH_4 produced in grasslands and manure management systems can contribute up to 25% of the emissions generated at the farm level, and therefore it is important to identify strategies to reduce the fluxes of these gases, especially in grazing systems where mitigation strategies have received less attention. This review describes the main factors that affect the emission of GHG from manure in bovine systems and the main strategies for their mitigation with emphasis on grazing production systems. The emissions of N_2O and CH_4 are highly variable and depend on multiple factors, which makes it difficult to use strategies that mitigate both gases simultaneously. We found that strategies such as the optimization of the diet, the implementation of silvopastoral systems and other practices with the capacity to improve soil quality and cover, and the use of nitrogen fixing plants are among the practices with more potential to reduce emissions from manure and at the same time contribute to increase carbon capture and improve food production. These strategies can be implemented to reduce the emissions of both gases and, depending on the method used and the production system, the reductions can reach up to 50% of CH_4 or N_2O emissions from manure according to different studies. However, many research gaps should be addressed in order to obtain such reductions at a larger scale.

Keywords: global warming, climate change, nutrient excretion, nitrification, mitigation, nitrogen losses

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INTRODUCTION

Greenhouse gas (GHG) concentrations in the world have increased rapidly since pre-industrial times due to human activities, with negative effects on the climate(IPCC (Intergovernmental Panel on Climate Change), 2013). Methane (CH_4) concentrations have doubled while nitrous oxide (N_2O) concentrations in the atmosphere are 20% higher than pre-industrial levels (IPCC (Intergovernmental Panel on Climate Change), 2013). Agriculture is considered one of the main sources of CH_4 and N_2O, two high warming potential gases. Within the agricultural sector, animal production contributes 14.5% of human-induced emissions (Gerber et al., 2013) and produces ∼37 and 65% of global emissions of CH_4 and N_2O, respectively (Steinfeld and Wassenaar, 2007).

Within livestock, cattle production systems can be broadly classified into confined, mixed -in which cattle can be in-house during part of the day or the year, and grassland-based systems (Seré and Steinfeld, 1995). In confined and semiconfined systems, manure can be stored and processed to be disposed in the field, whereas in grazing systems, manure is deposited directly on pastures and is degraded under environmental and grazing conditions (Uchida et al., 2011). Manure (feces and urine) managed and deposited on grasslands and pastures is the second largest source of GHG emissions after enteric methane and is responsible for ∼7% of agricultural emissions of CH_4 and N_2O worldwide (Aguirre-Villegas and Larson, 2017).

Nitrous oxide is the third most abundant GHG and accounts for 6% of all radiative forcing (Myhre et al., 2013). Despite its low concentration in the atmosphere compared to CH_4 and CO_2, N_2O has a significant effect on global warming, as it has a lifespan of ∼120 years and 265 times higher radiative potential than CO_2 (IPCC (Intergovernmental Panel on Climate Change), 2014; U. S. E. P. A and United States Environmental Protection U. S. E. P. A. United States Environmental Protection Agency, 2021). In addition, it contributes significantly to the depletion of stratospheric ozone (Myhre et al., 2013).

Grasslands around the world emit about 2.2 Tg of N_2O–N, 74% of which comes from anthropogenic sources (Dangal et al., 2019). Deposition of animal feces and urine is the biggest source of N_2O emissions per year in grasslands (54%), followed by manure application (13%), and nitrogen fertilizers (7%) (Dangal et al., 2019). Nitrification and denitrification are the main responsible mechanisms for the production of N_2O in soils, although nitrification-denitrification, codenitrification and chemodenitrification can also lead to the formation of N_2O given a microbial community and suitable environmental conditions (Hallin et al., 2018). Regarding methane, ruminant manure is responsible for the emissions of 109 million tons of this GHG to the atmosphere per year, of which 86% comes from cattle. Three main factors affect the amount of CH_4 emitted by manure: the type of storage, the climate, and the composition of manure (Opio et al., 2013). While most of CH_4 emissions from manure occur during storage under anaerobic conditions, in tropical regions manure can also be a generator of a considerable amount of emissions of this gas at the grassland level (Montes et al., 2013; Cai et al., 2017).

However, it is important to mention that although these gases play an important role in global warming, as they are predominantly flow pollutant gasses, they differ in their impact from CO_2 that is a stock pollutant with a very long-term persistence in the atmosphere and consequently with a greater cumulative effect on the climate (Lynch et al., 2021). In addition, grasslands around the world also hold a large mitigation potential for building and conserving soil carbon and could capture as much as 0.5 Pg C per year to 1 m depth, as they cover ∼52.5 million km^2 equivalent to 40.5% of the land area (Gerber et al., 2013; Lorenz and Lal, 2018).

This article reviews the magnitude of typical N_2O and CH_4 emissions from manure, analyses the factors affecting them, and discuss potential mitigation strategies in bovine production systems, with an emphasis on tropical and subtropical regions where grazing systems are predominant.

NITROUS OXIDE AND METHANE EMISSIONS IN GRAZIN SYSTEMS

Nitrous Oxide Emissions

Ruminants are poor nitrogen converters, because only 5–30% of ingested nitrogen are up taken by the animal and the remaining 70–95% are excreted *via* feces and urine (Luo et al., 2010). Therefore, nitrogen loads in animal excreta, often exceed plant demands and are vulnerable to losses *via* gaseous emissions and leaching (Selbie et al., 2015). This is more critical as the proportion of nitrogen in animal urine has increased with increasing nitrogen intake; although it has remained relatively constant in feces (Jarvis et al., 1995). According to Jaimes and Correa (2016) the efficiency in the use of N by lactating cows varies between 8.96 and 27.82% in Colombia, which, according to the number of animals per unit area can generate the application of up to 374 kg of N/ha/yr from manure (Correa et al., 2012). Likewise, Rivera et al. (2018) found that cows excreted 72% of ingested nitrogen in tropical dairy systems, generating the deposition of 46.8 kg N/animal/yr from manure and 42.9 kg from urine when the diet had on average 14% crude protein (CP). For this reason, improving the efficiency in the use of this nutrient by ruminants may be a viable alternative not only to increase animal productivity but also to reduce GHG emissions by reducing N excretion. It must be noted however that in extensive grazing and pastoralist systems cattle can be undernourished, and the scarce nutrients can be used more effectively by animals (Manzano and White, 2019).

Grazed pastures are systems with a wide range of environmental and management conditions that can result in the emission of N_2O (Wecking, 2021). A large proportion of total farm N_2O emissions in grazing systems often occurs from relatively small areas (Luo et al., 2017). These sites can be located where animals congregate (feeding bins, water troughs and gateways), occur after additional irrigation or result from soil compaction due to trampling and in the soil underneath excreta patches (Roesch et al., 2019). The magnitude of N_2O emissions depends on the interplay between prevailing soil microclimate, microbial activity, plant composition, biomass, and excreta composition, that in turn is defined by animal type and feed intake (Wecking, 2021). All these factors can alter the spatial heterogeneity of soil respiration and, hence, cause impact also on resulting N_2O emissions (Shi et al., 2019). Nitrous oxide emissions from a single application of cattle urine and feces can be as high as 16.8 kg N_2O–N/ha/yr and 5.57 kg N_2O–N/ha/yr, respectively (Luo et al., 2018). A meta-analysis by López-Aizpún et al. (2019) showed that, when reporting urine derived N_2O emissions, it was important to account for differences in animal

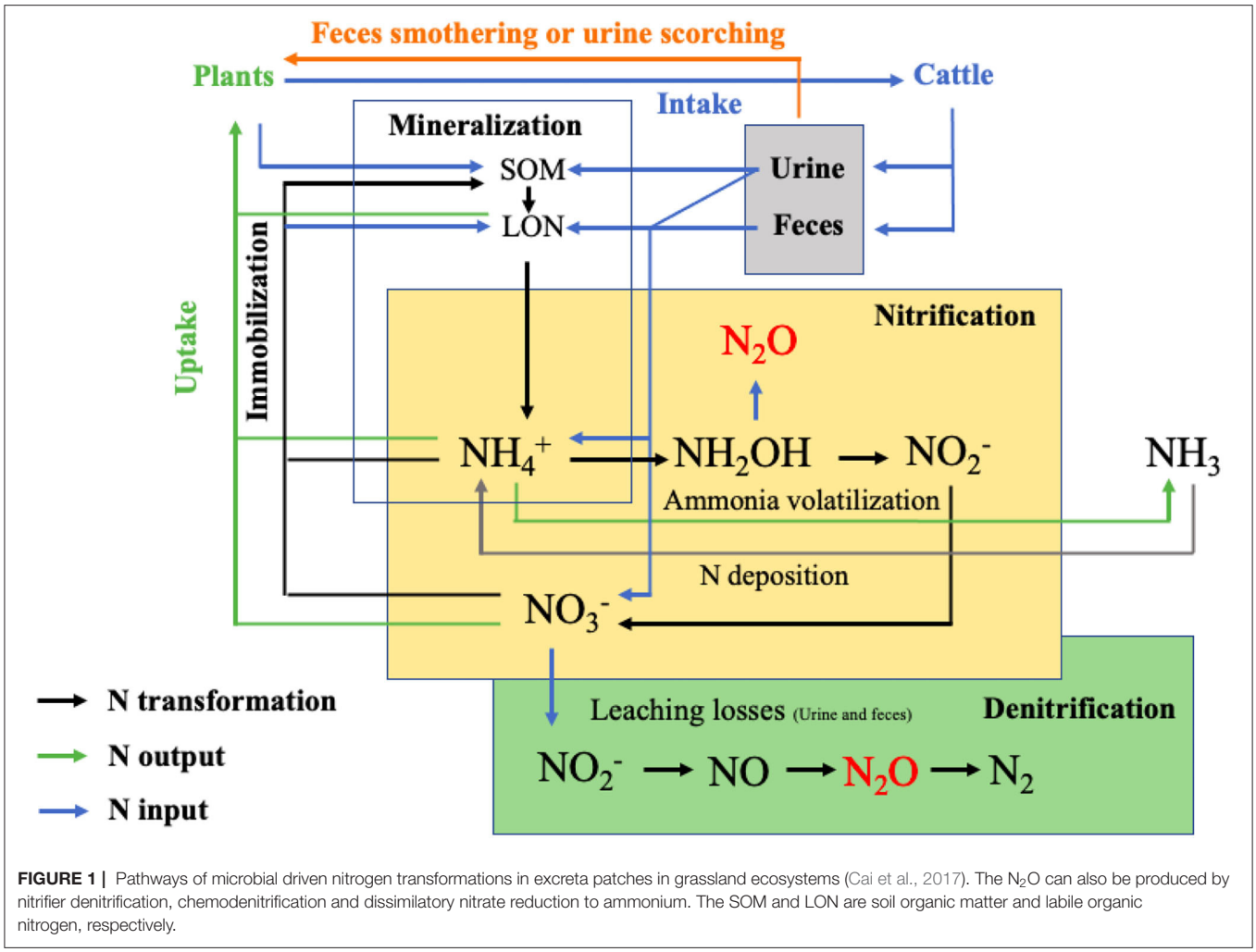

FIGURE 1 | Pathways of microbial driven nitrogen transformations in excreta patches in grassland ecosystems (Cai et al., 2017). The N_2O can also be produced by nitrifier denitrification, chemodenitrification and dissimilatory nitrate reduction to ammonium. The SOM and LON are soil organic matter and labile organic nitrogen, respectively.

diet, sex and breed, in addition to urine composition and nitrogen loads.

Factors Conditioning Nitrous Oxide Emissions From Manure

The two main processes that generate N_2O: nitrification and denitrification, are strongly influenced by climate and soil factors (Chen et al., 2008). The production of N_2O depends on the availability of substrates for both processes, i.e., NH_4^+ for nitrification and NO_3^- for denitrification (Zaman et al., 2007). The most important factors are the presence of oxygen, temperature, pH, humidity, salinity, and soil management; in the case of denitrification, it also depends on the carbon available for heterotrophic processes (Dalal et al., 2003). In addition, these factors are regulated by climate, vegetation, chemical, and physical properties of soil (apparent density, organic C, pH, and clay content), and agricultural management practices (Uchida et al., 2011). Each of these factors is discussed in more detail below.

The process of nitrification was first described by Schloesing and Muentz in 1877. Nine years later, Gayon and Dupetit discovered denitrification (Elmerich and Newton, 2007). An overarching framework capturing the production and consumption of N_2O and NO by nitrification and denitrification within a conceptual model was published by Firestone and Davidson (1989) and has been acknowledged as the "*hole in the pipe model*" (Wecking, 2021). However, recent research suggests that a range of other biotic and abiotic pathways might also lead to the emission of N_2O e.g., heterotrophic nitrification, nitrifier denitrification, chemodenitrification, coupled nitrification-denitrification, co-denitrification, and anaerobic NH_3 oxidation–apart from potential other yet still undiscovered processes in the nitrogen-cycling network (Kuypers et al., 2018). **Figure 1** shows the pathways of microbial driven nitrogen transformations in excreta patches in grassland ecosystems.

Irrespective of the underlying process, most nitrification and denitrification pathways in soil lead to the net emission of N_2O (Myrold, 2005). Only under certain conditions denitrifier activity, and to some extent that of nitrifiers, stimulate the uptake of atmospheric N_2O. Soils are more

likely to act as a sink for N_2O when the soil mineral nitrogen is low and when high soil moisture or other factors prevent microbial access to alternative oxygen (O_2) sources (Philippot et al., 2009).

Among the factors affecting N_2O emissions, the availability of C and N is critical, particularly when these elements are in labile organic form (van Groenigen et al., 2005). There is a linear relationship between the N input, either by fertilizer or by manure, and the emission of N_2O in agricultural areas (Dobbie et al., 1999); although, according to Zebarth et al. (2008), this relationship can also be exponential. In relation to soil properties, the moisture content is perhaps the variable with greatest influence on N_2O emissions (Saggar et al., 2004). Saturation values of 60–70% moisture promote the generation of N_2O since they limit the O_2 diffusion, resulting in denitrification processes (Saggar et al., 2004). Nitrous oxide emissions are therefore higher in wet soils and after dry conditions, N_2O production begins immediately after applying water to the soils (Chirinda et al., 2019).

Temperature is another factor influencing the level of emissions. When soil water content is close to the maximum retention capacity, N_2O emissions respond to temperature changes (Machefert et al., 2002). However, some studies have shown no significant relationships between emission and temperature variations mainly due to the higher influence of moisture content in the flow of gases (Singurindy et al., 2009). Soil type can also influence N_2O emissions, mainly through their effect on drainage level and moisture content (Luo et al., 2010). Poorly drained soils have higher N_2O emissions than well-drained soils (Oenema et al., 2007). Poor drainage and changes in the physical properties of soil such as compaction can affect transformations from N to N_2O because they affect the soil oxygen diffusion (Oenema et al., 1997) and can increase N gaseous losses (van der Meer, 2008).

Animal grazing with its trampling can favor this condition compared with grasslands without animal occupation. Compaction can also be increased when grazing takes place during winter and/or when there is inadequate management of animal stocking rates as this causes loss of structure and drastic decrease of porous space (Luo et al., 2010). pH can also affect the mechanisms that control N_2O emissions. A study of denitrifying enzymes found a link between soil pH and soil emission rate (van der Weerden et al., 1999), as denitrification processes decrease as soil pH tends to acidity. According to Dalal et al. (2003), the optimal pH for nitrification activity is 7, while for denitrification the optimal pH is 7.0–8.0.

Nitrous Oxide Emissions in Feces and Urine

Most studies comparing N_2O–N emission factors of feces and urine under grazing conditions suggest higher values for urine (van Groenigen et al., 2005; López-Aizpún et al., 2020). However, authors such as Sherlock et al. (2003) have reported similar values for both components, while Wachendorf et al. (2008) found higher emissions of N_2O–N in livestock feces, suggesting the need of disaggregating emission factors based on the type of excreta (Luo et al., 2010; López-Aizpún et al., 2020). According to Meng et al. (2014) and Sordi et al. (2014), the fraction of N lost as N_2O–N in the urine is greater than that of feces because only a relatively small fraction of N in manure is in an unstable condition and this depends on the diet of the animals.

According to the Intergovernmental Panel on Climate Change (IPCC) guidelines, it is estimated that the generation of N_2O by manure deposited in the grasslands corresponds to 2% of the total excreted N (IPCC (Intergovernmental Panel on Climate Change), 2006). However, some studies have found that this value may be considerably lower, to such a point that by 2019 this same body changed that value to 0.4% to estimate direct emissions and another 0.27% for indirect emissions by leaching and volatilization (IPCC (Intergovernmental Panel on Climate Change)., 2019). In a study carried out in New Zealand, Luo et al. (2008) reported emission factors (EF) for urine applications between 0.2 and 1.59%, depending on the season. This variability in EF highlights the importance of determining country-specific or climate region emissions. This variability can be caused by multiple factors such as: (i) moisture, carbon content, pH and soil structure; (ii) environmental conditions such as temperature and rain (due to its influence on soil moisture); (iii) quantity and availability of nutrients in soil and excreta; (iv) plant species present; and (v) soil management (Oertel et al., 2016; López-Aizpún et al., 2020).

Countries like New Zealand have advanced in disaggregating EF by type of livestock (bovines and sheep), type of excreta (feces and urine) and climate (wet and dry). In this country, EF for urine and feces are 1 and 0.25% respectively, and both have been implemented in the national inventory of agricultural greenhouse gases (van der Weerden et al., 2020). New Zealand values, applied to all major classes of livestock (sheep, cattle, and deer), are similar to those found by Chadwick et al. (2018) in studies in the United Kingdom (average urine and feces of 0.69 and 0.19%, respectively) and Ireland where EF of 1.18 and 0.31% have been found for these two emission sources, respectively (Krol et al., 2016). Similarly, Rivera et al. (2018) found EF between 1.37 and 1.77% for feces, and between 0.3 and 3.47% for urine in tropical conditions of Colombia, which are higher than those reported by Sordi et al. (2014) in Brazil who found values from 0.19 to 0.33% and 0.12 to 0.19% for urine and feces, respectively. **Figure 2** represents the final distribution of N in urine and feces, showing higher amounts of N leached and higher amounts of NH_3 in urine than in feces, which could lead to higher emissions in urine patches.

Methane Emissions

The production of CH_4 occurs *via* the microbial degradation of the proteins, organic acids, carbohydrates, and soluble lipids present in excreta (Khan et al., 1997). According to the IPCC-Tier 1 (2006), 1 kg of CH_4 is emitted from dung annually per adult head of cattle in grazing systems, but according to others reports these values may be lower (0.45–0.67 kg/animal/day), and can be highly variable (IPCC (Intergovernmental Panel on Climate Change)., 2019).

In the soil, CH_4 is produced under anaerobic conditions by methanogens and is converted to CO_2 by methanotrophs under

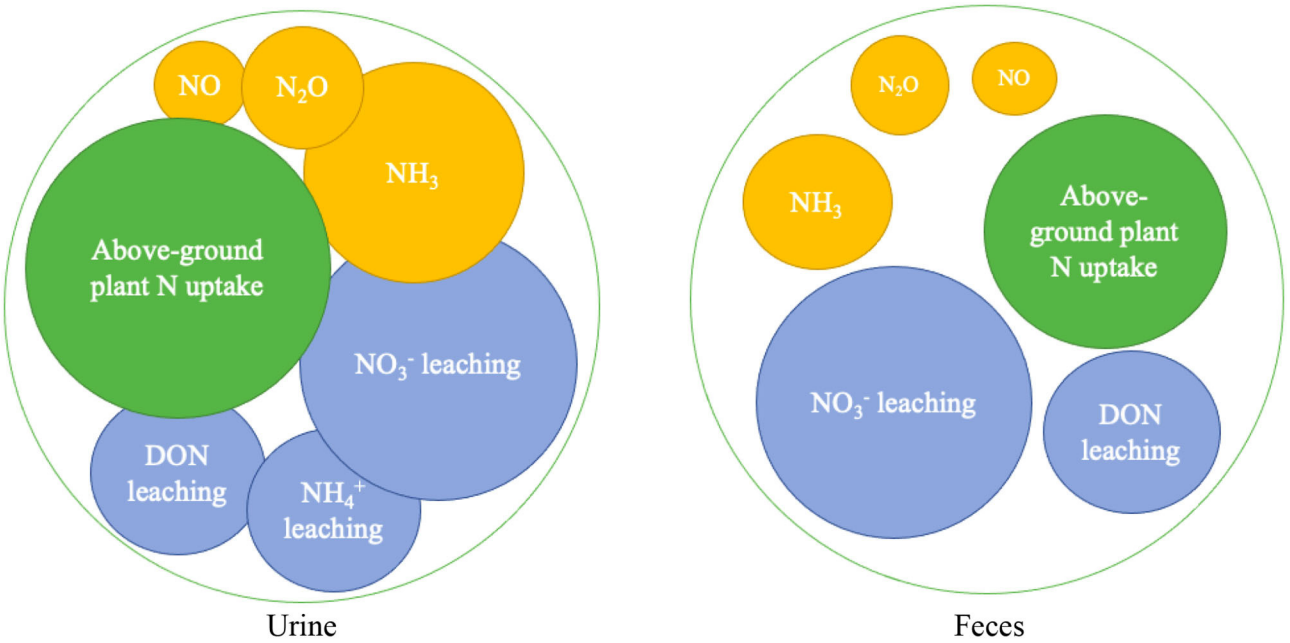

FIGURE 2 | Fate of nitrogen losses from cattle urine and feces patches distinguished into a leaching (blue), gaseous (orange) and plant uptake (green) component. DON stands for dissolved organic nitrogen. The size of the circles can be put into perspective compared to the two large black circles that reference a patch nitrogen load of 1,000 g. Figure adapted from Cai and Akiyama (2016) and Wecking (2021).

both aerobic and anaerobic conditions, and the net CH_4 flux in the soil-atmosphere system represents the balance between these two microbial processes (Le Mer and Roger, 2001). The impact of excreta deposition on CH_4 emission thus mainly depends on its relative impact on CH_4 production and CH_4 oxidation activities in the patch (Cai et al., 2017).

Around 15–30% of total global CH_4 emissions could be derived from soil source (Yanan et al., 2018). Ruminant manure is responsible for the emissions of 109 million tons of this GHG to the atmosphere per year, of which 86% comes from cattle. Three main factors affect the amount of CH_4 emitted by manure: the type of treatment, the climate, and the composition of manure (Opio et al., 2013). While most of CH_4 emissions from manure occur during storage under anaerobic conditions, in tropical regions manure can also be a generator of a considerable amount of emissions of this gas at the grassland level (Montes et al., 2013; Cai et al., 2017).

Even though pastures can emit CH_4, under certain conditions, upland soils including those covered by grasslands are also an important sink for atmospheric CH_4 as they can oxidize it at a faster rate than croplands (between 3 and 6 kg of CH_4/ha/yr), although at a slower rate than uncultivated soils (Boeckx and Van Cleemput, 2001). This is caused by the oxidative activity of methanotrophs and ammonium oxidizing bacteria (Shukla et al., 2013). The oxidative capacity is nevertheless affected, among other factors, by water content and inorganic N in the soil, by the use of inorganic fertilizers and by the NH_4^+ released during urine urea hydrolysis (Le Mer and Roger, 2001; Saari et al., 2004). During the rainy season CH_4 emissions rise due to increased anaerobic conditions caused by water saturation but, when soil moisture is reduced due to decreased rainfall, these are replaced by oxidative processes with predominance of aerobic bacteria that generate negative methane flows and act as CH_4 sinks (Visscher et al., 2007).

Factors Conditioning Methane Emissions

As for N_2O, microbial processes that determine methane emissions into the atmosphere in grazing systems are conditioned by soil factors such as redox potential, pH, temperature, organic carbon and nitrogen content (Towprayoon et al., 2005). These factors can affect the proliferation of some soil microorganisms and, in turn, promote or limit bacterial metabolism through its impact on synthesis and enzymatic activity. The process of methanogenesis is regulated by the concentration of O_2, the content of organic matter as a substrate, and the factors that determine its redox potential (Conrad, 1996). Organic matter is the main input for triggering methane production processes. The increase of available organic matter, and its subsequent decomposition in soils under anaerobic conditions, stimulates methanogenesis by providing a substrate for the production of acetate and hydrogen and causing soil reducing conditions (Sass et al., 1991).

In pastures, most of the organic matter comes from plants through leaves senescence and root decomposition, and the transformation and deposit by animal excreta (Waschütza et al., 1992). For this reason, forage species and their physiological status can also influence methane emissions (Kerdchoechuen, 2005). The strictly anaerobic methanogens, mainly *Methanobacteria, Methanococci* and *Methanopyri*, are sensitive to changes in soil water content (Malyan et al., 2016).

Methanogenic activity may be stimulated by urine deposition that create anaerobic conditions. Furthermore, increased soil pH resulting from hydrolysis of urine urea and decreased redox potential may also favor methanogenic activities (Le Mer and Roger, 2001). Emissions of CH_4 from excreta deposited by animals on pastures range from 7 to 27% of total emissions by ruminants (Kreuzer and Hindrichsen, 2006). However, these emissions may become less significant depending on environmental conditions and manure management (Oenema et al., 2007). When anaerobic conditions occur, ruminant manure in pastures release significant amounts of CH_4 (Misselbrook et al., 2001).

Methane Emissions in Feces and Urine

In an evaluation of methane fluxes of an intensive silvopastoral system (iSPS) with high density of Leucaena, an intensive pasture monoculture system and a secondary dry forest, Rivera et al. (2018) found that both the forest and the iSPS had negative CH_4 flows of −0.56 and −0.02 kg of CH_4/ha/yr respectively, probably due to the biodiversity of microorganisms found in their soils (Vallejo et al., 2010). Negative flows of CH_4 were also found during the dry season in three pasture systems in the north of Colombia (Espinosa-Carvajal et al., 2020). Methane flows are also influenced by the grass species and fertilization level. Pastrana et al. (2011) in a study evaluating three accessions of *Brachiaria humidicola* (Rendle) Schweickerdt found that methane emissions were increased from sink (−23.6 $\mu g\ m^2/h$) when nitrogen fertilization was zero, to emit 107.9 $\mu g\ m^2/h$ with application of 150 kg of N/ha/yr and 59.7 $\mu g\ m^2/h$ with 300 kg of N/ha/yr. CH_4 emissions were also affected by the *B. humidicola* accession.

CH_4 emissions from excreta deposited by animals on pastures range from 7 to 27% of total emissions by ruminants (Kreuzer and Hindrichsen, 2006). However, these emissions may become less significant depending on environmental conditions and manure management (Oenema et al., 2007). According to recent IPCC estimates, 0.49 ± 0.43 g of CH_4 per kg of dry matter (DM) of manure can be generated on average (IPCC (Intergovernmental Panel on Climate Change)., 2019). In addition, urine deposited directly in the grasslands increases emissions of this gas by up to 100 times compared to grasslands without urine patches (Oertel et al., 2016). The diversity of conditions that change CH_4 emissions has generated great dispersion in the results (Andueza et al., 2017), as there are reports of up to 1 g of CH_4 per kg of DM of manure generated, value that can be up to five times higher when manure is incorrectly stored under anaerobic conditions (Sneath et al., 2006). According to Chadwick (2005) manure emissions can range from 0.4 to 9.7% of the total C content deposited by manure.

Finally, Life Cycle Analysis (LCA) studies have identified that manure emissions (CH_4 and N_2O) can be considerable under different production conditions. For example, Rivera et al. (2014), who evaluated the LCA in two dairy systems in Colombia, found that manure emissions accounted for 30% as CO_2-eq of the total emitted on the farm and 22% of the total emitted throughout the LCA. In another study in dairy production systems, Rivera et al. (2016) found that manure emissions accounted for 6.5% of all farm-level emissions. According to this study, emission levels from manure depend mainly on the amount of excreted N given by the consumption of this nutrient in the diet, by the type of manure management, and by the rainfall regimen and use (or not) of irrigation in the grasslands.

NITROUS OXIDE AND METHANE EMISSION MITIGATION STRATEGIES

Opportunities and Tradeoffs

Opportunities to reduce N_2O and CH_4 emissions from livestock manure are diverse and can be addressed to different parts of the animal production cycle to control the production and emission of these two gases (Montes et al., 2013). Given the different environmental and metabolic conditions that influence N_2O and CH_4 flows from soil and manure, it is difficult to implement efficient mitigation alternatives that target both gases simultaneously (Montes et al., 2013). However, as presented in **Table 1**; **Figure 3**, measures related to improving production efficiency and improving soil protection with adequate cover and introduction of trees can contribute simultaneously to reduce emissions of both gases and, in addition, improve carbon sequestration.

The success of mitigation measures can be estimated based on the optimal conditions under which nitrification and denitrification processes occur, in the case of N_2O and from the dry matter degradation processes of manure for CH_4. Since CH_4 is produced under anaerobic conditions, while N_2O production requires sufficient oxygen levels, some practices that reduce CH_4 production tend to increase N_2O emissions. **Table 1** presents a list of mitigation alternatives with their potential impact and limitations.

According to de Klein and Eckard (2008) and Wecking (2021), strategies to mitigate N_2O emissions from grazing systems should include two complementary approaches: (1) to manage the sources of nitrogen uptake at the animal level, and (2) to control N_2O production *in situ* through soil and pastoral management. Beukes et al. (2010) determined that measures such as improved animal genetics, reduction of nitrogen fertilization, and improved grazing management had the potential to decrease GHG emissions from pasture-based systems by 27–32%. Future mitigation should ideally be focused on finding combined strategies that avoid any offsetting effect of the desired mitigation benefits and also, help to improve the efficiency of nitrogen cycling through the soil-plant-animal system while, at the same time, reducing emissions (Cai et al., 2017).

The first approach includes improving management and feeding practices, supplying nutrients, in particular protein sources, according to animal requirements, and increasing animal productivity and nitrogen efficiency per kilogram of animal product through breeding and genetic manipulation (de Klein and Eckard, 2008; Wecking, 2021). For the second approach, mitigation strategies addressed

TABLE 1 | Mitigation alternatives and potential for reducing CH_4 and N_2O emissions in grazing systems.

Strategy	Mitigation potential	Possible negative/Limiting aspect	GHG mitigated	References
Urease and nitrification inhibitors	15–45%	Labor cost, economic cost, difficult to use, and production of other GHGs.	N_2O	de Klein and Monaghan, 2011; Di and Cameron, 2012
Time of application of manure in the field	17%	Labor cost, economic cost.	N_2O	VanderZaag et al., 2011
Use of plant species that can inhibit nitrification	60%	Labor cost, economic cost.	N_2O	Byrnes et al., 2017
Use of SPS as a strategy to improve soil conditions in terms of cover and biodiversity	57%	Labor cost, economic cost.	N_2O	Chirinda et al., 2019
Integrate manure with fertilizer and crop rotation	60%	Labor cost, productivity decline.	N_2O	Nguyen et al., 2017
Dietary manipulation	35–55%	Economic cost, productivity decline, and difficult to use in non-intensive systems.	CH_4 and N_2O	Klausner et al., 1998; Hristov et al., 2011; Lee et al., 2012; Lombardi et al., 2021
Legumes as an alternative to N fertilizer	15–45%	Productivity decline, Limited forage species in some places or climates.	N_2O	Li et al., 2013
Improved soil cover and biological integrity	25–65%	Economic cost, labor cost.	CH_4 and N_2O	Chirinda et al., 2019
Application of biochar and liming material	54%	Economic cost, labor cost, economic cost, and difficult to use in non-intensive systems.	N_2O	Cayuela et al., 2014

at soil and grazing management are manifold and include managing the intensity and timing of grazing events (van der Weerden et al., 2018), increasing pasture productivity and soil carbon storage (Whitehead et al., 2018), and improving nutrient, fertilizer, and manure management (Kim and Giltrap, 2017). Removing animals from pasture areas can reduce treading damage, prevent leaching and gaseous losses of N, and thus preserve soil conditions (Luo et al., 2017). However, negative side effects of stand-off pads can be the accumulation of manure and reduced production that might outweigh the mitigation benefits. van der Weerden et al. (2018) showed that controlled grazing was beneficial on poorly drained soils where it contributed to reducing N_2O emissions, whereas the approach was not suitable on imperfectly-drained pasture.

Nitrous oxide and CH_4 emissions differ between intensive and non-intensive systems. Intensive systems can emit more GHG because they have a higher stocking rate, offer commercial feed, use fertilizers and irrigation. However, under tropical and subtropical conditions extensive systems are predominant; non-intensively managed pastures occupy 66% of the total grassland area around the world (Klein Goldewijk et al., 2017). Mitigation strategies must be in accordance with the type of system to achieve cost-effective reductions under grazing conditions. The main mitigation strategies are presented in more detail below:

Dietary Manipulation

The most promising options for reducing GHG emissions at the livestock management level include improving animal production through dietary changes. Nitrogen (N) excretion rates, which affect N_2O emissions from manure, are based on dry matter consumption (DMC) and its N content (Vergé et al., 2012). Therefore, dietary manipulation to optimize protein consumption, and thus improve the efficiency of N utilization, is one of the most effective measures to reduce emissions from manure (Novak and Fiorelli, 2010). The more nitrogen used by an animal, the less will be excreted; it is recommended that an adjusted amount of nutrients be offered in the diet to meet the animal's requirements, thus, avoiding increased excretion (Schils et al., 2008). This condition can occur in both high-supply N systems such as dairy production, as well as in tropical systems where the N supply in the feed is reduced. According to Klausner et al. (1998), in dairy systems, feeding of lactating cows with rations based on their production decreases the excretion of N by up to 34%. According to these authors, the reduction occurs by optimizing microbial fermentation in rumen which significantly improves the use of N.

Since urine is the main source of volatile N emissions, manipulating the N excretion pathway becomes an important N_2O and NH_3 mitigation tool. Urea is the main nitrogenous component of ruminant urine reaching 60–80% of total urinary

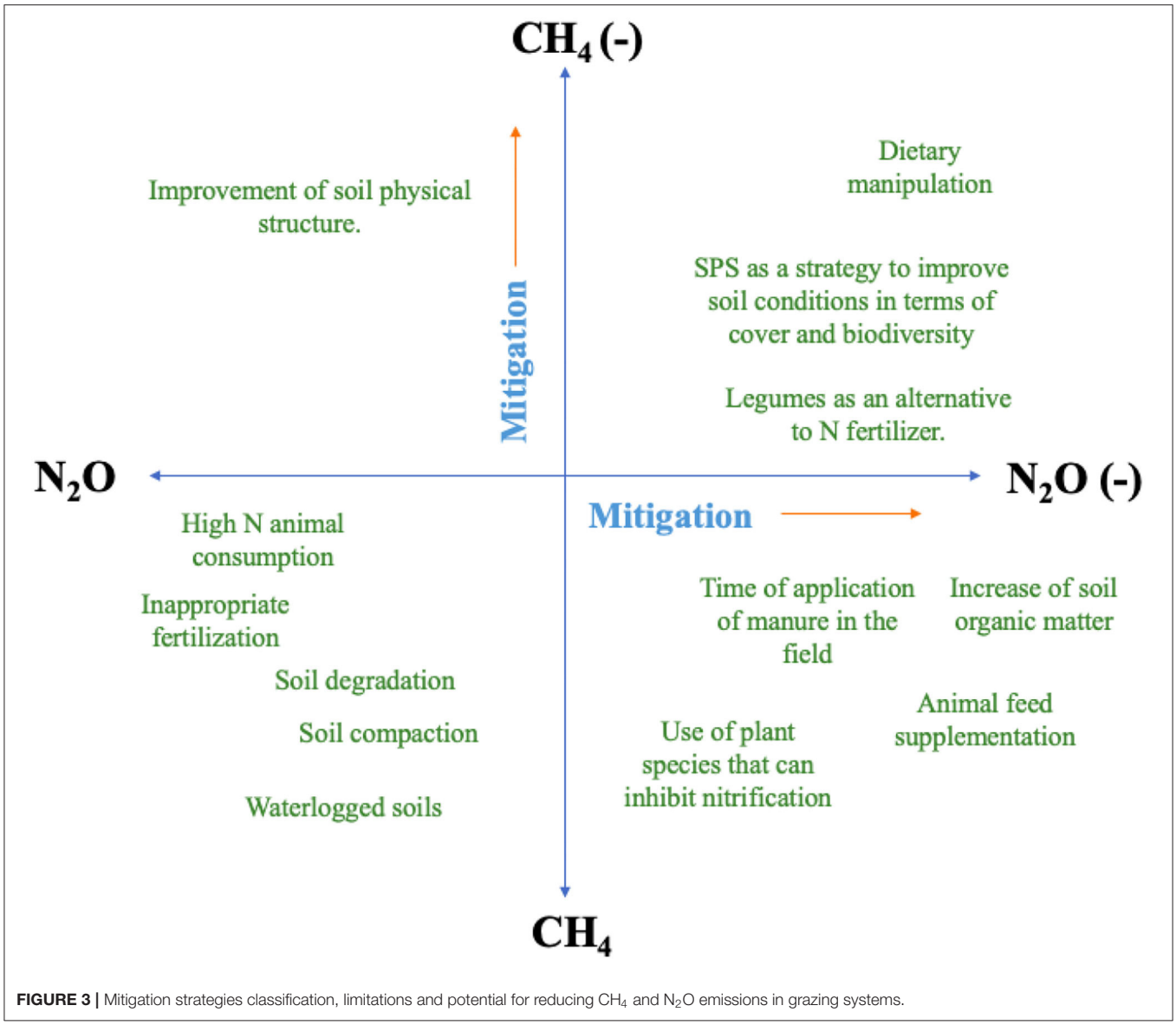

FIGURE 3 | Mitigation strategies classification, limitations and potential for reducing CH_4 and N_2O emissions in grazing systems.

N in high-production dairy cows (Montes et al., 2013) and decreasing proportionally as dietary CP decreases (Colmenero and Broderick, 2006). In low-protein diets, ureic N may drop to 46–53% of total urinary N (Hristov et al., 2011; Lee et al., 2012). For this reason, decreasing the concentration of CP in the diet is an effective method to mitigate N emissions from manure (Hristov et al., 2013). Similarly, emissions of manure deposited in the soil are reduced because low CP diets generate manure with a slower N mineralization rate (Powell et al., 2011). Optimizing N supply to animals can achieve between 12 and 21% less N excretion and 15–33% less N volatilization losses in livestock fed according to the physiological status of the animals (Erickson and Klopfenstein, 2010).

de Klein and Eckard (2008) concluded that N_2O reduction should be part of an integrated approach to improving efficiency in the use of N in animal production systems. According to these authors, current technologies could offer up to 50% reduction in N_2O emissions from a confinement system, but only up to 15% of a grazing system. In intensively operated pastoral systems, supplementation of cattle with N-low foods such as maize or silage, which generally reduce the concentration of N in the diet, can reduce urinary N losses and, consequently, NH_3 and N_2O emissions in manure and soil by 8–36% (de Klein and Monaghan, 2011).

Also, plants species such as *Lolium perenne, Trifolium repens,* and *Plantago lanceolata,* that may exhibit diuretic properties have the potential to reduce the urinary-N loading in individual urine patches by increasing the urination frequency of grazing animals (de Klein et al., 2019; des Roseaux et al., 2020). Although the increased urination frequency results in greater coverage of urine-affected soil, total paddock-scale N_2O emissions from urine patches are not likely to be higher if the total amount of

urinary-N excreted remains the same. In fact, they could be lower if the N_2O emission factor reduces with N loading rate (de Klein et al., 2019).

Diet manipulation can also reduce CH_4 emissions from manure. In a study by Lombardi et al. (2021) the supplementation of grazing beef steers with maize grain lowered CH_4 emissions of dung from 4.0 to 1.7 g CH_4-C/m^2. Dung from supplemented animals had higher N, starch, and DM content, which resulted in lower CH_4 emissions compared with dung from non-supplemented animals. Results from this study indicated that the initial water content may control the CH_4 emissions, since rainfall events after dung crusting did not increase the CH_4 fluxes from dung patches (Zhu et al., 2018). On the other hand, these authors state that supplementation with maize grain can thus have dual benefits in cattle production, through maximizing body weight gain in grazing steers (18% more) by improving the efficiency of utilization of nutrients (dietary N in particular) and through decreasing the total amount and/or the intensity of GHG emissions. In addition, the improvement in N utilization efficiency may reduce N_2O emissions from urine deposition (Cai et al., 2017).

This mitigation route can be used especially in grazing systems with daily rotation, where there is a greater control in feeding. In these systems animals are usually supplemented during milking or at certain times of the year (for example in summer or winter, according to the area). Also, the manipulation of the diet can be done to certain groups of animals that may demand more nutrients or simply by using pastures with various forage species that can be supplemented or whose nutrient supply is in accordance with the physiological state of the animal. This could be applied to both intensive and non-intensive systems (use of species that favor an adequate energy:protein balance).

Although diet supplementation might be difficult under very extensive systems, it is possible in more managed systems such as those under rotational grazing.

Improved Soil Cover and Biological Integrity

Maintaining a more diverse environment with healthy soils and good pasture cover is another strategy that can help reduce emissions. Chirinda et al. (2019) found lower urine patch emission factors in seven locations in South America (0.42 vs. 0.18%) when the grasslands had greater plant cover compared to areas with poor cover or degraded pastures. The results indicate that, under rainy conditions, adequate plant cover, through good pasture management, helps reduce urine-induced N_2O emissions. According to these authors, higher emissions in low-covered soils are due to grass degradation that can stimulate or restrict N losses. For example, low plant cover can reduce N sinks for deposited excreta and therefore increase N vulnerability and loss through microbial soil and leaching processes (Chirinda et al., 2019). However, low plant cover may also be associated with fewer exuded plant roots that decrease microbial activity and N_2O emissions (Henry et al., 2008).

Improved soil cover and pasture management also contributes to maintain or increase soil organic matter (SOM) (Aryal et al., 2018), which plays a critical role in determining the N_2O emission response to urea deposition (Clough et al., 2020). Increasing SOM increases cation exchange capacity, reducing the soil solution NH_4^+ concentration that in turn, reduces soil solution NH_3 and associated inhibition of NO_2^- oxidation, thus reducing urea-derived N_2O emissions (Breuillin-Sessoms et al., 2017). Soil buffer capacity increases with increasing soil organic matter alleviating increases in soil pH following urea deposition and associated solubilization of organic matter, and the formation of dissolved organic C and N (Breuillin-Sessoms et al., 2017).

On the other hand, excessive grazing without time for grass recovery increases the risk of soil compaction, an indicator of grass degradation. Compaction reduces soil porosity and pore continuity, decreases aeration, restricts plant growth, and increases soil N_2O emissions in urine patches (van Groenigen et al., 2005). In addition, soil acidification, which could also be an indicator of pasture degradation, has been shown to increase N_2O emissions as acidic conditions generally reduce plant growth and inhibit the activity of the enzyme N_2O reductase, which is responsible for transforming N_2O into dinitrogen (N_2) (Robinson et al., 2014).

Implementation of Silvopastoral Systems

The incorporation of shrubs and trees in pastures in the called silvopastoral systems (SPS) can also contribute to reduce emissions by improving soil cover and health and by increasing the quality of the diet (Chará et al. 2019). These systems have demonstrated effects on the physical, chemical and microbiological properties of the soil both by the provision of shade and higher amount of heterogeneous biomass that is deposited on the soil in the form of leaves, branches, fruits, and exudates and by improving the root microbiome allowing the modification of microorganism populations in the soil that can regulate nitrification and oxidation processes (Vallejo et al., 2010, 2012). Silvopastoral systems can also contribute to increase SOM (Aryal et al., 2018) which contributes to reduce GHG flows as mentioned previously. Cubillos et al. (2016) found in a study including SPS of different ages, that these arrangements have significantly lower potential for ammonia nitrification compared to monoculture systems (between 15 and 20%), similar to those observed in forest patches, which is why N_2O flows are expected to be reduced under these systems. According to these authors, in SPSs ammonia oxidizing bacteria and archaea limit the rate in nitrification and the resulting N_2O emissions could be reduced.

On the other hand, such systems can modify emissions in feces by the presence of dung beetles that limit the interactions of manure with mineral soil, restricting substrates for nitrification and denitrification processes (Slade et al., 2016). Studies in different regions of Colombia have shown that SPSs have greater abundance, diversity, and activity of beetles than treeless pastures (Giraldo et al., 2011; Montoya-Molina et al., 2016). According to Slade et al. (2016) the presence of beetles in livestock systems reduces N_2O emissions by 14.7% and CH_4 emissions by 17%.

Another effect of these systems is the increased N partitioning into dung relative to urine as this has shown to reduce N_2O emissions from pastures, since the emission factor for dung is lower than that for urine (Luo et al. 2018). Feeding

animals condensed tannin (CT)-rich diets can also increase N partitioning into dung (Carulla et al., 2005). The inclusion of CT either as a dietary supplement or in forages fed to ruminants reduced urinary-N excretion, increased the amount of N excreted in the dung and improved N retention in the animal (Misselbrook et al., 2005). Since N_2O emissions are traditionally higher in urine, this may be a mitigation pathway. Silvopastoral systems use forage species such as *Leucaena leucocephala, Tithonia diversifolia,* and *Gliricidia sepium*, which contain significant amounts of tannins in their leaves and stems (Barahona et al., 2006; Rivera et al., 2021). Rivera et al. (2018) compared an iSPS and a traditional system and found reductions in emission factors (N_2O) of 23% and 10 times less for feces and urine, respectively.

Finally, plant morphological factors that can affect soil N cycling and N_2O emissions include the effect of the root system on a plant's ability to access water and nutrients, and plant canopy-effects on the dispersion of urine voided by grazing animals (de Klein et al., 2019). When roots are present, root morphology can affect soil structure and hydrology, both of which influence conditions that govern the reduction of N_2O to N_2 and diffusion of gases to the soil surface (Chapuis-lardy et al., 2007). Root morphology can also affect plant N uptake and thus availability for soil nitrification and denitrification processes (Abalos et al., 2014). These authors found that combining two grasses, *L. perenne* and *Poa trivialis*, which is a high fertility responsive grass like *L. perenne*, produced the greatest amount of biomass and the lowest N_2O emissions. They suggested this was due to the complementarity of the root foraging strategies of these two species, where *P. trivialis* may access N hotspots not previously emptied by *L. perenne*. This combination, together with its very high total root biomass, is thought to increase mineral N uptake, thereby lowering soil nitrate content and subsequent N_2O emissions (Abalos et al., 2014).

Plant species diversity and interactions can also influence N uptake and N_2O emissions (Niklaus et al. 2016). Increasing plant species richness from 1 to 16 grassland species has been found to reduce N_2O emissions in the absence of N fertilizer (Niklaus et al., 2016), due to more efficient soil inorganic N uptake. However, this pattern was not observed when the diversity included a large proportion of legumes (de Klein et al., 2019).

Although pasture species with increasing root mass or rooting depth have greater ability to take up N, the winter-activity of pasture species such as *Lolium multiflorum* Lam.—i.e., the ability of roots to take up N under cooler conditions—appeared to be more important than specific root architecture (e.g., deep roots) for reducing N leaching losses (Woods et al., 2016). Woods et al. (2016) found that winter-active Italian ryegrass had the greatest N uptake and lowest N leaching, whereas the opposite was found for the tap-rooted *L. perenne*. The high N leaching losses from *M. sativa* in this study were attributed to poor winter herbage growth and the limited depth of the lysimeters (0.7 m) used for this deep rooting species.

Legumes as an Alternative to N Fertilizer

Biological nitrogen fixation (BNF) in association with forage legumes provides an alternative N source for grazing systems (Li et al., 2013). In tropical and subtropical conditions, legumes such as *Desmodium ovalifolium, Leucaena leucocephala, Centrosema pubescens, Stylosanthes guianensis, Cajanus cajan*, among others, can fix between 120 and 150 kg N/ha/yr. For SPS with *Leucaena leucocephala* planted in rows in Australia, nitrogen fixation rates range between 36 and 61.9 kg/ha/y (Radrizzani et al., 2011; Conrad et al., 2018) while for an iSPS with high density of *L. leucocephala* in Mexico ranged between 77.1 and 80 kg N/ha over a period of 100 days (Sarabia-Salgado et al., 2020). This fixed N becomes available slowly over time to the grass in pastures after its release into soil *via* exudates from living legume roots, by mineralization of senesced legume tissues and in excreta after consumption by grazing animals (Ledgard et al., 2009), generating lower losses of N that can be converted into N_2O (Schmeer et al., 2014). Rising costs of fertilizer N and environmental regulations governing stocking densities and fertilizer N use on farms is increasing interest in the use of legumes in pastures. A review by Andrews et al. (2007) concluded that herbage and milk production from legumes-based pastures (perennial ryegrass with 20% white clover (*Trifolium pratense*) in herbage DM on an annual basis) are likely to be similar to that from a perennial ryegrass pasture receiving annual input of 200 kg/ha of N fertilizer and around 70% of that obtained with perennial ryegrass receiving an annual input of 350–400 kg/ha of fertilizer.

The N_2O emissions induced by the growth of legume crops/forages may be estimated solely as a function of the above-ground and below-ground N inputs from crop residues (Li et al., 2013). Accordingly, N_2O emissions from legume-based grasslands are much lower than fertilized grasslands. For example, Ruzjerez et al. (1994) reported up to five-fold more N_2O emission from heavily N fertilized grasslands than from their legume-based counterparts in New Zealand. A data synthesis indicates that the average soil N_2O emissions from field-grown legumes, N fertilized grass pastures and crops, and unfertilized soils are 1.29, 3.22, and 1.20 kg N/ha/yr, respectively (Li et al., 2013).

Use of Forage Species With Potential for Biological Inhibition of Nitrification

Within soil GHG emission mitigation strategies, especially for N_2O flows, the use of nitrification inhibition grass species (BNI) is an option to reduce gas production (Byrnes et al., 2017; Teutscherova et al., 2019). According to Byrnes et al. (2017) and Beeckman et al. (2018) the use of nitrification inhibitors, whether synthetic or plant-based (biological nitrification inhibitors -BNI) reduce soil nitrification rates and therefore NO_3^- leachate and N_2O emissions. In addition, this reduction in the nitrification rate contributes to increase the efficiency in the use of N in the system (Yang et al., 2016).

Species such as *Brachiaria humidicola* (Byrnes et al., 2017), *Sorghum bicolor, Oryza sativa* and *Triticum aestivum* have demonstrated their ability to decrease N_2O flows (Subbarao et al., 2009; Zhu et al., 2012). Byrnes et al. (2017) reported that in urine patches with *B. humidicola* cv. Tully, N_2O emissions were 60% lower than in *B. hybrid* cv. Mulato (32 vs. 80 mg N_2O–N m^2, respectively). These authors also found that the

high content of NO_3^- had a positive relationship ($p < 0.05$) with the number of copies of the *amo*A gene of ammonia oxidizing archaea and bacteria at sites with higher emissions. Studies regulating soil N dynamics associated with plant and microorganism interaction such as BNI and, more recently, inhibition of biological denitrification (BDI), have increased, as they represent an ecological, sustainable, and cost-effective strategy compared to the use of synthetic inhibitors (Subbarao et al., 2017).

On the other hand, root exudates can also affect the availability of soil mineral N, as C in these exudates may temporarily increase microbial immobilization of N (Fisk et al., 2015). Carbon from root exudates may thus reduce N_2O emissions derived from urine deposition due to immobilization of urine-N. Indeed, a number of studies have demonstrated that excess N in urine patches does not immediately become available for nitrification and denitrification (Bol et al., 2004). Instead, it can be immobilized into organic matter or fixed on clay particles.

Application of Biochar and Liming Material

Biochar is a carbonaceous material produced during the thermal decomposition of different materials (wood, plant litter, crop residues, animal manure or waste products) under low-oxygen conditions (Cai et al., 2017). Biochar may inhibit nitrification by compounds such as α-pinene and ethylene and constrain denitrification through physical and biochemical regulations, including improved soil aeration, increased sorption of substrates for denitrification, and increased soil pH to facilitate complete denitrification to N_2 (Cayuela et al., 2014). A meta-analysis reports a 54% reduction of N_2O emissions (with a confidence interval from −60 to −48%) following biochar addition to agricultural soils (Cayuela et al., 2014).

Biochar can also decrease CH_4 emission or increase CH_4 oxidation *via* increasing soil aeration and reducing soil bulk density, but some compounds contained in biochar may also inhibit the activity of methanotrophs and increase CH_4 emission (van Zwieten et al., 2010).

Since increased pH can enhance the activity of N_2O reductase, lime application should be able to reduce N_2O emissions. Liming has also been shown to enhance nitrification (Khan et al., 2011). Therefore, the effect of liming on N_2O emission depends on its net effect on N_2O reductase and nitrification. Generally, liming can increase N_2 emission and reduce the ratio of N_2O to N_2 for emission from urine patches, but there are contradictory results about its effect on N_2O emission (McMillan et al., 2016). In addition, owing to the decreased Al_3^+ toxicity as pH increases, liming has shown to increase soil CH_4 oxidation and reduce CH_4 emission; this effect might be more pronounced in acid soils (Kunhikrishnan et al., 2016). However, it should be noted that even though liming has merit for lowering N_2O emissions, the increased nitrification after lime application may also potentially enhance a risk of N_2O production from denitrification when the soils become anaerobic, due to the possible accumulation of the resultant NO_3^- from nitrification (Barton et al., 2013).

Given the many uncertainties of the effect of liming on N_2O emission, caution should be exercised in using liming as an option for mitigating N_2O from excreta patches, and the effect of liming on the emission of CO_2, CH_4, and NH_3 from excreta patches should also be considered (Cai et al., 2017).

Application of Nitrification and Urease Inhibitors

Since N_2O emission from excreta patches mainly results from nitrification and denitrification, thus any inhibitor that can suppress these two processes could be used to mitigate N_2O emissions from excreta patches Cai et al. (2017). Nitrification inhibitors (NIs) such as dicyandiamide (DCD), 3, 4-dimethylpyrazole phosphate (DMPP), nitrapyrin, and pyrazole derivatives (PD), can inhibit the conversion of NH_4^+ to NO_2^-, decrease NO_3^- production and the subsequent denitrification (Barneze et al., 2015). Additionally, Urease inhibitors (UIs) can slow down the conversion of urea to NH_4^+ and NH_3 and decrease the availability of NH_4^+ for nitrification (Cai et al., 2017). The efficacy of NIs in reducing N_2O emission varies widely depending on the application rate, timing and method (Cai et al., 2017). Application of DCD significantly decreases N_2O emissions from cattle urine (by 4.24 ± 1.10 kg N/ha) and cattle feces (by 0.66 ± 0.61 kg N/ha) patches (Cai et al., 2017). However, NIs may decrease CH_4 oxidation (or increase CH_4 emission) by the inhibitory effect of accumulating NH_4^+ and possibly directly affect methane monooxygenase (MMO), presumably due to the close structural relationship between ammonia monooxygenase (AMO) and MMO activities (Le Mer and Roger, 2001; Hatch et al., 2005). Therefore, the effect of NIs on CH_4 emission from both urine and feces patches needs to be further studied under a wide range of conditions, since uncertainties about the differential effect of urine and feces deposition on CH_4 emission are large (Cai et al., 2017).

Although this mitigation pathway is effective, its applicability is difficult under grazing conditions, since these are systems where the excretion of urine and feces is dispersed; also, the costs could be high and may not outweigh the benefits.

According to Adhikari et al. (2021) in intensively managed dairy pastures, urine deposited by cattle during grazing covers relatively small proportion of the total grazed area. Maire et al. (2018) reported that urine patches represent only about 6–12% of the grazed area in a single dairy cow grazing event. Therefore, there is a large potential for limiting the risk of NIs entry into these systems if these compounds can be targeted directly to urine patches, avoiding the need for their application across often large areas of pasture unaffected by urine deposition during grazing. Most N_2O emissions from a urine patch occur within the first few days to weeks of its deposition, depending on soil and climatic conditions (Selbie et al., 2015). Technologies are therefore needed that can identify and treat urine patches shortly after deposition to inform optimum mitigation options for emission reductions (Marsden et al., 2017). Approaches to identifying urine patches include visually monitoring cows in the field, automated monitoring using electromagnetic induction, electrical conductivity measurements and optical sensing (Misselbrook et al., 2016), ground-based sensing and airborne technologies, such as remotely piloted areal systems (RPAS), LiDAR and satellites using hyperspectral and

near infra-red imaging, and temperature sensors (Dennis et al., 2013). The application rates for DCD, DMPP and nitrapyrin in previous field experiments involving urine ranged from 5 to 80, 1 to 5, and 1 to 10 kg/ha, respectively (Adhikari et al., 2021).

MANAGEMENT AND STORAGE OF MANURE AS A MEANS OF DECREASING CH_4 AND N_2O EMISSIONS

According to IPCC (Intergovernmental Panel on Climate Change). (2019), in bovine systems in tropical and subtropical areas only 4 to 15% of manure receives some type of management. Considering the manure handling chain, mitigation options involve adequate handling, storage, and application, especially in those systems where animals are kept in confinement permanently or during part of the day or the year. According to Montes et al. (2013) there are many options for mitigating emissions of N_2O and CH_4 during storage. For CH_4 mitigation, during solid manure storage, composting can be an efficient mitigation option, if it is properly managed. Samer (2015) found that adding straw to solid manure reduces CH_4 emissions, but under certain conditions, it could increase N_2O emissions.

In general, the most effective methods are anaerobic digestion and composting, which in turn have the advantage of generating products that replace fossil fuels and chemical fertilizers. Generally speaking, to mitigate N_2O emissions from manure deposits, the following are the best methods: (i) maintain anaerobic deposits (e.g., compact and covered); (ii) adopt a liquid manure system compared to a deep-bed system (although it has the drawback of increased water use); and (iii) add straw to immobilize ammonium. On the other hand, to mitigate CH_4 emissions, the following are the best methods: (i) anaerobic digestion (Chadwick et al., 2011); (ii) water removal in manure (opposite to N_2O mitigation); (iii) minimize the volume of liquid manure stored during the summer months; (iv) cooling; and (v) aeration of solid manure and composting heaps. According to these recommendations, apart from anaerobic digestion, there are no options to tackle both gases simultaneously, but there are some general strategies to reduce GHG emissions by manure management. Although some of the strategies proposed are efficient in reducing emissions, their actual mitigation potential must be evaluated against possible tradeoffs as they may rely on high use of electricity, fossil fuels, labor or water, or reduce productivity. For example, cooling, constant washing of excreta or the use of specialized structures, could increase costs or increase emissions of other gases such as CO_2, or generate other negative environmental impacts such as eutrophication and acidification.

FINAL REMARKS

Integrated production systems such as silvopastoral systems are strategic to reduce emissions of both CH_4 and N_2O through the reduction in the use of external inputs (i.e., fertilizer and feed supplements), soil protection and improvement of its structure and aeration, and efficient use of nutrients in the production process. An efficient production system that provides nutrients to animals according to their requirements not only contributes to reducing emissions but also allows for more efficient production. Although the reduction of emissions for integrated systems can be as high as 50%, the uptake of these alternatives is still very low and many research gaps remain to make these reductions more generalized.

With regard to mitigation practices, it is important to note that these may result in an "*emission exchange*" or increase in the flows of some GHGs. Therefore, due to numerous interactions, mitigation practices should not be evaluated in isolation but as a component of the bovine production system (Montes et al., 2013). Optimizing the animal diet to improve the efficiency of N use, balance N input with production level and maintaining fiber digestibility while reducing enteric fermentation of CH_4, are important steps to reduce N_2O and CH_4 emissions from manure. In addition, the use of BNI fodder, as well as providing adequate soil cover offered in well-managed systems, are strategies alternatives to reducing N_2O emissions (**Figure 3**).

Finally, it is important to mention that some strategies, if applied separately, have different limitations and drawbacks as mentioned in **Table 1**. For this reason, it is important to advance in studies focused on evaluating the economic impact of mitigation strategies, and to determine their impact on animal and food production. It is also important to work on the estimation of CH_4 emission factors and to evaluate mitigation strategies for this gas that has received less attention compared to N_2O.

AUTHOR CONTRIBUTIONS

JR and JC were involved in the conceptual development of the article. JR led the general construction of the texts and the literature review. JC contributed to manuscript revision and proposed some mitigation strategies. All authors contributed to the article and approved the submitted version.

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