Research Topics

Conservation of invertebrates in agricultural landscapes

Edited by
Christopher Williams, Mike Gormally, James Moran
and Rory Mc Donnell

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Conservation of invertebrates in agricultural landscapes

Topic editors
Christopher Williams — Liverpool John Moores University, United Kingdom
Mike Gormally — University of Galway, Ireland
James Moran — Atlantic Technological University, Ireland
Rory Mc Donnell — Oregon State University, United States

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EDITED AND REVIEWED BY
Orsolya Valkó,
Hungarian Academy of
Sciences, Hungary

*CORRESPONDENCE
Christopher D. Williams
✉ chris.david.williams@gmail.com

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Editorial: Conservation of invertebrates in agricultural landscapes

Christopher D. Williams[1]*, Rory J. Mc Donnell[2], James Moran[3] and Mike Gormally[4]

[1]School of Biological and Environmental Sciences, Liverpool John Moores University, Liverpool, United Kingdom, [2]College of Agricultural Sciences, Crop and Soil Science, Oregon State University, Corvallis, OR, United States, [3]Agro-ecology and Rural Development, Marine and Freshwater Research Centre, Atlantic Technological University, Galway, Ireland, [4]Applied Ecology Unit, School of Natural Sciences, University of Galway, Galway, Ireland

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Editorial on the Research Topic
Conservation of invertebrates in agricultural landscapes

Invertebrates play a central role in our food production systems and underpin the functioning of our agricultural landscape as pollinators, natural pest control, detritivores, and nutrient cyclers. There is a pressing need to consider the conservation of these oft-neglected creatures in agricultural landscapes, which make up a high proportion of the Earth's terrestrial surface (∼38% of the global land surface according to the UN FAO, with approximately one third of this area under crop production). As Wilson has commented, invertebrates are "the little things that run the world" (Wilson, 1987). Invertebrates contribute to many different ecosystem services including, but not limited to, pollination, decomposition including dung removal, nutrient cycling, and pest control, all of which have important implications for the successful management of agricultural ecosystems. Many different management interventions on farmed landscapes can have profound implications on the abundance, species richness, diversity, and composition of invertebrate communities. This Research Topic aims to highlight some of the myriad ways that agricultural systems interact with invertebrate communities.

With the widespread press coverage of colony collapse disorder in Honey Bee colonies (a disorder that is still somewhat mysterious in its cause), Honey Bees are an iconic species that feature heavily in insect conservation. There has been a lot of high-profile research lately on the lethal and sublethal effects of neonicotinoid pesticides on honeybees (Tsvetkov et al., 2017). In our Research Topic, Carlson et al. review 36 relevant papers that use hazard quotients (HQ) of pesticides to estimate hazards to honeybees. They conclude, "that HQ calculations should be used cautiously in future studies and more research should be dedicated to field level exposure models." Staying with pollination biology, but this time wild pollinators, Cortina et al. provide a detailed complex analysis of the factors affecting plant-pollinator interactions in grassland habitats in Central Texas. They use indices of network connectance, specialization and robustness to investigate contemporary (last 10 years) and historic (last 90 years) landscape effects on these metrics. Surprisingly, pollinator richness is positively correlated with degree of landscape urbanization. They conclude, "[o]verall, our results demonstrate that historic grazing regimes, current urbanization levels, and distinct phenological periods can simultaneously drive plant-pollinator community composition and network dynamics in shrinking but critical grassland ecosystems." Schoch et al., in this Research Topic, provide a comprehensive study of 74 wildflower strips over seven years and looked at total insect abundance, richness and, also, the subset of pollinators. They found that wild bee abundance, richness and diversity were negatively affected by the amount of semi-natural farmland habitats in the landscapes (a dilution effect). However, "[o]n the other hand, semi-natural habitats with elevated ecological quality (i.e., biodiversity promotion areas with high botanical and structural diversity) enhanced total insect and pollinator abundance in flower strips. Furthermore, pollinator abundance and wild bee abundance in specific were positively affected by the flower coverage of the strips."

As well as pollination, another important ecosystem service provided by insects is pest control. Typically, generalist predators have not received as much attention as specialists. In this Research Topic, Smith et al. use elegant molecular gut content analyses of two generalist predatory true bug species (*Geocoris* sp. and *Nabis* sp.) in their control of the serious thrip pest (*Frankliniella occidentalis*) in conventional and organic potato crops. They provide evidence that alternative prey and predator interference influence levels of predation. Viticulture is one of the most intensive forms of agriculture and whereas vineyards rely heavily on soils in that the so-called terroir of a wine is most heavily influenced by soil health, many viticultural practices adversely impact on soil health. These impacts are reviewed by Giffard et al. Staying with vineyards, the paper by Schindler et al. provides an assessment of the effects of the pesticide Indoxacarb on the parasitoid wasp community of vineyards and surrounding natural areas. Sticky trap and vacuum sampling revealed movement of wasps at the vineyard edge. They also showed that wasp communities recovered within 2 weeks after spraying. However, they note, "[t]he results indicate an effect of Indoxacarb on the parasitoid wasp community, particularly on parasitoids of lepidopterans, the target group of Indoxacarb." In contrast to Schindler et al.'s study on vineyards, Pandey et al. provide evidence of a large negating effect of pesticides on natural enemy communities in *Brassica* crops in Australia. They note, "[t]he effects on natural enemy numbers of the presence of adjacent perennial native vegetation was weaker than the effect of pesticide regime for all taxa except Staphylinidae."

Soil health is important to various ecosystem services, most notably nutrient recycling, but what about its effects on pest control? Sacco-Martret de Préville et al. investigated the effects of soil conservation vs. conventional management in Winter Wheat on the generalist predators (Ground Beetles, Carabidae) and specialist biological controls (parasitoid Wasps) in their provision of a pest control service to reduce Aphid numbers. They note, "[s]oil conservation system hosted more abundant and diverse carabid beetles' assemblages, and received higher aphidophagy service in June than conventional system. However, neither parasitoid abundance, nor parasitism rates, were affected by soil management. Aphid infestation and its associated damage did not depend on soil management either."

Agri-environmental schemes (AES) are an important element of land sharing and assessing their efficacy is an urgent priority to inform best management. Jeanneret et al. in a large study of 478 fields in three regions of Switzerland over 4 years assessed AES for spider species. They showed an overall positive effect of AES on alpha diversity and indicator species analysis highlighted the importance of woody habitats. Prairie strips are an analogous system in the US Midwest. Kemmerling et al. showed that dung beetle abundance, spider abundance and richness, pollination and decomposition decreased with distance from prairie strips. There doesn't have to be a trade-off, however, between biodiversity and production since, "[c]rop yield in one treatment with prairie strips was equal to that of the highest intensity management, even while including the area taken out of production." Nutrient management of grasslands can have a profound effect on soil mesofauna (Nematoda, Orabitida, and Collembola). Birkhofer et al. showed that community composition of soil mesofauna responds to a nutrient pulse despite differences in long-term nutrient management. Low and middle-income countries like Morocco do not have the benefit of good baseline data on insect pollination. To address this shortfall, El Abdouni et al. conducted insect surveys in 22 crops in four eco-regions for 2 years. They recorded an impressive 53,361 pollinator interactions, 37,091 of which were in crops. They compare diversity and compositional trends among crops and eco-regions.

This Research Topic can present only a small subsample of the exciting cutting-edge research on the *Conservation of Invertebrates in Agricultural Landscapes*. Not all Research Topics were covered e.g., we had no contributions on dung recycling service-providers. However, we hope that this Research Topic of original research and reviews will stimulate further work to be published on this exciting Research Topic in Frontiers and other outlets.

Author contributions

CW wrote the first draft of the manuscript, which was corrected by all co-authors and approved.

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Conflict of interest

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

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ORIGINAL RESEARCH
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Parasitoid Wasp Community Dynamics in Vineyards Following Insecticide Application

Bracha Schindler*[1,2]*, Efrat Gavish-Regev*[1] *and Tamar Keasar*[3]*

[1]The National Natural History Collections, The Hebrew University of Jerusalem, Jerusalem, Israel, [2]Department of Evolutionary and Environmental Biology, University of Haifa, Haifa, Israel, [3]Department of Biology and Environment, University of Haifa–Oranim, Tiv'on, Israel

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Edited by:
Christopher Williams,
Liverpool John Moores University,
United Kingdom

Reviewed by:
Carlo Duso,
University of Padua, Italy
Johannes Stökl,
University of Bayreuth, Germany

****Correspondence:***
Tamar Keasar
tkeasar@gmail.com

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In order to integrate parasitoid wasps in agroecosystems as biological control agents, we need to understand how insecticides affect the parasitoids in the crops and their surroundings. We investigated the non-target effect of Indoxacarb, an insecticide commonly used against European grapevine moth, on parasitoid wasp communities in vineyards. We focused on characterizing: 1. The dynamics of common wasp species, and 2. Wasp abundance and species richness in the vineyard center, edge, and nearby natural area. Seven vineyards, with neighboring natural areas, were sampled before, and up to 2 weeks after, Indoxacarb applications over 2 years. We expected initial negative effects of spraying in the vineyard with some effect of Indoxacarb drift into the natural habitat, followed by wasp recovery, first in natural areas, then at the vineyard edge and finally in the center. Sticky traps were hung at the vineyard edge and center to evaluate migration into and out of the vineyard. Vacuum sampling was used to obtain parasitoid total abundance and species richness, and the abundances of four common species (43% of the wasps collected). From the vacuum samples we found that total wasp abundance and richness declined after spraying in the vineyards' margins and center but rose over time in the natural area. Vineyard wasp abundance was restored to pre-spraying levels within 2 weeks. Among the abundant species, *Trichogramma* sp. and *Telenomus* sp., which parasitize lepidopteran hosts, declined after spraying, and *Trichogramma* sp. recovered more quickly than *Telenomus* sp. Two other abundant species, *Lymaenon litoralis* and *Oligosita* sp., did not decline after spraying. In the sticky traps, wasp abundance increased at the vineyard edge but not center after spraying, suggesting that there was migration of wasps at the vineyard edge, into or out of the crop. The results indicate an effect of Indoxacarb on the parasitoid wasp community, particularly on parasitoids of lepidopterans, the target group of Indoxacarb. The results also indicate a potential for recovery of the parasitoid community through migration from neighboring natural vegetation.

Keywords: avaunt insecticide, conservation biological control, parasitoid wasp diversity, pesticides, vineyard

1 INTRODUCTION

Conservation of biological control agents, such as parasitoid wasps, in agricultural areas can aid in integrated pest management schemes. However, to successfully integrate chemical and biological control it is necessary to determine the effects of pesticides on natural enemies and balance insecticide use with conservation of natural populations of predators and parasitoids (Janssen and van Rijn 2021). Parasitoid wasps in particular can serve as good indicators of effects of pesticides, since they are particularly sensitive to pesticides compared to other natural enemies (Mates et al., 2012).

Most tests of pesticide non-target effects are done in laboratory settings, focusing on the effect of a specific pesticide on a specific species of parasitic wasp. The ecological realism of such studies is often low. Laboratory experiments generally lack potential sheltered locations where wasps could avoid direct pesticide exposure and the wasps lack the ability to escape pesticides in space (Macfadyen et al., 2014). Also, the wasp populations tested may lack genetic variability which could be protective and may be affected by conditions of laboratory rearing (Macfadyen et al., 2014). On the other hand, abiotic stressors (such as suboptimal temperature or humidity), which may interact with pesticides to exacerbate parasitoid mortality, are usually eliminated from laboratory tests. Further, toxicology assays under laboratory conditions generally test one species at a time, and do not consider community-level effects of pesticides. Field studies can serve as a more realistic model of the effects of pesticides on entire parasitoid communities. For example, a study of the effects of Deltamethrin, a pyrethroid, in wheat fields indicated a decrease in density of wasps in the center of fields compared to edges, with recovery within 12 days (Longley et al., 1997). Another study of wheat fields sprayed with Dimethoate, an organophosphate, showed a decline in wasps of the genus *Aphidius* in sprayed and neighboring unsprayed areas of the fields, up to 20 days after insecticide application (Holland et al., 2000). In contrast, another wheat field study where Fenvalerate, a pyrethroid, and Dimethoate were sprayed found no effect on aphid parasitoids throughout the season following pesticide applications (Giller et al., 1995). An additional study in various field crops found negative effects of the insecticides Demeton-S-methyl, an organothiophosphate, and Cypermethrin, a pyrethroid, on abundance of aphid parasitizing wasps, and less so of the insecticide Pirimicarb, a carbamate (Smart et al., 1989). While these examples suggest some potential harmful side effects of pesticides to parasitoid assemblages, communities-level field studies remain few and scattered. In particular, how parasitoid communities recover after spraying remains poorly explored.

To address this gap, here we focus on Indoxacarb use in vineyards and its impacts on the resident parasitoid assemblages. Indoxacarb is an oxadiazine insecticide used against the European grapevine moth, *Lobesia botrana* Denis and Schiffermüller, 1775. It is applied as a spray to control lepidopterans at the larval stage and works by blocking voltage-gated slow-inactivated sodium channels, disrupting the nervous system (von Stein et al., 2013). It is one of the most commonly applied pesticides in vineyards (Nash et al., 2010). Laboratory experiments have been used to test the effects of Indoxacarb on specific parasitoid wasp species, and it has been shown to be harmful to *Cotesia plutellae* Kurdjumov, 1834, a larval endoparasitoid, in the adult stage (Haseeb et al., 2004), adult *Trichogramma pretiosum* Riley, 1879, an egg parasitoid (Scholz and Zalucki 2000), and *Aphidius colemani* Viereck, 1912 adults, which parasitize aphids (Bostanian and Akalach 2004). Fecundity and longevity of females were also reduced in *C. plutellae* and species in the genus *Trichogramma* (Haseeb et al., 2004; de Paiva et al., 2018; Gallego et al., 2019). Effects on emergence after insecticide application on immature stages varied among studies. There was a reduction in emergence of *Trichogramma achaeae* Nagaraja and Nagarkatti, 1970 (Gallego et al., 2019) and *Trichogramma cacoeciae* Marchal, 1927 from host eggs (Asma et al., 2018), but no effect on *Trichogramma pretiosum* (de Paiva et al., 2018) and *Aphidius colemani* emergence from eggs and aphid mummies, respectively (Bostanian and Akalach 2004). Altogether, the effect of Indoxacarb in laboratory experiments was highly dependent on the species and stage affected, and the dose the individual receives. Laboratory tests of Indoxacarb's effects on predatory mites and bugs also yielded mixed results (Bostanian and Akalach 2006). The consequences of Indoxacarb applications for parasitoid communities under field conditions have not been evaluated yet.

One factor which may protect from harmful effects of pesticides could be the availability of field margins. A study testing for effects of pesticides on parasitism in field margins found none (Bakker et al., 2021), suggesting that the margins may serve as a source for recolonization of fields. The possibility of migration from non-sprayed habitats has been raised by several studies (Longley et al., 1997; Mates et al., 2012; Bakker et al., 2021), and this could allow recovery of parasitoid communities in agricultural habitats. However, it is necessary to examine the dynamics of the parasitoid community over space and time to further understanding of the effects of pesticides and mechanisms of recovery.

The aims of this study are to characterize dynamics of populations of common parasitoid wasps after insecticide application in vineyards and the distribution of parasitoid wasps in crop and non-crop habitat. Specifically, we tested the effect of Indoxacarb application on parasitoid abundance and diversity, and abundance of dominant species. We asked: 1) What is the effect in vineyard center, vineyard border, and natural habitat? 2) What is the effect in herbaceous and vine vegetation in the vineyard? 3) What is the effect over the course of 2 weeks after pesticide application? 4) Do wasps migrate between the vineyard and the natural habitat in response to insecticide application?

2 METHODS

We sampled the parasitoid communities in wine-producing vineyards in Binyamina, Israel (32°30′14″N, 34°56′30″E-32°32′12″N, 34°57′24″E) during the 2019 and 2020 grape-growing seasons. The climate at the study area is Mediterranean, with cool rainy winters and hot dry summers.

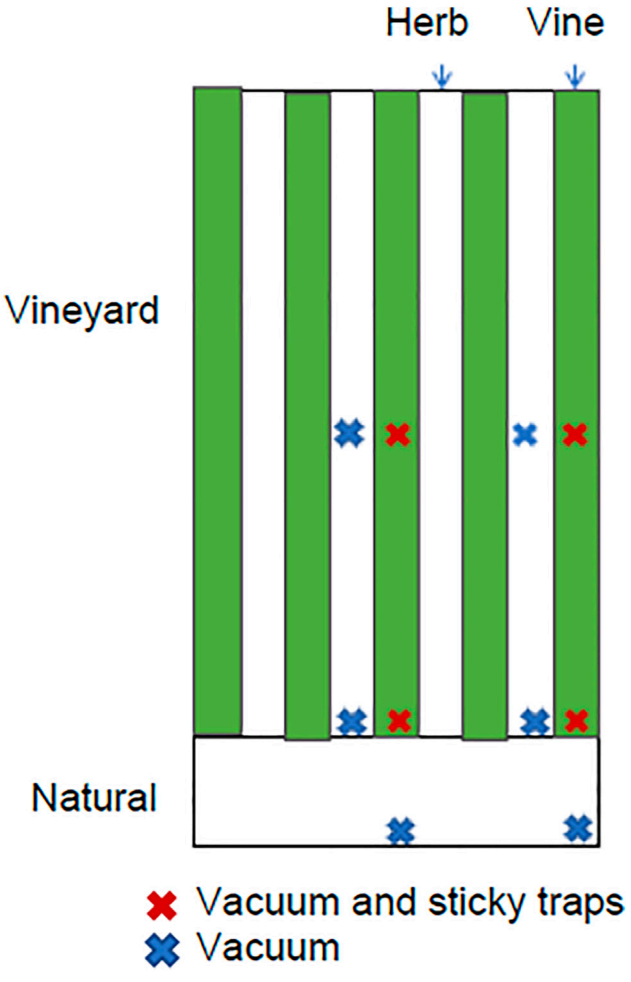

FIGURE 1 | Location of vacuum and sticky trap sampling points in each vineyard.

Pre-spray samples were taken 0–24 days (median 9 days) before Indoxacarb application. In the first year (2019), post-spray samples were taken twice or three times in the week after spraying, and in the second year (2020) samples were taken five to six times in the 2 weeks after spraying (**Supplementary Table S1**). For analysis, we chose sets of samples that were taken before and after application of Indoxacarb, without other insecticides, and with sampling for at least a week after spraying, with no other insecticides applied during that time. In 2019, five vineyards were sampled, one was sprayed twice, in mid-June and late July, and was included as two replicates. In 2020, four vineyards were included. Altogether, there were ten replicates across 2 years. Further information on the vineyards' characteristics and on additional pesticides applied to them is provided in **Supplementary Tables S1, S2**.

Insects were sampled using vacuuming and sticky traps. A vacuum sampler (Vortis, United Kingdom) was passed through vegetation for 15 seconds. Sampling was done in ten locations at each vineyard: At the edge of the vineyard in the vine and groundcover herbaceous vegetation, 25 m into the vineyard, also in the vine and herb, and in a natural area near the vineyard, 10 m from the vineyard. Sampling was done in two such transects (**Figure 1**). The dominant species in the groundcover vegetation are listed in the **Supplementary Table S3**; most were summer flowering annuals, about half were weeds, and the most common family was Asteraceae. Sticky traps were hung at the edge and in the center of the vine at a height of ~1.2 m (**Figure 1**), for two-three days at a time. Additionally, to explore effects of spraying at a greater distance from the vineyard, sampling was done at distances of 10–50 m from one vineyard, in 10 m intervals, in the second year.

Vacuum samples were stored in 75% ethanol. Parasitoid wasps were sorted out from each sample and identified to morphospecies, to obtain species richness per sample. The most abundant species were identified to genus or species. The keys used for identification of dominant species were Doutt and Viggiani 1968; Gibson et al., 1997; Goulet and Huber 1993; Huber et al., 2009; Masner 1980.

The effects of Indoxacarb spraying on parasitoid wasp abundance and species richness were analyzed in a generalized linear model with a Poisson distribution, including location of sample, vineyard, time in relation to spraying, year, and the interaction of location and time from spraying as factors. The abundances of the dominant species were also analyzed in generalized linear models with the same explanatory variables. We used two models for each species. The first model compared all vineyard locations to nature locations, and the second model included only vineyard locations with vegetation type and location in vineyard as factors. Thus, it was possible to determine what aspect of location affected populations. Both models also included year, time in relation to spraying, and interactions as factors. Wasp abundance per day in sticky traps was log transformed to fit a normal distribution and was analyzed in a general linear model with time in relation to spraying, location, year, and interactions as factors. The correlation between distance from the vineyard and wasp abundance, species richness, and *Telenomus* sp. abundance was tested for each date in relation to spraying, using a Pearson correlation. The analysis of the transect into the natural area focused on *Telenomus* sp. because the abundance of other species was low in this area.

To avoid confounding effects between date of sampling and time after spraying, samples included different vineyards which were sprayed at different dates. This might affect the results if there are seasonal trends in parasitoid abundance. To test for this possibility, we correlated the dates of pre-spraying sampling and the abundance of all wasps and of the dominant species using Spearman correlations. All analyses were done using IBM SPSS version 24 software.

3 RESULTS

3.1 Overall Parasitoid Abundance and Species Richness

Wasp abundance declined during the first and second days after spraying in most vineyard locations except the vine at the border of vineyards, and gradually recovered over the next days

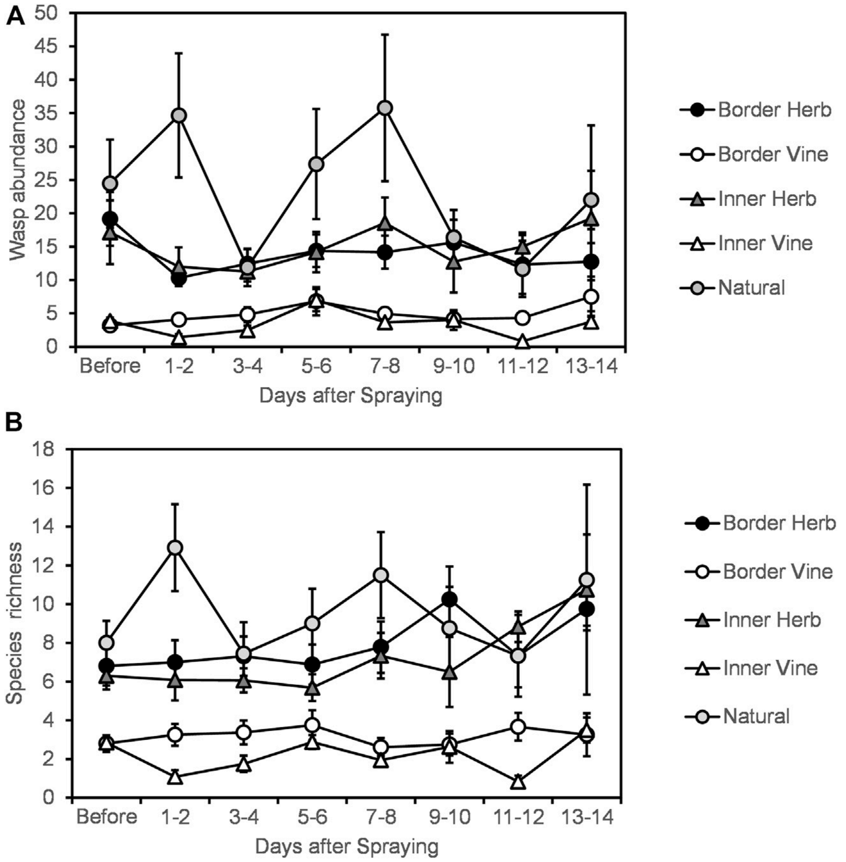

FIGURE 2 | (A) Average per-sample parasitoid wasp abundance and **(B)** average per-sample species richness before and after Indoxacarb application in and near vineyard: border of vineyard (border) and 25 m into vineyard (inner), in the vine and herbaceous vegetation (herb), and in a natural area 10 m outside the vineyard. Error bars indicate standard errors.

TABLE 1 | Results of generalized linear model of factors affecting wasp abundance and species richness.

Source of Variation	**—**	**Wasp abundance**		**Species richness**	
	df	χ^2	***P***	χ^2	***P***
Location	9	958.4	<0.001	388.8	<0.001
Vineyard	7	989.1	<0.001	136.2	<0.001
Time after spraying	7	134.9	<0.001	35.1	<0.001
Year	1	7.1	<0.008	10.1	0.001
Location*Time after spraying	58	417.4	<0.001	110.1	<0.001

(**Figure 2A**). In the natural area there was an initial increase in the first and second days after spraying, followed by large fluctuations in wasp abundance in the later post-spraying samples (**Figure 2A**). Species richness declined only in the inner vineyard vine during the first and second days after insecticide application, while increasing in the natural habitat (**Figure 2B**). There was also an interaction between location and time of sampling, so different locations within and around the vineyard responded differently to Indoxacarb application (**Table 1**). Specifically, abundance and richness were highest in nature, lower at the border of the vineyard in both the vine and herbaceous vegetation, and lowest in the inner vineyard locations in both vine and herbaceous vegetation (**Figure 2**). In the second year of the experiment, species richness and wasp abundance were lower than in the first year. Thus, there was an effect of time in relation to spraying and of location of sampling on wasp abundance and species richness, as well as an effect of vineyard and year (**Table 1**).

Sticky trap samples showed that wasp abundance increased at the border of the vineyard, but not in inner vineyard locations, one to 2 days after spraying (**Figure 3**). Location in vineyard alone, and time after spraying alone, did not affect wasp abundance; only the interaction of these two factors had an effect (**Table 2**). Also, the different locations in the vineyard had an interactive effect with year of sampling (**Table 2**). Namely, in the second year of sampling there was a greater difference in abundance between border and inner locations compared to the first year.

3.2 Dominant Parasitoid Species

Four dominant species made up 43% of parasitoid wasp assemblages in vacuum samples; *Trichogramma* Westwood,

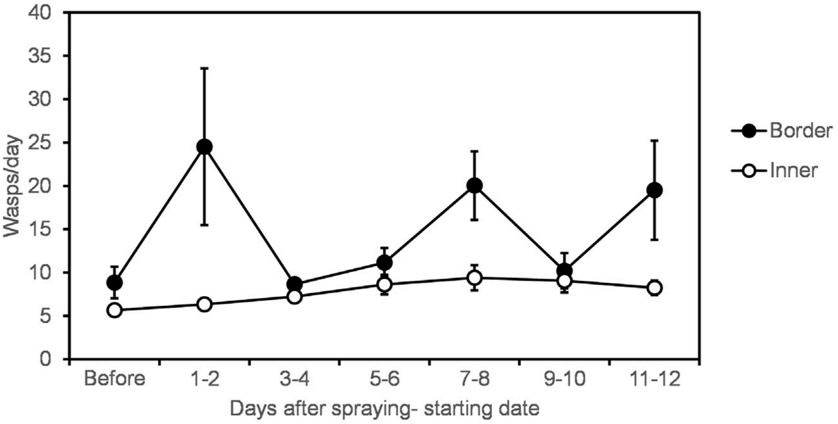

FIGURE 3 | Average wasp abundance per day of trap placement in vineyard border and 25 m into vineyard, over time after Indoxacarb application. Data from both years were pooled. Error bars indicate standard error.

TABLE 2 | Results of repeated measures general linear model of factors affecting abundance of wasps per day on sticky traps.

Source of Variation	df	F	*P*
Time after spraying	6	2.0	0.28
Location	1	7.3	0.19
Year	1	3.2	0.20
Time after spraying*Location	6	3.5	0.04
Location*Year	1	10.1	0.04

1833 sp. (17% of all parasitoid wasps collected), *Telenomus* Haliday, 1833 sp. (10%), *Lymaenon litoralis* Haliday, 1833 (9%), and *Oligosita* Walker, 1851 sp. (7%). The following trends are reflected in the interaction between time after spraying and the vineyard/nature habitats: All species except *Trichogramma* sp. were more abundant in the natural area than in the vineyards. *Trichogramma* sp., which dominated the vineyards in 2019, declined in abundance after Indoxacarb application in this year (**Figure 4A**). *Telenomus* sp., which dominated the vineyards in 2020 and the natural areas in both years, declined for several days after spraying as well (**Figures 4B–D**). Both *L. litoralis* and *Oligosita* sp., mostly occupied the natural areas and did not show a clear decline though there were changes in population throughout the sampling period (**Figure 4**). All species except *Telenomus* sp. showed a different response to spraying in vineyard locations compared to natural areas (**Table 3**, top). Both *Trichogramma* sp. and *Telenomus* sp. declined after spraying in 2019, but *Trichogramma* sp. recovered more quickly (**Figure 4**). There was however a difference in response between years (**Table 3**, top), and *Trichogramma* sp. did not decline after spraying in 2020 (**Figure 4**). There was an increase in other dominant species as *Telenomus* sp. declined, particularly in the natural areas (**Figures 4B,D**). Within the vineyard, *L. litoralis* and *Trichogramma* sp. responded differently to spraying in different locations and vegetation types (**Table 3** bottom). Both species declined most in herbaceous vegetation in 2019, but in 2020 increased after spraying. *L. litoralis* also showed a greater decline in inner vineyard locations than in edge locations. *Telenomus* sp. was consistently negatively affected, and *Oligosita* sp. showed no effect (**Figure 4**).

3.3 Natural Habitat at Increasing Distance From a Vineyard

Measurements of wasp abundance, species richness, and *Telenomus* sp. abundance in the natural area, up to 50 m from one of the vineyards, increased for a few days after spraying, and then declined again. However, there was no correlation between distance from the vineyard and these measures on any day after spraying or before spraying, except for wasp abundance which was positively correlated with distance from the vineyard on the third and fourth days after spraying (**Figure 5**; **Table 4**).

In the test for correlation between pre-spraying date and parasitoid abundances, total abundance, *Telenomus* sp. and *Oligosita* sp. abundance were not correlated with date. *L. litoralis* and *Trichogramma* sp. abundance were negatively correlated with date (**Supplementary Figure S1**).

4 DISCUSSION

Parasitoid richness and abundance were highest in natural habitat, higher in the herbaceous vegetation than in the vines, and higher in the vineyard margins than at their center (**Figure 2**), i.e., vineyard habitats that were close or similar in vegetation to the natural areas were richer in parasitoids. Similar patterns of parasitoid abundance and diversity were observed in previous surveys of vineyards in Israel's Mediterranean region (Shapira et al., 2018), but not in desert vineyards (Segoli et al., 2020). The difference between the natural and the vineyard habitats became more marked in the first 2 days after Indoxacarb application, as parasitoid abundance and richness declined in the vineyards but not in the natural habitat. Yet, all areas in the vineyards also showed a recovery in total

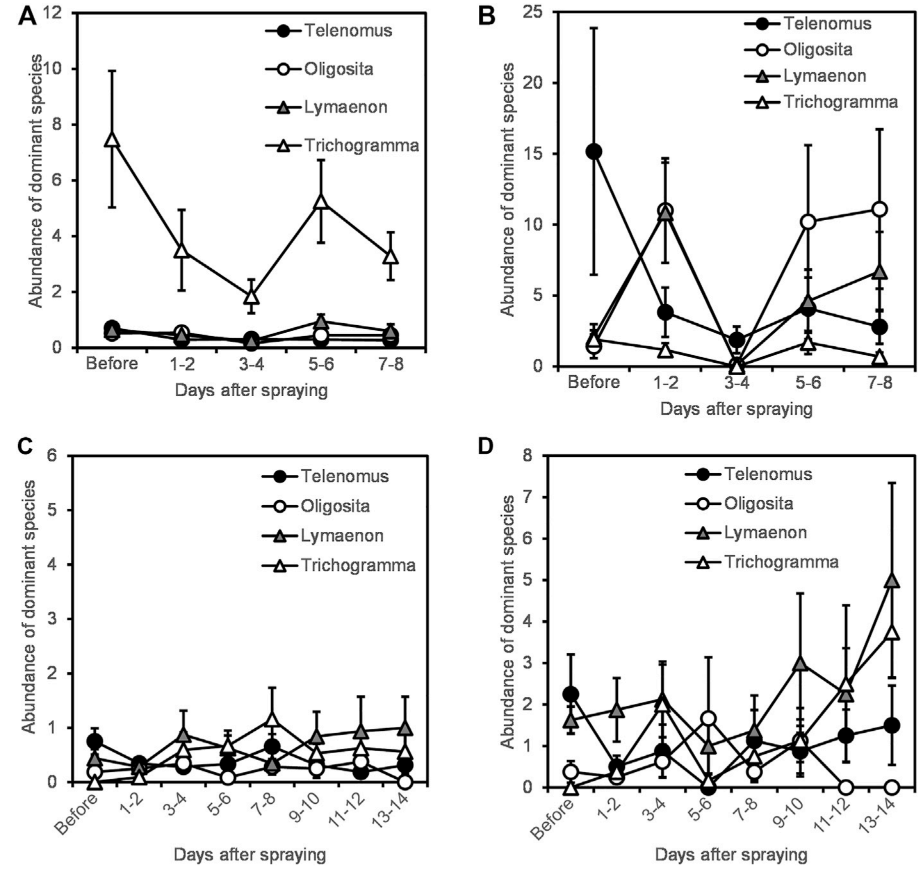

FIGURE 4 | Abundance of the four dominant species in vineyard and natural areas in each of the 2 years of study. **(A)** Vineyard, 2019. **(B)** Nature, 2019. **(C)** Vineyard, 2020. **(D)** Nature, 2020. Note that we used different *y*-axis scales for 2019 and 2020 for visual clarity.

TABLE 3 | Results of generalized linear models of factors affecting abundance of the four dominant wasp species. Model 1 included a comparison of all vineyard locations to natural locations and model 2 compared vegetation types and locations within the vineyard.

Source of Variation	***Telenomus***			***Lymaenon litoralis***			***Trichogramma***			***Oligosita***		
	df	**χ2**	***P***	**df**	**χ2**	***P***	**df**	**χ2**	***P***	**df**	**χ2**	***P***
Model 1												
Vineyard or Nature	1	167.4	<0.001	1	103.8	<0.001	1	0.6	0.428	1	71.3	<0.001
Time after spraying	7	80.5	<0.001	7	48.4	<0.001	7	43.1	<0.001	6	23.3	0.001
Year	1	16.1	<0.001	1	3.7	0.055	1	42.6	<0.001	1	35.36	<0.001
Location*Time after spraying	7	8.4	0.296	7	30.2	<0.001	7	21.6	0.003	5	23.8	<0.001
Model 2												
Location in Vineyard	1	0.0	0.996	1	8.9	0.003	1	6.2	0.013	1	0.338	0.561
Veg. type	1	0.1	0.711	1	77.0	<0.001	1	127.8	<0.001	1	45.1	<0.001
Time after spraying	7	23.0	0.002	7	16.2	0.023	7	52.8	<0.001	6	2.7	0.841
Year	1	1.9	0.166	1	0.7	0.387	1	317.2	<0.001	1	8.8	0.003
Location*Time after spraying	7	8.6	0.284	7	16.3	0.023	7	21.8	0.003	6	9.8	0.133
Veg. type*Time after spraying	6	6.5	0.366	4	1.5	0.820	5	49.9	<0.001	4	0.3	0.991

abundance and in species richness within 2 weeks. These findings may result from a combination of demographic processes: mortality and emigration of adult parasitoids due to insecticide exposure can lead to population declines. The subsequent recovery in their abundance may reflect the emergence of new adult cohorts within the vineyard and recolonization from nearby natural areas. A study of beetles, for example, found that recovery patterns differed among families, with some recovering from within fields and some from boundaries (Jepson and Thacker 1990). Our sampling design did not allow assessment of *in-situ*

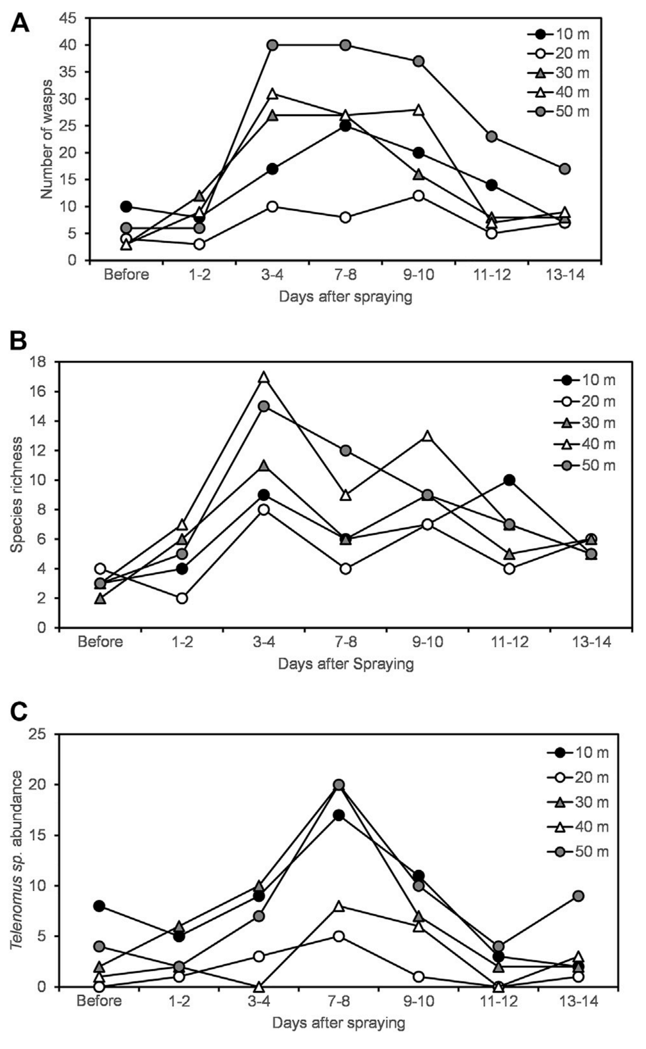

FIGURE 5 | (A) Number of wasps **(B)** species richness **(C)** abundance of *Telenomus* sp. in a natural area near one of the vineyards at varying distances from the vineyard across time after spraying.

recovery within the vineyards. We did, however, find evidence for parasitoid migration out of the vineyard following Indoxacarb applications.

The increases in wasp capture on sticky traps suggest that there was migration to the natural habitats and subsequent migration back to the vineyard. The trend towards increasing abundance that was observed in the natural habitat transect in days 3–10, particularly at greater distances from the vineyard, provides additional suggestive evidence of migration to and recovery from the natural habitat. The distance of 50 m from the vineyard which was sampled represents a reasonable distance that the wasps could have traveled since a previous study has indicated that *Anagrus erythroneurae* Trjapitzin and Chiappini, 1994 wasps, which are similar in size to the common species in this study, were observed traveling 30 m in 6 days (Irvin et al., 2018). The mechanism driving adult wasp migration from the vineyard to natural habitats could be a repellent effect of the insecticide, which has been shown to increase parasitoid mobility (Desneux et al., 2007). These results provide support for the suggestion in previous studies that parasitoid recovery occurs from non-sprayed areas that contain potential habitat for parasitoids (Mates et al., 2012). Spatial analysis of recovery of predatory beetles after insecticide application has also shown gradual increase from unsprayed edges towards the sprayed center of fields over time (Duffield and Aebischer 1994). Further, in a study in corn fields, the presence of strips of natural vegetation reduced the negative effect of pesticide applications on predatory beetles (Lee et al., 2001). Since the vineyards in this study were small, the center of the vineyards was close to the margins, potentially allowing rapid recovery (Kattwinkel et al., 2015).

Heterogeneous vegetation within and around vineyards improves biodiversity and pest control (Paiola et al., 2020). Our study supports this idea by providing evidence that natural enemies migrate between vineyards and neighboring natural habitats after insecticide applications. Increased presence of natural habitat has been shown to increase biocontrol, though by predators and not parasitoids, when pesticide pressure was low, suggesting that if pesticides are sprayed more frequently, there is insufficient time for recovery from natural habitats (Ricci et al., 2019). Another study where low toxicity pesticides were used found no effect of agrochemical use, but a positive effect of plant diversity and complexity, on arthropod diversity, suggesting that the combination of low pesticide intensity and presence of appropriate habitat for arthropods promote higher arthropod diversity in agricultural habitats (Geldenhuys et al., 2021).

Of the four abundant species, *Telenomus* sp. and *Trichogramma* sp. were more affected by spraying than

TABLE 4 | Pearson correlation between wasp community measures and distance from the vineyard on different days since spraying. N = 5 (10, 20, 30, 40, 50 m from the vineyard's edge) for each test.

Time after spraying	Wasp abundance		Species richness		*Telenomus* abundance	
	r	P	r	P	r	P
Before	–0.48	0.41	–0.22	0.72	–0.35	0.56
1–2	0.09	0.88	0.58	0.31	–0.37	0.55
3–4	0.9	0.04	0.86	0.06	–0.26	0.67
7–8	0.68	0.21	0.86	0.06	0.20	0.74
9–10	0.79	0.11	0.65	0.24	0.12	0.85
11–12	0.43	0.47	-0.21	0.74	0.18	0.78
13–14	0.82	0.09	-0.29	0.64	0.79	0.11

Oligosita sp. and *Lymaenon litoralis*. All the abundant species are egg parasitoids; *Telenomus* sp. parasitize Lepidoptera and Hemiptera, *Trichogramma* sp. parasitize Lepidoptera, *Oligosita* sp. parasitize Hemiptera and Thysanoptera, and *Lymaenon litoralis* parasitize Hempitera. *Telenomus* sp. and *Trichogramma* sp. may be most affected because their hosts are targeted by Indoxacarb, in addition to the toxic effect by contact on adult wasps that could be experienced by all species. The decline within the first 2 days in total wasp abundance suggests a direct toxic effect, while the decline in species parasitizing Lepidoptera suggests an indirect effect on the parasitoids via their hosts, if the insecticide is toxic to the moth eggs, which are parasitized by the wasps. On the other hand, egg parasitizing species may be protected by the chorion of their hosts while they develop and survive if they emerge after the effect of pesticide residue subsides (Loch 2005; Bueno et al., 2017). For *Telenomus* sp. and *Trichogramma* sp., emergence of adults from eggs that were parasitized before spraying would occur up to 8–13 days later (Navasero and Oatman 1989; Reda Abd el Monsef 2004), within the sampling time in the second year of the study.

Interestingly, *Trichogramma* sp. was among the most abundant species inside the vineyards, though this is a genus that has been shown to be relatively sensitive to pesticides (Theiling and Croft 1988), and specifically to Indoxacarb (Scholz and Zalucki 2000; Asma et al., 2018; de Paiva et al., 2018; Gallego et al., 2019; Madhusudan and Bhushanam 2020). It is, however, a genus that has been previously found in high densities inside vineyards, reflecting abundance of lepidopteran hosts (Reda Abd el Monsef 2004).

Our study documented the consequences of Indoxacarb applications that were administered by the farmers, rather than our own controlled manipulations of the spraying schedules. This observational approach did not allow us to set up Indoxacarb-free plots as matched controls to the sprayed vineyards. Untreated control plots are important for detecting seasonal population trends that are independent of the insecticide's effects (Longley et al., 1997). For example, population declines that occur both in insecticide-treated plots and in their matched control plots should not be attributed to the insecticide, but instead to the species' seasonal phenology. In our study, we reduced the potential confound between Indoxacarb-induced and seasonal population changes by sampling Indoxacarb-treated vineyards at various dates along the whole grape-growing season (**Supplementary Table S1**). In addition, we found no seasonal trend in the pre-spray total abundance of all parasitoids combined, suggesting that their post-spray decline reflects mortality associated with Indoxacarb exposure.

5 CONCLUSION

Overall, the dominant species in the natural and vineyard habitat, as well as overall parasitoid abundance and species richness appeared to recover within 2 weeks. The natural habitat present near the vineyards could provide a non-sprayed shelter for parasitoids repelled by the pesticide and could be a source to replace vineyard wasp communities. Thus, preservation of some natural habitat near vineyards, along with infrequent pesticide applications, could contribute to conservation of natural parasitoid communities, as shown here. In fact, a retrospective analysis of a large pest control dataset from vineyards found that natural habitats around the vineyards reduce pest outbreaks, as well as the need to apply chemical insecticides (Paredes et al., 2021). A further question to be explored would be the dynamics of the community outside the vineyard in space and time after spraying, and how the frequency of sprayings affects the community over an entire season.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://tamarkeasarlab.weebly.com/data-sets.html

AUTHOR CONTRIBUTIONS

BS and TK planned the study. EG-R and TK provided funding and equipment. BS conducted field and laboratory work, analyzed the data and wrote the paper. All authors edited and revised the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2021.785669/full#supplementary-material

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Alternative Prey and Predator Interference Mediate Thrips Consumption by Generalists

Olivia M. Smith[1], Eric G. Chapman[2], Michael S. Crossley[1], David W. Crowder[3], Zhen Fu[3], James D. Harwood[4], Andrew S. Jensen[5], Karol L. Krey[3], Christine A. Lynch[3], Gretchen B. Snyder[3] and William E. Snyder[1]*

[1] Department of Entomology, University of Georgia, Athens, GA, United States, [2] Department of Entomology, University of Kentucky, Lexington, KY, United States, [3] Department of Entomology, Washington State University, Pullman, WA, United States, [4] Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China, [5] Northwest Potato Research Consortium, Olathe, CO, United States

Edited by:
Christopher Williams,
Liverpool John Moores University,
United Kingdom

Reviewed by:
Raphael Didham,
Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia
Inga Reich,
MKO - Planning & Environmental Consultants, Ireland

***Correspondence:**
William E. Snyder
wesnyder@uga.edu

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Generalist predators' complex feeding relationships make it difficult to predict their contribution to pest suppression. Alternative prey can either distract predators from attacking pests, weakening biocontrol, or provide food that support larger predator communities to enhance it. Similarly, predator species might both feed upon and complement one another by occupying different niches. Here, we use molecular gut-content analysis to examine predation of western flower thrips (*Frankliniella occidentalis*) by two generalist predatory bugs, *Geocoris* sp. and *Nabis* sp. We collected predators from conventional and organic potato fields that differed in arthropod abundance and composition, so that we could draw correlations between abundance and biodiversity of predators and prey, and thrips predation. We found that alternative prey influenced the probability of detecting *Geocoris* predation of thrips through a complex interaction. In conventionally-managed potato fields, thrips DNA was more likely to be detected in *Geocoris* as total abundance of all arthropods in the community increased. But the opposite pattern was found in organic fields, where the probability of detecting thrips predation by *Geocoris* decreased with increasing total arthropod abundance. Perhaps, increasing abundance (from a relatively low baseline) of alternative prey triggered greater foraging activity in conventional fields, but drew attacks away from thrips in organic fields where prey were consistently relatively bountiful. The probability of detecting *Geocoris* predation of thrips generally increased with increasing thrips density, but this correlation was steeper in organic than conventional fields. For both *Geocoris* and *Nabis*, greater *Nabis* abundance correlated with reduced probability of detecting thrips DNA; for *Nabis* this was the only important variable. *Nabis* is a common intraguild predator of the smaller *Geocoris*, and is highly cannibalistic, suggesting that predator-predator interference increased with more *Nabis* present. Complex patterns of thrips predation seemed to result from a dynamic interaction with alternative prey abundance, alongside consistently negative interactions among predators. This provides further evidence that alternative prey and predator interference must be studied in concert to accurately predict the contributions of generalists to biocontrol.

Keywords: alternative prey, predator interference, complementarity, biodiversity and biocontrol, organic farming

INTRODUCTION

The ability of generalist predators to switch among different prey species often is exploited in conservation biological control (Symondson et al., 2002). For example, plantings of wildflowers or other perennial refuges can provide pollen, nectar, and habitat for prey that can help build predator abundance and diversity (Patt et al., 1997; Blitzer et al., 2012; Balzan et al., 2016; Gurr et al., 2017). However, to be effective, predators must willingly leave the refuge and move into adjacent crop fields (Blaauw and Isaacs, 2012). For example, Middleton and MacRae (2021) found that several kilometers of wildflower plantings around potato (*Solanum tuberosum* L.) fields yielded a dramatic >50% increase in predator abundances in the refuge. However, these natural enemies did not readily leave the refuge for the cropping fields such that predation of Colorado potato beetle (*Leptinotarsa decemlineata* Say) eggs was not increased (Middleton and MacRae, 2021). A seemingly simpler approach would be to increase abundance of prey other than pests in the cropping field itself, so that predators can be conserved in-place (Agustí et al., 2003). Settle et al. (1996) found that plant thatch and reduced insecticide applications allowed generalist predator populations to build in Indonesian rice paddies, feeding on detritus-feeding prey, before plants emerged and were colonized by herbivores; the predators then switched to attacking pests as detritivores declined and herbivores increased (see also Brust, 1994; Stoner et al., 1996; Johnson et al., 2004). However, here too success is not guaranteed. For example, Halaj and Wise (2002) found that adding straw mulch to cucurbit plantings built densities of detritus-feeding prey and greatly enhanced generalist predator abundance, but pest control was not improved because the predators never switched to attacking herbivores. These examples highlight that alternative prey can indirectly enhance the biocontrol effectiveness of generalists in some situations, but disrupt it in others (Eubanks and Denno, 2000a,b; Harmon and Andow, 2004; Koss and Snyder, 2005; Symondson et al., 2006).

Another complexity when considering generalist predators as biocontrol agents, is that they often feed on one another in addition to pests (Rosenheim, 1998; Paul et al., 2020). Intraguild predation is most disruptive when a predator both infrequently feeds on the pest and heavily attacks the pest's key natural enemy (Finke and Denno, 2004; Ives et al., 2005). It is important to note that biological control can be disrupted even when predators do not commonly feed on one another, if a predator species reduces its foraging activity to avoid becoming a victim of intraguild predation (Preisser et al., 2005, 2007). Often, prey and predator abundance and diversity interact to determine how often intraguild predation occurs (Finke and Denno, 2002). When predator abundance is relatively high and herbivores are uncommon, intraguild predation offers a way for predators to escape food limitation (Hironori and Katsuhiro, 1997). However, when prey is relatively plentiful, generalists might often encounter and kill herbivorous or detritus-feeding prey rather than natural enemies (Lucas et al., 1998). More broadly, a high diversity of other prey might allow predators to move into separate feeding niches that lead to fewer predator-predator encounters, and thus less intraguild predation (Schmitz et al., 1997; Letourneau et al., 2009; Schmitz, 2009; Dainese et al., 2017; Jonsson et al., 2017; Greenop et al., 2018). Of course, in the field, abundances of alternative prey and intraguild predators might widely vary from site to site and throughout the year, leading to complex indirect effects on pest suppression by generalists (Snyder, 2019).

Here, we use molecular gut-content analysis to track predation of herbivorous western flower thrips (*Frankliniella occidentalis*) by the predatory bugs *Geocoris* sp. and *Nabis* sp. [molecular identification failed to reveal a confident species determination for either predator; Krey et al. (2021)] in potato (*S. tuberosum*) fields. The crops were managed by growers using organic or conventional management practices, which creates site-to-site differences in predator and prey communities (Koss et al., 2005; Crowder et al., 2010; Krey et al., 2021). Both predator taxa are generalists that presumably feed on a broad diversity of arthropods, with thrips typically being among the most abundant herbivores at our study sites (Krey et al., 2021). Our central hypotheses were that (1) greater arthropod abundance and/or diversity would increasingly draw attacks away from thrips, reducing the probability of detecting thrips predation by *Geocoris* and *Nabis*, but that (2) this could be counteracted by reduced predator-predator interference in fields with more robust arthropod communities, indirectly enhancing foraging efficiency by the generalists.

MATERIALS AND METHODS

Our project had three complementary components. First, we developed a species-specific PCR primer that allowed detection of DNA of *F. occidentalis*. Second, we surveyed densities of *Nabis* and *Geocoris* predators, thrips, and other arthropods that might serve as prey, in organic and conventional potato fields managed by cooperating commercial growers (see Krey et al., 2021). Third, during these arthropod community surveys we used molecular gut content analysis to test a subsample of *Nabis* and *Geocoris* adults for the presence of *F. occidentalis* DNA, using model fitting to attempt to link detection of thrips DNA to management and arthropod community metrics.

Primer Design

To design primers to test for *F. occidentalis* consumption, all of the thrips cytochrome *c* oxidase subunit I (COI) sequences available on GenBank were downloaded with the search criterion "thrips and (coi or co1 or cox1)" which resulted in ∼567 hits (search conducted in September, 2011). We also generated 34 COI barcode sequences from thrips specimens collected in Washington potato fields. After removal of duplicate sequences and sequences that would not align (using MUSCLE; Edgar, 2004) with the barcode region (Hebert et al., 2003), and adding ours, we were left with an alignment that included 530 operational taxonomic units (OTU). After using maximum likelihood (Garli 0.95, default settings; Zwickl, 2006) to build a tree from these terminals, OTUs were arranged in the data set in a similar fashion to the relationships shown in the maximum likelihood tree. This facilitated easy searches for DNA sites that were different from the other species (especially closely-related ones), and therefore potentially specific to *F. occidentalis*. Seven pairs of primers were initially designed such that the 3′ base was as unique to *F. occidentalis* as possible. Primer properties (e.g., self-complementarity, melting temperature, % GC-content) were examined using *Primer3* (Rozen and Skaletsky, 1998). Initial testing showed that one pair worked better than the others, so we optimized it for amplification of *F. occidentalis* (see below). The primers we identified were Frank-84-F (5′-CTTTTAAACTATTTATTAGAAATGAC-3′) and Frank-323-R (5′ GTTCCTGCACCATCTTTTGAT-3′) (from 12 COI alleles we generated from *F. occidentalis*; GenBank accession numbers: MZ677036-MZ677047). The numbers in the primer names reflect the position of the 5′ base relative to an alignment of the barcode region of COI (Hebert et al., 2003) amplified using the Folmer et al. (1994) COI primers. These primers produce a 240 bp amplicon.

Study System

Potato fields in eastern Washington state host a diversity of herbivores. Key pests that are the subject of insecticide applications are the green peach aphid [*Myzus persicae* (Sulzer)], which is an important virus vector, and the Colorado potato beetle which is a defoliator (Koss et al., 2005). Western flower thrips and a diverse group of leafhoppers are among the most abundant herbivores in these fields (Krey et al., 2021), and are sometimes, although relatively rarely, controlled using insecticides (Kaur, 2021). The detritus-feeding fly *Scaptomyza pallida* (Zetterstedt) reaches remarkable abundances in these fields, often making up >50% of all arthropods, and appears to be a key alternative prey for generalist predators (Krey et al., 2021). *Geocoris* are among the most abundant natural enemies, sometimes making up >50% of all predators (Koss et al., 2005). *Nabis* are less abundant, typically representing ca. 10% of the predator community, but are relatively large predators that attack larger insects such as *Geocoris* (Krey et al., 2021). Other common predators include coccinellid and carabid beetles and a diverse community of spiders (Koss et al., 2005; Crowder et al., 2010; Krey et al., 2021).

Previously, we described arthropod communities in the same fields considered here, while examining predation of aphids by the same predator individuals (Krey et al., 2021). We found that abundances of *Nabis* and *Geocoris*, and also total predator abundance, predator richness, and overall arthropod richness, were significantly higher in organic than conventional fields (Krey et al., 2021). All other arthropod community attributes that we considered (i.e., abundances of aphids [all adults were *M. persicae*], western flower thrips, Colorado potato beetles, and *S. pallida*; total arthropod abundance; and predator evenness) did not significantly differ between organic and conventional potato fields (Krey et al., 2021).

Arthropod Survey and Predator Collections in Commercial Potato Fields

We sampled from 6 organic and 6 conventional fields in the first year (2009), 9 organic and 8 conventional fields in the second year (2010), and 6 organic and 6 conventional fields in the third year (2011), with all fields managed by cooperating growers in the Columbia Basin of central Washington in Adams, Benton and Grant counties (see Krey et al., 2017, 2021). All organic fields met organic standards defined by the United States Department of Agriculture, and were the standard ca. 50 ha circles, under center pivot irrigation, typical of the region. In this region, potatoes are rotated with other crops such that no field was sampled twice. Predators were collected in July–early August of each year, which is the approximate midpoint of the growing season (Krey et al., 2017), from 50 haphazardly selected plants using a D-vac suction-sampling device using previously described methods (e.g., Koss et al., 2005; Krey et al., 2017, 2021). Briefly, we haphazardly identified 5 groups of 10 potato plants per field, walking in a zigzag pattern from the field edge toward the center of the field, for sampling. We held the collecting cone over each plant, gently shaking the foliage for 20 s and changed collecting bags between each group of 10 plants (Koss et al., 2005). D-vac bags containing arthropods were immediately placed on dry ice, and up to 80 individuals of *Geocoris* and *Nabis* were removed using forceps, placed individually in 95% EtOH in 1.5-mL microcentrifuge tubes on ice for transport, and then transferred to a −80°C freezer to await DNA extraction; Chapman et al. (2010) found that this methodology avoids contamination of predators with prey DNA.

Following the removal of predators for gut-content analysis, all other remaining arthropods from each D-vac bag were retained from vacuum samples and stored in a −20°C freezer before being sorted to allow us to describe overall prey community structure (predators removed from samples for gut-content analysis were included in predator-density estimates for each field). Arthropods were generally identified to family, but sometimes to genus or species for pests, as described in Krey et al. (2017). D-vac bags were washed with a 10% bleach solution and air-dried before being re-used, to further minimize the risk of cross-contamination of DNA from one sampling period to another.

Molecular Gut-Content Analysis

In total, we tested between 5 and 71 *Geocoris* per field (mean = 48.7 ± 2.32 SE) and between 1 and 82 *Nabis* per field (mean = 30.5 ± 2.73 SE). Total DNA was extracted from these crushed field-collected predators using the QIAGEN DNeasy Blood & Tissue Kit following the manufacturer's animal tissue protocol (QIAGEN Inc., Chatsworth, CA, United States). PCRs (25 μL) consisted of 1× Takara buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of each dNTP, 0.25 mM of each primer, 0.625 U Takara Ex Taq^{TM} (Takara Bio Inc., Shiga, Japan), and template DNA (3 μL of total DNA). PCRs were carried out in Bio-Rad PTC-200 and C1000 thermal cyclers (Bio-Rad Laboratories, Hercules, CA, United States). The optimized thermal cycling protocol was an initial denaturation at 94°C, followed by 45 cycles of 94°C for 45 s (denaturing), 53°C for 45 s (annealing) and 72°C for 30 s (extension). Electrophoresis was used to confirm amplification using 10 μL of PCR product in 1.5% SeaKem agarose (Lonza, Rockland, ME, United States) stained with GelRed (0.1 mg/μL; Phenix Research, Chandler, NC, United States).

Data Analyses

We used the extensive literature on ecological interactions among arthropods in potato fields in our study region, described above, to construct a set of putative models (**Supplementary Table 1**). Based on this known arthropod community structure and interaction network, the factors we considered in our modeling effort were (i) abundances of the key possible prey species *M. persicae*, *L. decemlineata*, *F. occidentalis*, and *S. pallida;* (ii) abundances of the focal predators *Geocoris* sp. and *Nabis* sp.; (iii) total abundance, species richness, and evenness of predators; and (iv) total abundance, richness, and evenness of all arthropods (**Supplementary Table 1**). Richness was calculated as the sum of species and evenness using the metric Evar, without rarefaction, as described in Crowder et al. (2012). We examined the impact of arthropod community metrics and farming system on the probability of detecting predation of western flower thrips by *Geocoris* and *Nabis* using GLMMs with a binomial distribution and logit link function in the glmmTMB package in R (Brooks et al., 2017). Models included random effects of field and year. First, we made a simple comparison of the likelihood that thrips DNA was detected in *Nabis* and *Geocoris* predators collected from organic versus conventional farms. Next, we constructed 35 candidate models that tested the relative importance of each of the arthropod community metrics and their potential additive and interactive effects with farming system (**Supplementary Table 1**). We *z*-score transformed arthropod community metrics prior to running our models. We checked assumptions using the DHARMa package in R and did not detect any issues (e.g., overdispersion) (Hartig, 2021). While we considered all combinations of arthropod community metrics and farming system, we did not consider all possible combinations of arthropod community metrics because (1) they are often highly correlated, which would cause multicollinearity issues (**Supplementary Figure 1**), and (2) the possible candidate model set considering all possible combinations is quite large. We then ranked models based on Akaike Information Criterion (AICc) and identified those that were most supported (ΔAICc < 2.0) (Burnham and Anderson, 2002). Briefly, AICc is a statistical technique intended to select a "best" model among a series of candidate models. AICc has a second order bias correction for AIC [$\text{AICc} = \text{AIC} + (2K(K+1)/(n - \text{K} - 1)$] for when sample sizes are small but converges to AIC as sample sizes increase. Change (Δ) in AICc values are on a continuous scale of information relative to other models in the set, where low Δ values have higher relative support (Burnham and Anderson, 2002; Burnham et al., 2011). We assessed multicollinearity for candidate models using the performance package in R (Lüdecke et al., 2020). Multicollinearity was not an issue (VIF < 5).

RESULTS

Western Flower Thrips Primer

Western flower thrips primers were tested for specificity against 174 invertebrate morphospecies including: Araneae (14), Chilopoda (1), Coleoptera (29), Diptera (32), Hemiptera (40), Hymenoptera (36), Lepidoptera (6), Neuroptera (6), Orthoptera (1), Thysanoptera (1), Gastropoda (6) and Nematoda (2), 93 of which were collected from WA potato fields during this study (**Supplementary Table 2**). PCR of DNA extractions from all of these invertebrate species failed to produce an amplicon with the thrips primers. Examining an alignment of the primers and thrips COI sequences, at least one of these primers has a mismatch at the 1st or 2nd position of the 3′ end in all thrips species closely-related to *F. occidentalis*. A mismatch within the first two bases at the 3′ end of a primer usually prevents successful extension in PCR, as was confirmed while developing a general aphid COI primer using the same PCR reagents used herein (Chapman et al., 2010). Furthermore, there is a 3-base (single codon) deletion in COI that occurred early in the evolution of the thrips suborder Terebrantia, which contains ∼40% of extant thrips species (see Buckman et al., 2013), and includes *F. occidentalis*. The reverse primer (Frank-323-R) spans this region such that there is a 3-base insertion in insects outside of the Terebrantia relative to the primer. This insertion occurs between the 1st and 2nd 3′ bases and is probably the main reason that these primers did not amplify any of the taxa in **Supplementary Table 2**. Given these mismatches and the completely negative non-target test results above, we can be reasonably assured that our primer is specific to the strain of western flower thrips that occurs in Washington potatoes and false positives are reasonably accounted for. False negatives are difficult to address because they are difficult to define. A false negative could arise from (1) a meal of a prey item that has a mutation that stops the primer from annealing or extending (apparently rare from the above testing) or (2) collecting a

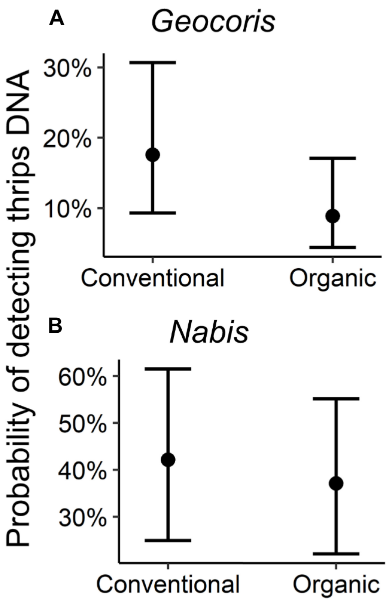

FIGURE 1 | Probability of detection of western flower thrips DNA in **(A)** *Geocoris* and **(B)** *Nabis* by farming system. Figure shows the predicted values from the models including farming system alone using the "plot_model" function in the sjPlot package in R. Bars show 95% confidence interval.

TABLE 1 | Model selection results for arthropod community and farm management (conventional = 0, organic = 1) that influence the probability of detecting thrips DNA in *Geocoris* guts.

Model	Factor 1 (first listed)	Factor 2 (second listed)	Factor 3 (interactions if tested)	ΔAIC_c*	df	Weight
Management * Thrips abundance	−0.30 (0.29)	0.18 (0.11)	**0.80 (0.29)****	0	6	0.28
Nabis abundance	**−0.40 (0.13)****			0.2	4	0.26
Management * Total abundance	−0.39 (0.31)	**0.58 (0.26)***	**−0.93 (0.27)*****	1.5	6	0.14
Management + Nabis abundance	−0.17 (0.30)	**−0.39 (0.13)****		2.5	5	0.082
Management * *Scaptomyza* abundance	−0.41 (0.34)	**0.51 (0.22)***	**−1.25 (0.38)*****	3	6	0.063
Thrips abundance	**0.29 (0.11)****			3.4	4	0.052

*Only models having >5% of model weights are shown. Numbers in columns "Factor 1 (first listed)" through "Factor 3 (interactions if tested)" indicate model estimate ± SE. "Factor 1" and "Factor 2" correspond to the first and second variable mentioned in the corresponding row. Variables were standardized using z-scores, and the standardized coefficients are shown. Bolded values indicate the individual model parameter's 95% confidence intervals do not overlap zero. Year and field were included as random effects. The next-best model not shown had $\Delta AIC_c = 4.1$ and weight = 0.036. *** 99.9% confidence intervals do not overlap zero, ** 99% confidence intervals do not overlap zero, * 95% confidence intervals do not overlap zero.*

**Akaike Information Criterion with a correction for small sample sizes.*

predator after the DNA in their gut contents has degraded past the point of detectability with our primers. The latter could arise after a variable time period after feeding depending on meal size and metabolic rate of the predator between the time of feeding and collection. Therefore, the rate at which we have detected feeding should be considered a lower bound on the actual predation rate.

Factors Impacting Predation

When ignoring arthropod community attributes or abundance of particular species, and making a simple comparison between organic and conventional potato fields, we found no differences in the probability of detection of western flower thrips DNA in *Geocoris* [$\beta = -0.33 \pm 0.34$ (SE), $P = 0.33$; **Figure 1A**] nor *Nabis* [$\beta = -0.21 \pm 0.48$ (SE), $P = 0.66$; **Figure 1B**] collected in the two farming systems.

However, we did find evidence for impacts of intraguild predation when examining our full model set. For probability of detection of western flower thrips DNA in *Geocoris*, three models had high support (i.e., ΔAICc < 2.0; **Table 1**). The best-supported model included an interaction between management (organic versus conventional) and thrips abundance, with probability of detection consistently increasing at sites with more thrips but with a steeper relationship in organic than conventional fields (**Table 1** and **Figure 2A**). The second-best model suggested there was a decreasing probability of thrips detection in *Geocoris* with increasing *Nabis* abundance (**Table 1** and **Figure 3A**). The third best-supported included an interaction between farm management and total arthropod abundance, with the probability of thrips detections increasing in conventional fields with relatively higher arthropod abundance, but the probability of thrips detection decreasing in organic fields with relatively higher arthropod abundance (**Table 1** and **Figure 2B**).

Patterns were relatively straightforward for detection of western flower thrips DNA in *Nabis*. Here, both well-supported models included *Nabis* abundance, with the probability of thrips DNA detection decreasing as *Nabis* abundance increased (**Table 2** and **Figure 3B**).

DISCUSSION

A simple comparison of predators collected from organic versus conventional fields did not show any significant difference in the probability of detection of western flower thrips DNA in either *Geocoris* or *Nabis* (**Figure 1**). However, this did not mean that farming system had no impact. Our model fitting efforts that considered several aspects of arthropod community structure alongside management, revealed several interesting interactions. *Geocoris* foraging in conventional fields were more likely to have thrips DNA detections with increasing total arthropod abundance (**Figure 2B**). The opposite pattern

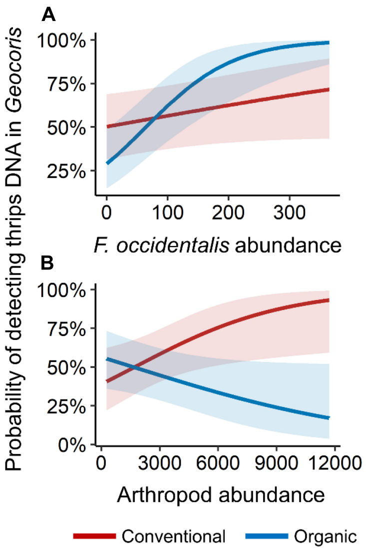

FIGURE 2 | Probability of detection of western flower thrips DNA in *Geocoris* versus **(A)** western flower thrips abundance and **(B)** total arthropod abundance. Figure shows the predicted values from the best-supported models using the "plot_model" function in the sjPlot package in R. Red lines indicate conventional fields (red bands are 95% confidence intervals) and blue lines indicate organic fields (blue bands are 95% confidence intervals). *X*-variables were standardized in the candidate model set but are plotted on the original scale for visualization.

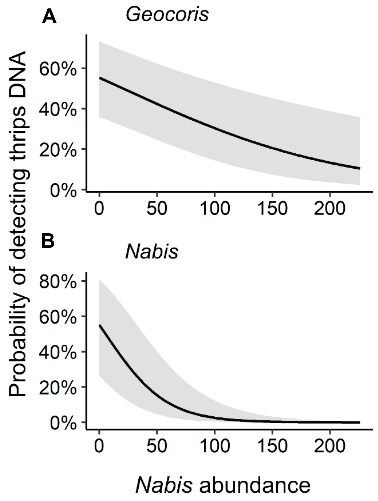

FIGURE 3 | Probability of detection of western flower thrips DNA in **(A)** *Geocoris* and **(B)** *Nabis* by *Nabis* abundance. Gray bands are 95% confidence interval. Figure shows the predicted values from the best-supported models using the "plot_model" function in the sjPlot package in R. *X*-variables were standardized in the candidate model set but are plotted on the original scale for visualization.

was found in organic fields, however, with greater arthropod abundance correlated with a lower probability of detection of thrips predation by *Geocoris*. One possible explanation is that, at the relatively low prey abundance and diversity typical of conventional potato fields (Krey et al., 2021), increases in prey trigger greater *Geocoris* foraging activity that leads a greater chance that they will find and eat thrips. In contrast, the plentiful prey in organic fields may instead draw attacks away from thrips as *Geocoris* feast on other prey. Another possibility is that very low arthropod abundances in conventional fields correlated with recent insecticide applications, with sublethal effects reducing overall predator foraging (Stark et al., 1995; Biondi et al., 2013). In either case, observations of *Geocoris* foraging behavior under low and high prey conditions, and in the presence versus absence of insecticide residue, would be needed to discern between these possible explanations. For *Geocoris* we also observed an interaction between western flower thrips abundance and the probability of detecting thrips DNA (**Figure 2A**). However, greater thrips abundance correlated with greater probability of thrips DNA detection, with the interaction perhaps simply resulting from a steeper relationship in organic than conventional fields (**Figure 2A**). In future work, it may be helpful to separate thrips collected in suction samples into life stages, or conduct open-field observations of predator foraging, to further delineate which thrips life stages were present and being attacked by which predator species. A final possibility is that organic fields were weedier, which might have complicated predator foraging to alter feeding relationships (e.g., Blubaugh et al., 2021) in organic versus conventional potato fields.

Interestingly, for both predator species, the probability of detection of thrips predation generally decreased in fields with higher *Nabis* abundance. This is consistent with greater predator-predator interference where *Nabis* was more abundant, leading either to reduced overall foraging or a switch away from predation on thrips. Previous work suggests that either explanation is possible. *Nabis* is an effective intraguild predator of *Geocoris* (Snyder et al., 2006), and also is highly cannibalistic (Takizawa and Snyder, 2011), such that predators might face heightened risk when foraging where *Nabis* is abundant. *Geocoris* in these fields do appear to feed more heavily on detritus-feeding *S. pallida* flies, rather than attacking aphids, in fields where other predator species are relatively more abundant; this suggests a feeding-niche shift when the threat of intraguild predation is higher (Krey et al., 2021). Altogether, these findings suggest another case where the contribution of generalist predators to biocontrol is reduced by altered foraging to reduce the risk of intraguild predation (e.g., Prasad and Snyder, 2006; Hosseini et al., 2021).

Molecular gut content analysis allows the inference of predation patterns under open field conditions, where predator-prey interactions naturally occur, without the constraints of caging or other artificial manipulations (King et al., 2008). However, the method does have its limitations that must be acknowledged. We could not discern how many thrips of what stages were consumed, if they were alive when consumed, or if the predator had eaten another natural enemy that had itself eaten thrips. Of course, scavenging or intraguild predation do not contribute to thrips suppression and might well weaken it (Juen and Traugott, 2005). All of the results reported here result from models that look for correlations among factors that differ among sites, but were not directly manipulated. It then remains uncertain whether the correlations reported here

TABLE 2 | Model selection results for arthropod community and farm management (conventional = 0, organic = 1) that influence the probability of detecting thrips DNA in *Nabis* guts.

Model	*Nabis* abundance	Management	*Nabis* abundance * Management	ΔAIC$_c$*	df	Weight
Nabis abundance	**−1.48 (0.26)*****			0	4	0.63
Nabis Abundance + Management	−**1.53 (0.27)*****	0.36 (034)		1.5	5	0.30
Nabis abundance * Management	−**1.48 (0.23)*****	0.34 (0.35)	−0.058 (0.41)	4.3	6	0.075

*Only models having >5% of model weights are shown. Numbers in columns Nabis Abundance through Nabis Abundance * Management indicate model estimate ± SE. Variables were standardized using z-scores, and the standardized coefficients are shown. Bolded values indicate the individual model parameter's 95% confidence intervals do not overlap zero. Year and field were included as random effects. The next-best model not shown had ΔAIC$_C$ = 18.8 and weight < 0.001. *** 99.9% confidence intervals do not overlap zero.*

** Akaike Information Criterion with a correction for small sample sizes.*

reflect true cause-effect relationships. Additionally, arthropod community metrics are often highly correlated (**Supplementary Figure 1**), making it difficult to isolate individual effects. Clearly, additional experimental work, ideally alongside observations of predator foraging behavior, are needed to further elucidate possible feeding relationships in this arthropod community. This would be particularly powerful if enough different fields could be sampled to construct and test the fit of Structural Equation Models, which could include explicit examination of indirect interactions suggested above (e.g., Blubaugh et al., 2021). Unfortunately, logistical constraints prevented us from sampling more fields in the study presented here.

Organic farming relies on natural processes, wherever possible, as an alternative to chemical interventions to control pests. This approach consistently leads to higher arthropod diversity in organic than conventional fields, including among natural enemies (Bengtsson et al., 2005; Hole et al., 2005; Crowder et al., 2010, 2012). Yet, pest abundance also generally is higher in organic fields, and greater natural pest suppression is not always apparent (Hilbeck and Kennedy, 1996; Macfadyen et al., 2009a,b; Schmidt et al., 2014; Muneret et al., 2018; Cloyd, 2020). The findings presented here suggest ecological complexities that might contribute to these general patterns. First, at the higher arthropod abundances typical of organic fields, growing abundance of possible prey correlated with reduced probability of detecting thrips DNA in *Geocoris*. Second, greater abundance of *Nabis* generally correlated with reduced probability of detection of thrips DNA in both *Geocoris* and *Nabis*. Higher predator abundance in organic fields, then, might lead to greater predator-predator interference that defuses any gains for pest suppression. So, robust arthropod communities may not necessarily translate into more effective biological control.

CONCLUSION

The effectiveness of generalist predators as biological control agents has long been questioned (DeBach and Rosen, 1991; Symondson et al., 2002). This is because the same polyphagy that allows predators to build their populations on detritus-feeding or other non-pest prey, can sometimes also distract them from attacking key herbivores (Harmon and Andow, 2004). Likewise, predators that feed heavily on other natural enemies might disrupt, rather than strengthen, net pest suppression (Rosenheim, 1998; Venzon et al., 2001; Finke and Denno, 2004; Janssen et al., 2007). We found evidence that these two disruptive interactions might reinforce one another, as detection of thrips DNA in predators was reduced both in the presence of abundant arthropod prey and with increasing abundance of predators perhaps drawn to those prey. This reinforces the complexity of feedbacks that might be seen in open field situations, where prey and predator abundance interact with one another in complex ways (Paul et al., 2020). Molecular gut content analysis, despite its limitations, may be a particularly powerful tool to detect these relationships against the high background arthropod diversity typical of real agricultural fields.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, MZ677017 – MZ677025, https://www.ncbi.nlm.nih.gov/genbank/, MZ677026 – MZ677035, and https://www.ncbi.nlm.nih.gov/genbank/, MZ677036 – MZ677047. Raw arthropod abundance and diversity, and gut content, data are provided in the **Supplementary Material** of this article.

AUTHOR CONTRIBUTIONS

OS and WS led writing of the initial draft. EC and JH led molecular work. OS and MC led data analysis. DC, ZF, KK, CL, and GS led field work. All authors contributed to manuscript editing and revision.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.752159/full#supplementary-material

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Competition and Facilitation Effects of Semi-Natural Habitats Drive Total Insect and Pollinator Abundance in Flower Strips

*Kevin Schoch[1], Matthias Tschumi[2], Stefan Lutter[1], Hans Ramseier[1] and Silvia Zingg[1]**

[1] School of Agriculture, Forest and Food Sciences, Bern University of Applied Sciences, Zollikofen, Switzerland, [2] Swiss Ornithological Institute, Sempach, Switzerland

Flower strips are an effective agri-environmental measure to promote functional biodiversity and ecosystem services in agricultural landscapes. In particular, tailored annual flower strips are increasingly implemented to foster insect pollination and biological pest control. While positive effects of flower strips on service providers and associated ecosystem services were recently demonstrated, little is known about how their effectiveness is affected by the surrounding landscape. We investigated how landscape composition and configuration, as well as flower strip traits influence the abundance of all insects, pollinators and natural enemies in 74 annual flower strips across 7 years (2014–2020). Landscape characteristics such as crop diversity, mean field size, area, and quality of semi-natural farmland habitats were assessed in a 1-km radius surrounding flower strips and combined with flower strip traits such as size, flower coverage, and flowering plant species richness to model insect abundance and diversity. Total insect and pollinator abundance, as well as wild bee abundance, richness, and diversity in flower strips were negatively affected by the share of semi-natural farmland habitats in the surrounding landscape, suggesting a dilution effect. On the other hand, semi-natural habitats with elevated ecological quality (i.e., biodiversity promotion areas with high botanical and structural diversity) enhanced total insect and pollinator abundance in flower strips. Furthermore, pollinator abundance and wild bee abundance in specific were positively affected by the flower coverage of the strips. Our results therefore suggest simultaneous competition and facilitation effects of semi-natural habitats on the landscape scale depending on their ecological quality. Annual flower strips will therefore be most effective in fostering services in landscapes of moderate to low complexity but with a high share of semi-natural habitats with increased ecological quality. For additional benefits for pollinator and wild bee abundance, flower strips should be designed to yield high flower cover. Our study thus highlights the importance of quality of ecological infrastructure and provides recommendations to maximize ecosystem services and biodiversity by means of flower strips at the landscape scale.

Keywords: agri-environment scheme (AES), biodiversity promotion area, agroecology, ecosystem service, natural enemies, functional biodiversity, habitat quality, landscape composition

Edited by:
James Moran,
Galway-Mayo Institute of Technology, Ireland

Reviewed by:
Zoltan Elek,
Hungarian Academy of Sciences (MTA), Hungary
Marco Ferrante,
University of the Azores, Portugal

***Correspondence:**
Silvia Zingg
silvia.zingg@bfh.ch

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INTRODUCTION

The ongoing agricultural intensification has resulted in severe declines of farmland biodiversity and associated ecosystem services (Robinson and Sutherland, 2002). Beyond the increase in external inputs such as fertilizers and pesticides, the simplification of the agricultural landscapes was identified as the predominant driver of these declines. This includes increases in average field size, reduction in land-use diversity, and crop rotation, as well as the removal of natural and semi-natural habitats such as hedges, waterbodies or small structural elements (Stoate et al., 2001). To counteract these declines, agri-environmental schemes (AES) were implemented across Europe, after the reform of the Common Agriculture Policy in 1992 (Herzog et al., 2017). As a popular measure, AES often include subsidies for the protection and creation of semi-natural habitats such as hedges, extensively managed grasslands, or flower strips. While earlier schemes mainly targeted at halting biodiversity declines, they are now increasingly focused on protecting and enhancing ecosystem services (Kleijn et al., 2011; Scheper et al., 2013; Ekroos et al., 2014). Recently, tailored annual flower strips were designed to flexibly enhance pollination and pest control services at the time and place they are needed (Tschumi et al., 2015; Tschumi et al., 2016; Ganser et al., 2019). In Switzerland, annual flower strips are eligible biodiversity promotion areas (BPA) and part of the official AES since 2015. The basic concept of annual flower strips is that pollinators and natural enemies are attracted to the flower strips from where they spill-over to adjacent agricultural crops to enhance the respective services (Tschumi et al., 2015; Tschumi et al., 2016; Albrecht et al., 2020).

While some studies have indeed shown high effectiveness of flower strips in enhancing natural enemies and pest control (Tschumi et al., 2015; Albrecht et al., 2020) and positive effects on pollinators and crop pollination (Rundlöf et al., 2018), others have found only weak or no effects at all (Haaland et al., 2011; Albrecht et al., 2020). Besides flower strip traits, the surrounding landscape influences the colonization of flower strips and spill-over to adjacent crops. Among the most influential landscape characteristics are the proportion of arable land (Haenke et al., 2009; Carvell et al., 2011), forest cover (Fabian et al., 2013), the presence of other semi-natural habitats providing floral resources (Aviron et al., 2011; Carvell et al., 2011; Scheper et al., 2015), and habitat heterogeneity (Fabian et al., 2013; Wix et al., 2019). Typically, the abundance of beneficial arthropods in flower strips increases with the share of semi-natural habitats (Krimmer et al., 2019). This is because newly established flower strips need to be colonized from perennial habitats and arthropods need overwintering sites that are often not found in annual flower strips. However, the positive correlation with landscape complexity was often seen to level out at a higher degree of landscape complexity which is explained by the decreasing contrast created by the flower strips to the surrounding landscape (Haenke et al., 2009; Carvell et al., 2011; Scheper et al., 2015; Wix et al., 2019). Agri-environmental measures such as flower strips were thus predicted to provide the highest benefit in landscape of intermediate complexity (Tscharntke et al., 2012). Moreover, some studies have even detected negative effects of semi-natural habitats on insect abundance in flower strips. These apparently counterintuitive effects can be explained by a competition between habitats leading to a dilution of insects among the floral resources in the landscape (Desaegher et al., 2021). Hereby, a high share of suitable habitats, may results in lower insect abundance in flower strips. It is likely that both, facilitation and dilution effects often act simultaneously and that the shift from one being dominant to the other may depend on spatio-temporal conditions and specific characteristics of flower strips and landscape features. However few studies have so far assessed positive and negative effects of local flower strip traits and landscape composition and configuration simultaneously.

Annual flower strips were predominantly designed to enhance ecosystem services but are now also implemented as biodiversity-promotion measure. However, most studies have only investigated one functional group and with the exception of a few taxa (see. e.g., Tschumi et al., 2016), their effect on species diversity is largely unknown. Species- and group-specific reactions to flower strip traits have often been documented (Wood et al., 2015; Moquet et al., 2018). Whilst some groups such as bumblebees, for example, react positive to flower coverage within flower strips, others such as solitary bees do not (Jönsson et al., 2015; Wood et al., 2015). Particularly wild bees often show differentiated patterns, as they are more specialized and depend upon key plants and other species-specific factors, rather than general flower strip traits (Nichols et al., 2019). To assess the full potential of specific flower strips and their eligibility as biodiversity-promotion measures, it is thus crucial that their effects on multiple functional groups and their diversity are evaluated.

While there is increasing awareness of the different mechanisms described above, few studies have evaluated flower strip traits and landscape characteristics on multiple service providing groups and diversity components simultaneously. Using a large dataset of 74 annual flower strips across 7 years (2014–2020), we analyzed the effect of flower strip traits and landscape characteristics on total insect, pollinator, natural enemy, and wild bee abundance, as well as wild bee species richness and diversity. In specific we hypothesized that (i) flower strip traits such as flower strip size, flower coverage, and flower diversity would positively affect service providing insect abundance and diversity, and (ii) landscape characteristics associated with increasing complexity such as mean field size, crop diversity, the abundance of other biodiversity promotion areas, and the edge length of woody areas would positively affect service providing insect abundance and diversity

MATERIALS AND METHODS

Flower Strips

Between 2014 and 2020, 74 flower strips were sown in the central Swiss plateau in the cantons of Bern, Freiburg, and Solothurn (mean altitude 548 m, range 413–981 m). The study area represents the typical agricultural landscape of the Swiss lowlands, characterized by a small-scale mosaic of intensively managed crop fields, meadows, and forest fragments in a comparably densely populated region (**Figure 1**). One to four different seed mixtures were sown in adjacent plots within one flower strip—hereafter called seed mixture plots (n = 196; see **Supplementary Material 1** for illustration and seed composition of the flower strips). Flower strips were established between April 20th and May 25th by local farmers and left in place for a minimum of 100 days. No pesticides and fertilizers were used in the flower strips. All flower strips had a minimum width of 3 m and a maximum area of 0.5 ha. We used flower strip size (in hectares), flower coverage (in %), and flowering plant species richness (number of flowering plant species) as explanatory variables in the data analysis (see **Table 1**). Flower coverage and flowering plant species richness (only species with $\geq$ 0.1% coverage) was estimated in four representative squares (1 m^2) in every seed mixture plot (simultaneously with the insect surveys—see below).

Landscape Characteristics

The surrounding landscape of every flower strip was described in a 1 km radius (**Figure 1**). Data on agricultural land-use provided by the cantonal agricultural offices allowed to calculate the share of agricultural and biodiversity promotion areas (BPA), BPA with high ecological quality, mean field size, and crop diversity for every landscape circle (see **Supplementary Figure 2.1**). BPA are extensively managed semi-natural habitats such as meadows, pastures, or hedges that are subsidized by the Swiss agri-environment scheme (Herzog et al., 2017). All farmers receiving direct payments have to manage at least 7% of their farmland as biodiversity promotion areas and today 98% of the Swiss farmland is managed under this scheme (Herzog et al., 2017). BPA with ecological quality, as defined by the Swiss Ordinance on Direct Payments, are BPA with high botanical and/or structural diversity and high ecological value (Herzog et al., 2017). A description of the different BPA types, management requirements and quality indicators can be found in the **Supplementary Table 2.2**. For crop diversity we used the Shannon–Weaver index (H) of the proportion of different crop types within each landscape circle (see **Supplementary Table 2.1**).

In addition to agricultural land use, we quantified forest area and total edge length for every landscape circle. Forest was defined as the summed area of densely and loosely stocked forests and smaller woody elements covered with trees and bushes (in hectares). Edge length was defined as the total length of the perimeter of forests, woody elements, and hedges (in km) and served as proxy for landscape heterogeneity (Martin et al., 2019). The data used to calculate these variables was obtained through the swissTLM3D dataset (Version 1.8 provided by the Federal Office of Topography Swisstopo) and verified using orthophotos from 2013 to 2017 (Bundesamt für Landestopografie Swisstopo, 2021). All spatial analyses were conducted in ArcGIS (Version 10.8.1).

Insect Abundance and Diversity

Insects were collected repeatedly within each seed mixture plot in intervals of 9–12 days after the start of flower blooming (between May and July). The number of surveys varied among flower strips with a minimum of two and a maximum of seven surveys but was equal for all seed mixture plots within a flower strip. All insects were collected with a sweep-net with a diameter of 40 cm. For each survey, the sweep net was swept 20 times on a standardized straight line at walking pace. Insect surveys were only done on days with favorable weather conditions (temperatures between 20 and 35°C and no rain or strong winds). Captured insects were frozen at −18°C and subsequently counted and categorized into the following groups: honeybees (*Apis* spp.), bumblebees (*Bombus* spp.), other wild bees, hoverflies (*Syrphidae*), parasitic wasps (*Ichneumonidae*), soldier beetles (*Cantharidae*), rove beetles (*Staphylinidae*), ladybirds (*Coccinellidae*), green lacewings (*Chrysopidae*), ants (*Formicidae*), other flies (*Diptera*), and all others (e.g., *Heteroptera* and *Orthoptera*). Larvae and imagines were summed within each group. In addition, all the wild bees from 80 seed mixture plots in 36 flower strips from the years 2015 to 2017 were identified to species level.

From these counts the following indicators for insect abundance and diversity were calculated: (i) Total insect abundance was defined as the mean number of all individuals, (ii) pollinator abundance as the mean number of honeybees, bumblebees, other wild bees, and hoverflies, (iii) natural enemy abundance as the mean number of parasitic wasps, hoverflies, green lacewings, and ladybirds, (iv) wild bee abundance as the mean number of bumblebees and other wild bees, (v) wild bee richness as the number of wild bee species, and (vi) wild bee diversity as the Shannon-Wiener diversity index of wild bees. The mean numbers were always calculated as the sum of all individuals divided by the number of surveys per seed mixture plot (see **Table 2**).

Data Analysis

To assess the impact of landscape characteristics and flower strip traits on insect abundance and diversity, we fitted linear mixed-effects models with total insect abundance, pollinator abundance, natural enemy abundance, wild bee abundance, as well as wild bee richness, and diversity as response variables. All explanatory variables described above (see also **Table 1**) were included as fixed effects in every model. To account for dependencies between observations, flower strip id and year were included in the models as crossed random effects. Pairwise correlations between explanatory variables were assessed using Pearson's correlation coefficient (r_s) and agricultural area was excluded from the models due to a strong negative correlation ($r_s > -0.60$) with forest area. All linear mixed-effects model were fitted using the following formula:

lmer [y ∼ flower strip size + flower coverage + flowering plant species richness + BPA area + BPA area with quality + forest area + edge length + (1| Flower strip ID) + (1| Year)].

To assess if the differing number of surveys had a significant effect on our response variables, we fitted an additional linear-mixed effects model for every response variable including number of surveys as (only) fixed effect and flower strip id and year as crossed random effects. As the number of surveys had no significant effect on any response variable, it was subsequently not included in the main models. We also tested for quadratic effects of BPA area and BPA area with ecological quality, yet as

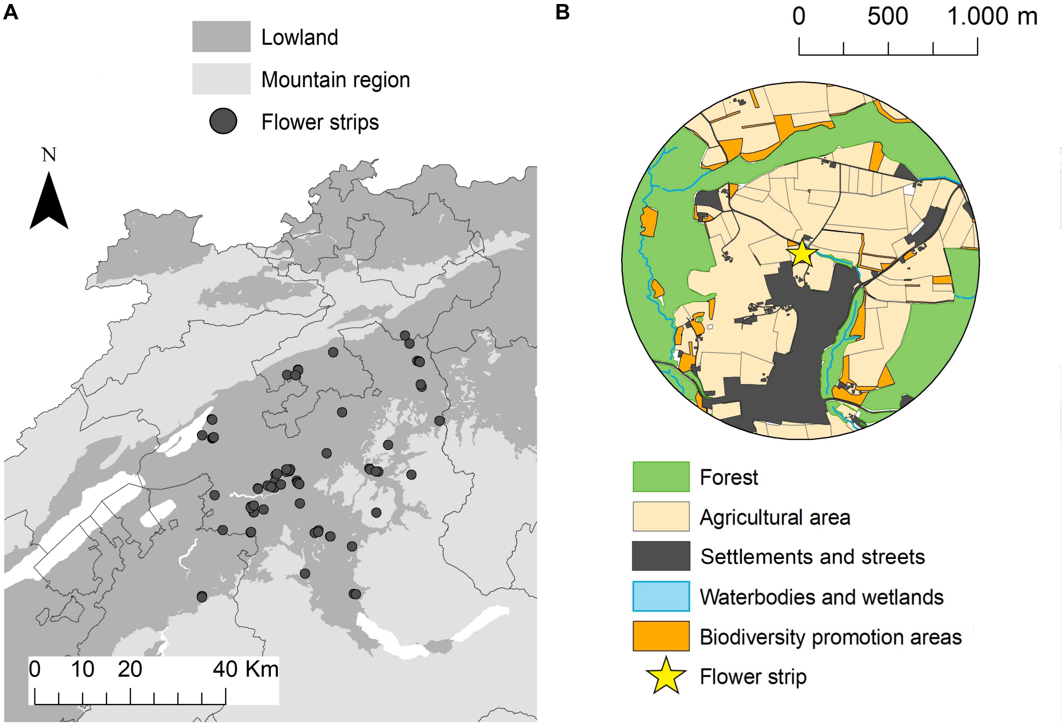

FIGURE 1 | Map of the study area with the (n = 74) flower strip locations **(A)**. Example of a study landscape with a 1 km radius surrounding flower strips in the center and different land use types **(B)**.

there were no significant relationships, quadratic effects were not included in the final models.

Models were fitted using the R package *lme4* (Bates et al., 2015) and model summaries calculated using the package *lmerTest* (Kuznetsova et al., 2020). Normality and homogeneity of the residuals were visually checked by using Tukey-Ascombe and QQ-plots. Spatial autocorrelation in the model residuals was checked with a Morans-I test [package *DHARMa;* (Hartig and Lohse, 2021)] and visually using bubble plots [package *sp;* (Pebesma et al., 2021)] but no remaining autocorrelation was detected ($p > 0.05$). Marginal effect plots were done using package *sjplot* (Lüdecke et al., 2021). The package *vegan* (Oksanen et al., 2021) was used to calculate the Shannon diversity indices. All statistical analyses were conducted in R Version 3.6.2 (R Core Team, 2021).

RESULTS

On average we found (mean ± SD) 181.5 (±111.3) insects, 31.6 (±19.1) pollinators, 10.6 (±9.0) wild bees, and 24.2 (±18.7) natural enemies per seed mixture plot (see **Table 2**). Hereby, pollinator communities were influenced in equal parts by (mean number of individuals ± SD) honeybees (10.8 ± 11.2), wild bees 9.5 (±8.7), and hoverflies (9.2 ± 6.5), whilst the relative share of bumblebees was rather low (0.9 ± 1.4). The community of natural enemies was dominated by the abundance of parasitic wasps (13.1 ± 16.4) and hoverflies (9.2 ± 6.5), while green lacewings and ladybirds were rare (1.3 ± 1.3 and 0.7 ± 0.8 individuals per seed mixture plot, respectively). Detailed information on the mean number of individuals for all species groups can be found in the **Supplementary Table 3.1**. In 80 seed mixture plots, a total of 3’882 wild bee individuals were identified to species level. Hereby 73 different species were detected, and the mean species richness was 3.2 (±1.4). The wild bee species *Lasioglossum malachurum*, *Lasioglossum pauxillum*, *Lasioglossum politum*, *Lasioglossum laticeps*, and *Bombus terrestris* were the five most frequently observed species and accounted for 68% of all identified wild bee individuals.

Biodiversity promotion areas area had a consistent negative effect on all response variables except natural enemy abundance (and with wild bee abundance only being $p = 0.065$; **Tables 3**, **4**; **Figure 2**). On the other hand, BPA area with ecological quality tended to have a positive effect on total insect abundance and pollinator abundance (**Table 4**; **Figure 3**). Finally, on the landscape scale, also forest area had a positive effect on total insect abundance (**Table 3**; **Figure 3**). From the flower strip traits, only flower coverage had a significantly positive effect on pollinator abundance and wild bee abundance and an almost significant effect on natural enemy abundance (**Tables 3**, **4**; **Figure 4**). In terms of effect size, an increase in 1 ha of BPA led to a decrease in total insect, pollinator and wild bee abundance between 1.9 and 3.1% from the average (**Figure 2**), whereas an increase in 1 ha of BPA with ecological quality led to a predicted increase of total insect or pollinator abundance between 2.4 and 2.9%.

TABLE 1 | Description of flower strip traits and landscape characteristics used as explanatory variables in all models with means ± standard deviations per flower strip and 1 km-radius landscape circles, respectively.

Explanatory variables		Mean (±SD)
Flower strip traits		
Flower strip size	Area of flower strip [ha]	0.1 (±0.1)
Flower coverage	Cover of flowering plants per seed mixture plot [%]	10.8 (±5.9)
Flowering plant species richness	Number of flowering plant species per seed mixture plot [#]	3.4 (±1.3)
Landscape characteristics		
Mean field size	Mean area of agricultural field units [ha]	1.0 (±0.2)
Crop diversity	Shannon-Wiener index of area of crop types	1.8 (±0.2)
BPA area	Total (sum) area of BPAs [ha]	16.9 (±6.1)
BPA area with quality	Total (sum) area of BPAs with ecological quality [ha]	6.1 (±4.1)
Forest area	Total (sum) area of forests and woody areas [ha]	61.2 (±43.7)
Edge length	Total (sum) perimeter of forests, woody areas, and hedges [km]	16.0 (±7.1)

BPA, Biodiversity Promotion Areas.

TABLE 2 | Description of abundance and diversity indicators used as response variables. Shown are means ± standard deviations per seed mixture plot.

Response variables		Mean (±SD)
Total insect abundance	Honeybees, bumblebees, other wild bees, hoverflies, parasitic wasps, soldier beetles, ladybirds, green lacewings, roove beetles, ants, other flies, and others	181.5 (±111.3)
Pollinator abundance	Honeybees, bumblebees, other wild bees, hoverflies	31.6 (±19.1)
Natural enemy abundance	Parasitic wasps, hoverflies, green lacewings, ladybirds	24.2 (±18.7)
Wild bee abundance	Bumblebees, other wild bees	10.6 (±9.0)
Wild bee richness	Number of wild bee species	3.2 (±1.4)
Wild bee diversity	Shannon-Index of wild bee species	0.8 (±0.3)

DISCUSSION

We found clear effects of landscape characteristics and flower strip traits on the abundance and diversity of different functional insect groups in annual flower strips. While all investigated groups and indices except natural enemies were negatively influenced by the area of BPA in the surrounding landscapes, BPA with elevated ecological quality had a positive effect on the abundance of total insects and pollinators in specific. In addition, forest area positively affected total insect abundance. Of the investigated flower strip traits, flower coverage was positively correlated with pollinator and wild bee abundance in flower strips.

Dilution Effects by Biodiversity Promotion Areas

The area of BPA in the surrounding landscape was negatively correlated with all biodiversity indicators, except for natural enemy abundance. This was unexpected, as we assumed that BPA in the landscape would increase insect abundance and diversity in flower strips. Previous research has mostly shown positive effects of semi-natural habitats on the abundance and diversity of ecosystem service providers at the landscape scale (Batáry et al., 2015; Holland et al., 2017; Zingg et al., 2019). Grassland habitats, especially extensively managed (low input) meadows which represented the most abundant BPA in our study, often harbor high pollinator species richness and abundances, as they provide mating, foraging and nesting sites (Pfiffner et al., 2018). In our case, however, a high proportion of BPA in the landscape apparently diluted insect abundance in flower strips. We suppose that in our case observed insect densities in flower strips more strongly reflect the distribution of individuals among alternative floral resources in the surrounding landscape than the total abundance in a given area (Haenke et al., 2009; Carvell et al., 2011). As indicated by the intermediate landscape-complexity hypothesis (Tscharntke et al., 2012), relative insect abundances in flower strips may be highest in landscapes with intermediate proportions of semi-natural farmland habitats and when moving from moderate to high landscape complexity, dilution effects may increasingly outperform facilitation. Indeed, most of our study landscapes would categorize as rather complex on an international scale, as they harbor on average 20% (±14) natural areas (i.e., forests) and an additional 5.4% (±1.9) semi-natural farmland habitats (i.e., BPA).

Irrespective of this result, it is the overall abundance of service providers in the landscape that determines the strength of provided ecosystem services. To support large and effective populations of service providers on the long-term, a network of diverse semi-natural habitats is indispensable (Sutter et al., 2018). Thus, landscape level greening, i.e., a high share of biodiversity promotion areas is crucial to maintain ecosystem services in the long term. Particularly annual flower strips are only temporary available habitats that can bypass resource scarcity but are plowed in autumn (Ramseier et al., 2016). Annual flower strips are therefore not sufficient to ensure a safe overwintering of arthropods and need complementing perennial habitats at spill-over distance (Ganser et al., 2019).

Facilitation Effect by Forest and Biodiversity Promotion Areas With High Ecological Quality

On the other hand, overall insect abundance in flower strips increased with the area of forest in the landscapes. Natural habitats are known to promote overall biodiversity and a share of at least 20% is often needed to maintain high species richness in agricultural landscapes (Tscharntke et al., 2012; Zingg et al., 2018). Nevertheless, none of the functional groups was correlated

TABLE 3 | Summary of linear mixed-effects models for total insect, pollinator, and natural enemy abundance with estimates (Est.), standard errors (SE), degrees of freedom (df), *t*-values (t), and *p*-values ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $\cdot p < 0.1$) for all fixed effects.

	Total insect abundance					Pollinator abundance					Natural enemy abundance				
Predictors	**Est.**	**SE**	**df**	***t***	***p***	**Est.**	**SE**	**df**	***t***	***p***	**Est.**	**SE**	**df**	***t***	***p***
(Intercept)	34.20	114.54	63.27	0.30	0.766	21.80	23.15	63.34	0.94	0.350	−9.60	24.31	63.38	−0.39	0.694
Flower strip size	−25.52	82.51	58.89	−0.31	0.758	4.85	17.07	62.80	0.28	0.777	−8.77	17.82	61.42	−0.49	0.624
Flower coverage	−0.04	1.07	176.63	−0.03	0.972	**1.03**	**0.18**	**162.87**	**5.64**	**<0.001*****	**−0.38**	**0.19**	**163.33**	**−1.96**	**0.052·**
Flowering plant species richness	−1.98	5.09	174.35	−0.39	0.697	0.13	0.91	184.55	0.14	0.888	0.74	0.96	183.52	0.77	0.441
Mean field size	14.54	39.72	59.76	0.37	0.716	1.57	8.17	61.58	0.19	0.848	9.27	8.54	61.09	1.09	0.282
Crop diversity	62.07	46.86	59.78	1.32	0.190	2.06	9.66	63.12	0.21	0.831	12.00	10.09	62.03	1.19	0.239
BPA area	**−3.48**	**1.59**	**59.95**	**−2.19**	**0.033***	**−0.90**	**0.33**	**62.23**	**−2.77**	**0.007****	−0.13	0.34	61.59	−0.37	0.710
BPA area with quality	**4.47**	**2.53**	**64.06**	**1.77**	**0.082·**	**0.93**	**0.51**	**65.01**	**1.84**	**0.070·**	0.09	0.54	65.42	0.16	0.872
Forest area	**0.51**	**0.24**	**59.05**	**2.12**	**0.038***	−0.02	0.05	61.95	-0.36	0.721	0.06	0.05	60.86	1.24	0.219
Edge length	0.72	1.46	60.42	0.50	0.622	0.14	0.30	62.69	0.46	0.645	0.01	0.31	61.85	0.03	0.977
Random effects															
τ_{00} Flower strip ID					323.18					161.27					174.29
Year					4422.03					48.13					93.36
N Flower strip ID					74					74					74
Year					6					6					6
Observations					196					196					196
Marginal R^2/Conditional R^2					0.110/0.752					0.180/0.774					0.065/0.765

For random effects, intercept variance (τ_{00}), and number of random effect levels (N) are shown. In addition, the number of observations and marginal and conditional R^2 are given. Kenward-Roger approximation was used to compute degrees of freedom and p-values. BPA, Biodiversity Promotion Areas. Significant p-values (<0.05) and trends (<0.1) are shown in bold.

TABLE 4 | Summary of linear mixed-effect models for wild bee abundance, wild bee richness, and wild bee diversity with estimates (Est.), standard errors (SE), degrees of freedom (df), *t*-values (t), and *p*-values ($^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $\cdot p < 0.1$) for all fixed effects.

	Wild bee abundance					Wild bee richness					Wild bee diversity				
Predictors	**Est.**	**SE**	**df**	***t***	***p***	**Est.**	**SE**	**df**	***t***	***p***	**Est.**	**SE**	**df**	***t***	***p***
(Intercept)	3.72	12.38	63.41	0.30	0.765	**5.47**	**2.80**	**24.17**	**1.95**	**0.063·**	**1.55**	**0.63**	**22.87**	**2.45**	**0.023***
Flower strip size	4.62	9.14	63.18	0.51	0.615	2.29	1.94	23.84	1.18	0.249	0.60	0.43	22.51	1.38	0.180
Flower coverage	**0.27**	**0.10**	**162.63**	**2.81**	**0.005***	−0.00	0.03	64.82	−0.17	0.868	−0.00	0.01	69.90	−0.55	0.585
Flowering plant species richness	0.28	0.49	184.82	0.59	0.558	0.09	0.12	62.14	0.75	0.454	0.01	0.03	61.48	0.43	0.672
Mean field size	−0.00	4.37	61.73	−0.00	1.000	−0.96	0.91	21.76	−1.06	0.300	−0.17	0.20	20.49	−0.83	0.419
Crop diversity	1.68	5.17	63.42	0.32	0.747	−0.29	1.25	24.91	−0.23	0.819	−0.15	0.28	24.09	−0.53	0.602
BPA area	**−0.33**	**0.17**	**62.40**	**−1.88**	**0.065·**	**−0.07**	**0.03**	**23.43**	**−2.18**	**0.040***	**−0.02**	**0.01**	**22.45**	**−2.25**	**0.034***
BPA area with quality	0.31	0.27	64.80	1.13	0.261	0.00	0.06	27.15	0.05	0.961	−0.00	0.01	26.31	−0.01	0.988
Forest area	−0.01	0.03	62.33	−0.55	0.586	−0.01	0.01	25.51	−0.87	0.393	−0.00	0.00	24.84	−0.69	0.494
Edge length	0.23	0.16	62.94	1.46	0.149	0.03	0.04	22.95	0.68	0.503	0.00	0.01	21.68	0.19	0.848
Random effects															
τ_{00} Flower strip ID					46.39					0.69					0.02
Year					11.61					0.74					0.03
N Flower strip ID					74					36					36
Year					6					3					3
Observations					196					80					80
Marginal R^2/Conditional R^2					0.094/0.746					0.143/0.682					0.120/0.509

For random effects, intercept variance (τ_{00}) and number of random effect levels (N) are shown. In addition, the number of observations and marginal and conditional R^2 are given. Kenward-Roger approximation was used to compute degrees of freedom and p-values. BPA, Biodiversity Promotion Areas. Significant p-values (<0.05) and trends (<0.1) are shown in bold.

with forest area. This suggests that forests facilitate insect species and groups that are attracted by resources offered in flower strips but are not considered as pollinators or natural enemies of crop pests. Although, forest edges can potentially act as a source of pollinators, the effect may further depend up on microclimatic conditions (Bailey et al., 2014), the vegetation

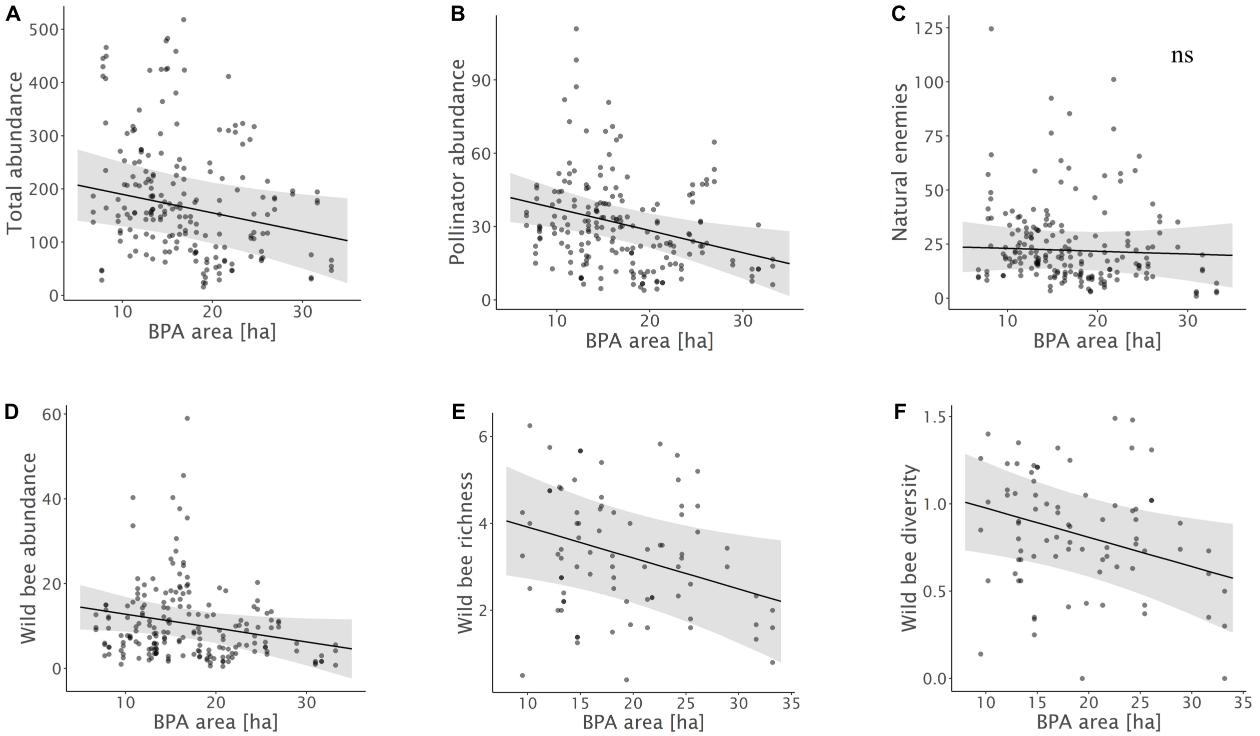

FIGURE 2 | Relationships between **(A)** total insect abundance, **(B)** pollinator abundance, **(C)** natural enemy abundance, **(D)** wild bee abundance, **(E)** wild bee species richness, **(F)** wild bee diversity and the total area of biodiversity promotion areas (BPA) in the 1 km radius landscape circles. Model predictions with 95% confidence intervals and raw data points are shown.

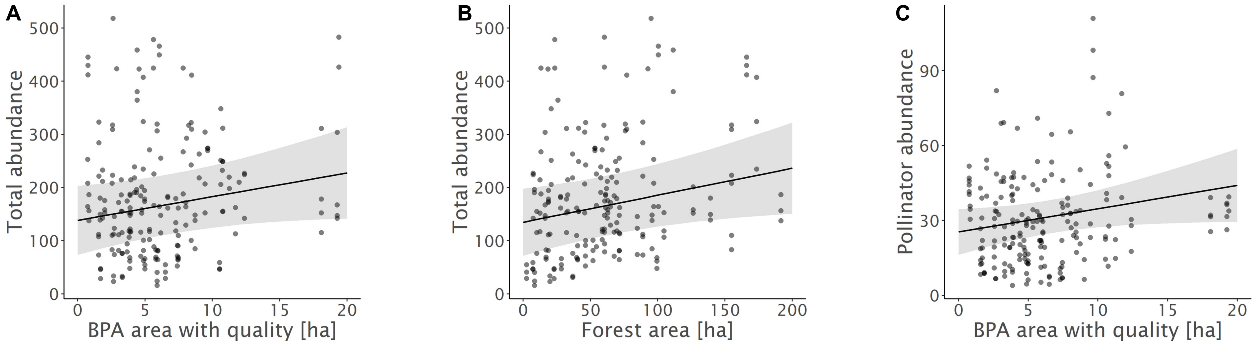

FIGURE 3 | Relationships between **(A)** total insect abundance and biodiversity promotion areas (BPA) with ecological quality, **(B)** total insect abundance and forest, and **(C)** pollinator abundance and BPA with ecological quality. Model predictions with 95% confidence intervals and raw data points are shown.

(Roberts et al., 2017), as well as the specific nesting behavior of a given species (Odanaka and Rehan, 2020). As highlighted by Ewers and Didham in their comprehensive review on habitat fragmentation and edge effects (2006), different invertebrate species are likely to vary strongly in their response to habitat edges and the composite of often contrasting responses may result in little to no effects on overall abundance and species richness observed.

In addition, also the share of BPA with ecological quality tend to promote overall insect and pollinator abundance. This stands in contrast to overall BPA area that had a negative effect but is more in line with our hypotheses. We assume, that the fulfilment of ecological quality requirements led to a pronounced improvement of landscape-scale habitat quality, capable to foster insect populations on the large scale and the long term. There are several reasons, why BPA with quality may represent an overall improvement of habitat quality for multiple insect groups: First, grassland BPA with high ecological quality harbor a higher botanical diversity, known to be a major driver of pollinator abundance and richness (Albrecht et al., 2007). Second, in grassland BPA with quality, the use of hay conditioners, knowing to tremendously reduce insect abundance

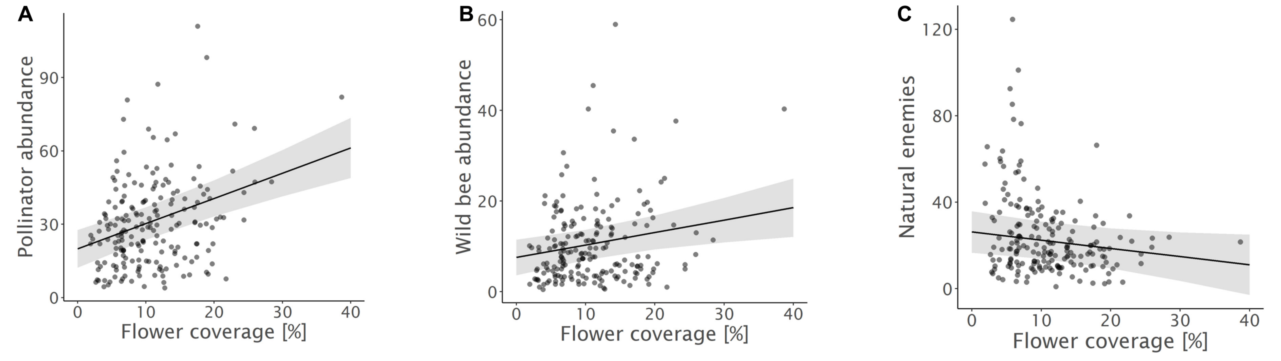

FIGURE 4 | Relationships between **(A)** pollinator abundance, **(B)** wild bee abundance, and **(C)** natural enemy abundance and flower coverage. Model predictions with 95% confidence intervals and raw data points are shown.

is not allowed (Frick and Fluri, 2001). Third, in woody BPAs with high ecological quality (e.g., hedges) additional structural elements such as piles of branches or stones and nest boxes for wild bees are offered. BPA with high ecological quality have thus previously shown to enhance different components of biodiversity at the landscape level and may thus represent an important key to foster pollinators and insects overall (Meichtry-Stier et al., 2014; Zingg et al., 2019). BPA with lower ecological quality, on the other hand, may not be sufficient to provide sufficient key resources for many insect groups.

High Flower Coverage Promotes Pollinator Abundance

Both, pollinator, and wild bee abundance increased with flower coverage inside the flower strips. This corroborates our hypotheses and shows that not only landscape, but also the flower strip configuration and ecological quality is decisive. Flower strips thus need to contain high flower density to support high pollinator numbers (Krimmer et al., 2019). Yet, no effect on species richness or diversity was observed. Species-specific reactions to flower strip traits have often been documented (Wood et al., 2015) and many wild bee species show a distinct response depending on floral and nesting resources (Pfiffner et al., 2019). Apparently, the annual flower strips investigated here attracted mainly generalistic wild bees (98.7% of the observed wild bee individuals were polylecthic species) and, as a consequence, the wild bee communities observed were dominated by few, but abundant species (68% of all individuals belonged to only five species). Indeed, the flower strips investigated here were often dominated by few flowering plants such as *Phacelia* or Buckwheat that seem to be mainly attractive to these few generalists. In this regard, it is not very surprising that no effect of flower coverage on species richness or diversity was observed. However, also flowering plant species richness had no effect on wild bee richness and diversity. This can be explained by the fact that the presence of key plants is more important for wild bee diversity than flower coverage or plant species diversity *per se* (Sutter et al., 2018; Warzecha et al., 2018). Although our seed mixtures (**Supplementary Table 1.1**) contained some of the key plants mentioned by Sutter et al. (2018) and Warzecha et al. (2018), such as *Phacelia tanacetifolia*, *Anthemis tinctoria*, and *Origanum vulgare*, *P. tanacetifolia* was the only key plant that properly emerged. Including additional key plants and improving their emergence may therefore be important to support species diversity and enhance the stability and resilience of ecosystem services (Warzecha et al., 2018).

No Effect of Configurational Complexity

From the parameters related to configurational complexity, neither field size, nor crop diversity or edge length had a significant effect on insect abundance or diversity. This contrasts our expectations based on the findings that pollinators and natural enemies and their services increase with configurational landscape complexity (Krimmer et al., 2019; Martin et al., 2019; Albrecht et al., 2020). A diverse cropping matrix with small fields can provide a temporal continuity of food, nesting sites and shelter and promote biodiversity, including pollinators and natural enemies (Bianchi et al., 2006; Hass et al., 2018; Sirami et al., 2019). A lack of effect of configurational complexity measures can be explained in three ways: First, all of our flower strips were established in rather heterogeneous agricultural landscapes with comparably small fields (mean ± SD: 1.0 ha ± 0.2), high crop diversity (mean ± SD: 15 ± 1.4 crop types according to **Supplementary Table 2.1**) and many patches of non-crop areas. Hereby the contrast, or gradient, for configurational complexity may have been insufficient to detect any ecologically relevant effect and corroborates earlier results from the Swiss lowland (Zingg et al., 2018). Second, field margins in the study area are often small to non-existent or of low ecological quality, as they are impacted by agrochemical drift from mostly intensively managed fields. As a result, the small field size in the Swiss lowlands does not necessarily correlate with more habitat for insects, and unproductive structural complexity in marginal areas may be low compared to nearby regions in neighboring countries (Tschumi et al., 2020). Finally, as highlighted above, the variable and often diverging responses of individual species to habitat edges and the complex effects of habitat edges on species interactions may simply have blurred an ecological signal in our functional groups (Ewers and Didham, 2006).

No Effect on Natural Enemy Abundance

Remarkably and in contrast to our expectations, none of the selected landscape characteristics and flower strip traits had a significant effect on natural enemy abundance (with the exception of a negative trend of flower coverage). A recent meta-analysis corroborates these findings by showing that flower strip traits and landscape context had little effect on biological control (Albrecht et al., 2020). One explanation might be that seed mixtures designed to promote pollination are not optimal for the promotion of other ecosystem services such as pest control (Windsor et al., 2021). Although in our study various seed mixtures were used, at least some of them where specifically designed to attract pollinators. Hereby, and in contrast to pollinators, natural enemies strongly depend on the distribution of their prey or hosts and cannot reproduce with floral resources alone. Another explanation for the lack of effects might be that the group of natural enemies consists of several groups with highly specialized and diverging life-histories. The communities of natural enemies in our study were mainly dominated by the abundance of hoverflies and parasitic wasps (see **Supplementary Material 3**). While hoverflies are rather generalist predators, parasitic wasps are highly specialized on few or single host species. Although the occurrence of both groups is driven by the availability of resources for larvae and adults (Moquet et al., 2018), they often have very differing requirements. Lumping various natural enemy groups together might have resulted in too much variance in their responses to landscape characteristics and flower strip traits to detect any biological signal.

CONCLUSION

Our results provide evidence that both flower strip traits and landscape characteristics are important for the abundance of insects overall and different functional groups in annual flower strips. This was particularly true for pollinators and wild bees in specific. The negative effect of BPA area with standard quality but positive effect of BPA area with high ecological quality, suggests that to support viable populations of insects, ecological quality (high botanical and/or structural diversity) of semi-natural habitats is decisive. This is similarly true for the flower strips themselves that need high flower coverage to be attractive. To enhance overall insect abundance and pollinators in specific, annual flower strips therefore need to be complemented by BPA of high quality and designed to yield high flower coverage. However, to enhance biodiversity of wild bees and likely many other taxonomic groups, flower strips may need to be left in place for multiple years, with high diversity perennial seed-mixtures that also contain key plants for specialized insect species. This study provides new insights into how landscape context and flower strip traits in combination influence insect communities and ecosystem service providers. Taking these findings into account can help improving the effectiveness of flower strips in promoting biodiversity and the delivery of associated regulatory ecosystem services such as pollination or natural pest control.

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.

AUTHOR CONTRIBUTIONS

SZ conceived and designed the study, helped with the statistical analysis, and had the lead in writing the manuscript. KS wrote the first draft of the manuscript, conducted the statistical analyses, and created the figures. MT helped with the statistical analyses, contributed to the writing, and provided critical feedback on the manuscript. HR and SL planned and conducted the data collection on the field and provided feedback on the manuscript. All authors approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.854058/full#supplementary-material

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Pesticide Regime Can Negate the Positive Influence of Native Vegetation Donor Habitat on Natural Enemy Abundance in Adjacent Crop Fields

Sunita Pandey[1,2]*, Anne C. Johnson[2], Gang Xie[3] and Geoff M. Gurr[2]

[1] Plant Quarantine and Pesticide Management Centre, Lalitpur, Nepal, [2] Gulbali Institute, Charles Sturt University, Orange, NSW, Australia, [3] Quantitative Consulting Unit, Charles Sturt University, Wagga Wagga, NSW, Australia

The benefits of non-crop vegetation to conservation biological control of insect pests in adjacent crops have often been demonstrated. Other studies have established that pesticide use can negatively impact natural enemies; but little is known about the outcomes from providing non-crop vegetation in systems with pesticide use. Here we conducted a natural experiment, sampling arthropods from within a set of four fields with varying pesticide use intensities that were otherwise similar and had perennial native vegetation adjacent to a single edge. Bayesian network analysis was applied to model the entire data set, then sensitivity analysis of numbers of arthropods captured in pitfall traps and sticky traps revealed that the overall effect of pesticide toxicity was large. Numbers of multiple arthropod taxa were especially strongly reduced in fields with pesticide regimes that had greater calculated toxicity scores. The effects on natural enemy numbers of the presence of adjacent perennial native vegetation was weaker than the effect of pesticide regime for all taxa except for Staphilinidae, for which it was equivalent. The benefit to in-crop numbers of natural enemies from the adjacent vegetation was strongest for ground active Araneae, Formicidae, and Dermaptera. Descriptive statistical analysis of the spatial distribution in the least heavily sprayed field suggested that the native vegetation was donor habitat for in-crop natural enemies, especially Hymenoptera, Dermaptera, and Formicidae, with numbers elevated close to the native vegetation, an effect that was apparent for around 100 m. Conservation of invertebrates in agricultural landscapes, including efforts to promote natural enemies for conservation biological control, are strongly impeded by "real world" pesticide regimes that include frequent applications and toxic compounds. Landscape features such as perennial native woody vegetation are potentially important refuges for a wide range of natural enemy taxa. The donor habitat effect of such refuges can elevate in-crop densities of these important ecosystem service providers over a scale of around 100 m, implying scope to enhance the strength of biological control in large fields (around 4 ha) by use of entirely wooded margins provided pesticide use is moderated.

Keywords: conservation biological control, refuge habitat, pesticide toxicity, Bayesian network, predator, parasitoid, habitat management, brassica

Edited by:
James Moran,
Galway-Mayo Institute of Technology,
Ireland

Reviewed by:
Francisco Sánchez-Bayo,
The University of Sydney, Australia
María Pérez-Marcos,
Instituto Murciano de Investigación y
Desarrollo Agrario y Alimentario
(IMIDA), Spain

Correspondence:
Sunita Pandey
pandey.sunita2009@gmail.com

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INTRODUCTION

Crop fields surrounded by non-crop vegetation such as hedgerows, shelterbelts, and riparian vegetation can benefit from pest biological control provided by enhanced numbers and diversity of natural enemies compared to fields with no such refuge habitat (Landis and Marino, 1999; Bianchi et al., 2006; Chaplin-Kramer et al., 2013; Morandin et al., 2014; Heimoana et al., 2017). Non-crop vegetation can protect and enhance natural enemies through the provision of appropriate microclimates and overwintering sites for shelter and food in the form of nectar, pollen or alternative hosts (Gurr et al., 2017; Shields et al., 2019; Gardarin et al., 2021). Perennial vegetation can provide a stable habitat for natural enemies from which to recolonize the disturbed habitat of crop fields (Asbjornsen et al., 2014; Schellhorn et al., 2014), supporting pest suppression (Perović et al., 2011; Macfadyen et al., 2015; Heimoana et al., 2017).

Non-crop vegetation does not, however, always increase natural enemy abundance or provide biological pest control service in adjacent crop fields (Tscharntke et al., 2016). There are several factors that potentially account for this, such as absence of effective natural enemies in the region, insufficient or inefficient source habitat, or disruptive agricultural practices (Tscharntke et al., 2016; Karp et al., 2018). Chemical pesticide use on crop fields in particular can interrupt the biocontrol service provided by natural enemies adjacent to non-crop vegetation (Jonsson et al., 2012; Zhu et al., 2017; Gagic et al., 2019). Increased agricultural pesticide use in cropping systems is hostile for natural enemies and low abundance of natural enemies results in ineffective biological control (Mansfield et al., 2006; Thomson and Hoffmann, 2006; Bommarco et al., 2011; Whitehouse et al., 2018). However, population recovery of natural enemies in crop fields is possible as a result of immigration from nearby non-crop vegetation (Duffield et al., 1996; Heimoana et al., 2017). Increasing diversity in cropping systems and reduced or selective insecticide use can enhance natural enemy populations, suppress crop pests and improve agricultural sustainability (Lu et al., 2012; Torres and Bueno, 2018; Lykogianni et al., 2021; Tscharntke et al., 2021). A review by Bommarco et al. (2013) identified that perennial vegetation can be incorporated into agricultural systems to provide a diverse habitat for natural enemies which can result in the suppression of pest populations, and reduced environmental impacts due to decreased use of chemical pesticides. Currently, the effect of interactions between non-crop vegetation and pesticide use on natural enemy activity in crop fields is poorly understood (Zhu et al., 2017; Thomine et al., 2022).

Agricultural landscapes often include portions of perennial non-crop vegetation, some of which is remnant natural vegetation and some intentionally established to manage soil erosion, salinity or other agri-environmental benefit. The inclusion of non-crop native vegetation in agricultural landscapes has been recommended for biodiversity conservation of native flora and fauna (Burghardt et al., 2009; Landis et al., 2012; Chrobock et al., 2013; Gill et al., 2014). However, few studies have explored the effects of perennial, woody vegetation in commercial agricultural fields on natural enemy populations (Thomson and Hoffmann, 2010, 2013; Morandin et al., 2014; Bianchi et al., 2015; Shields et al., 2016; Retallack et al., 2019), despite the benefits evident in laboratory and small field trial studies (Fiedler and Landis, 2007; Isaacs et al., 2009; Pandey et al., 2018; Pandey and Gurr, 2019).

This study used a natural experiment approach to assess natural enemy densities in a set of four fields that were growing a common crop (brassica vegetable), in one region and 1 year, each had one margin that was adjacent to woody vegetation, but that were being managed with differing levels of pesticide use intensity. This uncommon alignment of characteristics (we used all sites that in the region that met the foregoing criteria) provided an opportunity to assess the effects on natural enemies in a system in which pesticide toxicity load and presence of woody vegetation on one edge could be discriminated. We hypothesized that the benefit of adjacent vegetation on in-crop natural enemy densities would be strongest in fields with the lowest pesticide use intensity.

MATERIALS AND METHODS

Study Sites

The study sites consisted of four commercial brassica fields in Central West of New South Wales, Australia (**Figure 1**). This represented all possible available fields that met criteria for them to test the central hypothesis. Accordingly, sites each had an area of woody, perennial, native vegetation along one border with the opposite side of the field comprised of an unsealed farm road and another brassica crop. All fields were 3–4 ha in extent to allow for measurement of potential gradients in arthropod abundance in transects from the wooded margins. All four sites were in a simple landscape dominated by other commercial, conventionally managed (i.e., non-organic) crop fields. Each field contained a summer-autumn grown brassica vegetable crop (*Brassica oleracea* var. *botrytis*) that was managed by the farmer with conventional pesticides. Perennial woody native vegetation at all four sites was a *Eucalyptus* spp.-dominated zone, with a groundcover of grass family *Cynodon dactylon*, *Digitaria* spp., *Paspalum dilatatum*, *Microlaena stipoides*, *Aristida* spp., *Bothriochloa* spp., and herbaceous species *Rubus* sp., *Foeniculum vulgare*, *Conium maculatum*, *Datura stramonium*, *Portulaca oleracea*, *Verbena bonariensis*, *Chondrilla juncea*, *Sisymbrium officinale,* and *Chenopodium album*.

Pesticide Use

All crops were managed by the host farmer and for each site a "cumulative pesticide toxicity score" was calculated to measure pesticide load covering the time from field preparation to the last date of data collection. Crops were sprayed in response to the insect pest *Plutella xylostella*, *Pieris rapae*, *Hellula hydralis,* other lepidopteran pests and aphids. Pesticides were applied by farmers based on visual observations of pest infestation levels with decisions guided by their experience and consultation with their agronomist without influence from the researchers. Data on pesticide identity, rate and application frequency were collected from the spray application records

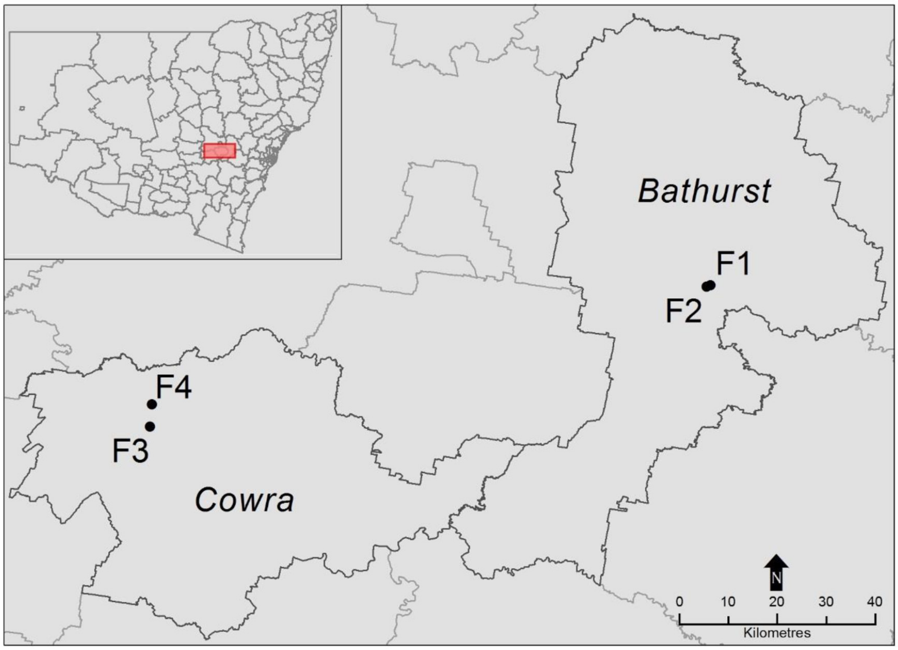

FIGURE 1 | Map of research sites in central west region of NSW, Australia.

provided by the farmers. Toxicity scores were calculated by multiplying a modified toxicity rating given to each pesticide chemical by the number of applications which was summed to give a "cumulative pesticide toxicity score" by following Thomson and Hoffmann (2006) and Markó et al. (2017). This approach has been found to be reasonable comparison of pesticide loads in fields with same type of crop and similar soil properties (Ockleford et al., 2017). The toxicity rating of each chemical was based on the International Organization for Biological Control (IOBC) Pesticide Side Effect Database,[1] which divides the acute toxicity of pesticides for non-target organisms into four risk categories from harmless (1) to harmful (4) (**Table 1**). This was modified by changing the scores to 0–3 to include only the influence of chemical pesticides that harm natural enemies (Thomson and Hoffmann, 2006). We also considered application rate [grams of active ingredient (a.i.)/ha] for toxicity rating of each pesticide. For example, methomyl 500 g/ha was given maximum score of 3, while methomyl 90 g/ha was rated 2. These a.i./ha of sites were compared with a.i./ha of the IOBC database and relevant peer-reviewed research papers that considered natural enemies-insecticide toxicity (Leggett, 1990; Elzen et al., 1998; Galvan et al., 2006; Wanumen et al., 2016; Deekshita et al., 2017). Sampling of natural enemies was always carried out after the relevant re-entry interval after each pesticide application.

Study Design

In each field, three parallel transects, 40 m apart, were established perpendicular from the woody vegetation toward the opposite edge of the field which was 140–180 m away depending on the field (**Figure 2**). For each transect there were 11 sampling points, which were positioned at 1, 5, 10, 20, and 50 m from each edge plus one point in the center of the field. The relative abundance of canopy and ground-active natural enemies was assessed at each sample point with a yellow sticky trap (17 × 10 cm) (Bugs for Bugs, Australia) mounted atop a 1,200 mm wooden stake and two round pitfall traps (12.5 cm deep and 8.5 cm diameter). Yellow sticky trapping is widely used for aerial arthropods such as parasitic Hymenoptera, reflecting efficacy, and low cost (Larsen et al., 2014; McCravy, 2018), whilst pitfall trapping is the most common sampling method for ground dwelling arthropods in cultivated land (McCravy, 2018). Pitfall traps were constructed using two cups, the outer cup was placed flush with the soil surface and inner cup was inserted into the first cup and filled with ∼250 ml of diluted ethylene glycol with a few drops of detergent. The traps were protected from rain and sun with a cover made from a plastic plate and wooden skewers. Traps were left in place for 4 days and then collected for quantification and identification of natural enemies. Natural enemy monitoring/sampling was carried out on four occasion's commencing 30 days after crop planting-out and at approximately 15-day intervals taking into consideration the constraint of re-entry periods after pesticide applications. The last observation was made 2 weeks before crop harvest (March 2017 for field 1 and 2 and May 2017 for field 3 and 4).

Arthropods collected in pitfall traps at a given transect position were placed in a plastic container and labeled. Similarly, each yellow sticky trap was labeled and wrapped in plastic food wrap film. The arthropods from the pitfall traps (ground active

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[1] http://www.iobc-wprs.org

TABLE 1 | Insecticides and fungicides applied during the growing season (Dec 2016–May 2017) and the toxicity as determined by IOBC and modified toxicity rating for each site (F1–F4).

Pesticide name	Active ingredients	Application dose	a.i./ha	Application dates by field (F) (day/month)				IOBC Toxicity	Modified toxicity (t)
				F1	F2	F3	F4		
Maldison	Malathion[a]	300 ml/ha	150 g/ha	31/12 04/02				4	3
Movento	Spirotetramat	200 ml/ha	48 g/ha	31/12 07/01				1	0
Delfin	*Bacillus thuringiensis* subspecies *kurstaki*	500 g/ha	500 g/ha	14/01 04/02 18/02				1	0
Orthene	Acephate[a]	700 g/ha	679 g/ha	19/01				4	3
Belt	Flubendiamide	100 ml/ha	48 g/ha		28/12 15/01			1	0
Lorsban	Chlorpyrifos[a]	2 l/ha	1,000 g/ha		06/01 15/01			4	3
Lannate	Methomyl[b]	2 l/ha	500 g/ha		28/12 06/01 25/01 05/02			4	3
Lannate	Methomyl[b]	400 ml/ha	90 g/ha			03/04		3	2
Durivo	Thiamethoxam and Chlorantraniliprole	30 ml/1,000 plants (seedling drench)	30 ml/1,000 plants			04/02		1	0
Success	Spinetoram	400 ml/ha	48 g/ha			20/04	07/04	1	0
Proclaim	Emamectin benzoate	250 g/ha	11 g/ha			27/03 12/04		2	1
Cropro Buzzard	Alpha cypermethrin[c]	400 ml/ha	40 g/ha	07/01 18/02		03/04		4	3
Gemstar	Polyhedrosis virus	500 ml/ha	500 ml/ha			03/04 12/04 20/04		1	0
Dipel	*Bacillus thuringiensis* subspecies *kurstaki*	2,000 g/ha	2,000 g/ha			12/04			0
Dimethoate	Dimethoate[a]	755 ml/ha	300 g/ha				07/04	4	3
Avatar	Indoxacarb	200 g/ha	60 g/ha				20/04 08/05	3	2
Transform	Sulfoxaflor	400 ml/ha	96 g/ha				08/05	3	2
Bravo	Chlorothalonil	3 l/ha	2,160 g/ha				07/04	1	0
Polyram	Metiram	3 kg/ha	2,100 g/ha				08/05	3	2
Cumulative Pesticide Toxicity Score (CPTS) = $\sum(f \times t)$				15	18	7	11		

[a] *Organophosphate.*
[b] *Carbamate.*
[c] *Pyrethroid.*

predators) were cleaned, counted, identified to a family level and placed in ethyl alcohol filled glass vial for further inspection within 48 h from collection. Yellow sticky traps were initially placed in freezers and all natural enemies later identified to super family or genus level for some taxa. Parasitoids were identified by following (Goulet and Huber, 1993) using a stereo dissecting microscope (10–20 ×) (Leica, SE305-A, Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Arthropod counts were totaled across the corresponding positions (distance from edge) for the three transects in each field.

Data Analysis

A Bayesian Network (BN) approach was used for data analysis so that the nuances of the complex interrelationships between the variables could be captured. Originally developed as a modeling tool from artificial intelligence since late 1980s, BNs have found applications across the sciences, industries and government organizations (Pearl, 1988; Kjærulff and Madsen, 2008). As probabilistic graphical models, BNs allow for effective modeling of physical, biological, and social systems operating under uncertainty (Kjærulff and Madsen, 2008; Korb and Nicholson, 2011). Formally, a BN model is a graphical representation, i.e., a directed acyclic graph (DAG), of a joint probability distribution of a set of random variables in which each variable is represented by a node and the dependency relationship is represented by a link/edge for two associated variables (Pearl, 1988; Kjærulff and Madsen, 2008). BNs are based on Bayes Theorem, a mathematical statement which expresses the interrelationships between the conditional, marginal, and joint probability distributions of random variables as defined in the following formula (Upton and Cook, 2006):

$$\Pr(B \mid A) = \frac{\Pr(A \mid B)\Pr(B)}{\Pr(A)} = \frac{\Pr(A, B)}{\Pr(A)},$$

where A and B are two random variables/events; Pr(A) and Pr(B) are the marginal probability distributions of A and B, respectively; Pr(B| A) is the conditional probability distribution of B given A; Pr(A| B) is the conditional probability distribution of A given B; and Pr(A, B) is the joint probability distribution of A and B.Since a BN model represents the joint distribution of all variables included in the model, anyone (or more than one) variable(s) may be selected as the target variable(s) (equivalent to the "outcome/dependent" variable in a regression model) to then perform inferential analysis by assuming different scenarios in terms of the "findings" of other variables. For example, by fixing the values of some variables (equivalent to those predictor variables in a regression model) we are able to estimate/predict the values (or the distribution of the values) of the remaining variable(s) (equivalent to the outcome/dependent variable in a regression model) in a BN model.

In the present study, the BN analysis investigated associations between field characteristics (pesticide toxicity score, distance from woody vegetation refuge, transect and sampling date) and number of arthropods caught of each taxonomic category or sampling method (pitfall trap or sticky trap) (**Figure 3**). The BN predictive model included the toxicity effect (reflecting the differing pesticide regimes among the four fields) and the refuge effect (representing the multiple sampling positions within each field at varying distances from the perennial native vegetation that bordered one edge and may have constituted a refuge for arthropods that later moved into the crop field). Other variables included the transect number and sample date as well as the natural enemy groups. By specifying different scenarios in terms of the toxicity effect and the refuge effect, this BN model could estimate/predict the abundance levels for each natural enemy groups. This BN model also allowed a sensitivity analysis to be run for each of the natural enemy groups to quantify the strength of the association between the abundance level and the toxicity effect and the refuge effect. Thus, the percentage values from such sensitivity analyses for a given variable are broadly analogous to the adjusted R^2, the goodness-of-fit measure from a regression analysis that provide an indication of the explanatory strength of one variable for another. The proprietary BN software package, Netica (Norsys Software Corp, 2021), was used for model construction and sensitivity analyses.

Descriptive analyses were conducted in R (R Core Team, 2020) and were used to describe the overall spatial pattern of arthropod catches in relation to the fields with contrasting levels of calculated pesticide toxicity and spatial position within fields in relation to the adjacent perennial native vegetation.

RESULTS

Natural Enemy Assemblages of Brassica Fields

A total of 14,459 natural enemies were caught; 11,123 on sticky traps and 3,336 in pitfall traps. Predators made up 34.7% (5,018) and hymenopteran parasitoids 65.3% (9,441). Predators were more numerous in pitfall traps (66.5%) compared to sticky traps (33.5%). All the parasitoids were caught on sticky traps. The most abundant predators comprised of Araneae (30.1%) (both canopy and ground), Formicidae (25.8%), Dermaptera (13.0%), Staphylinidae (12.8%), and Carabidae (9.3 %). Within Carabidae 95% of specimens were fully winged and small (<9 mm, length of the body excluding head). Less abundant taxa were Coccinellidae (1.41%), Syrphidae (0.5%), Dolichopodidae (0.33%), Melyridae (0.23%), Cantharidae (0.22%), Hemerobiidae (0.21%), Vespidae (0.09%), Nabidae (0.07%), and Cicindelinae (0.01%). Among total parasitoids, small-minute parasitoids comprised 97.3% followed by *Diadegma* spp. (1.62%), *Aphidiinae* (0.84%), Ichneumonoidea (0.12%), and *Cotesia* spp. (0.05%).

Effect of Pesticide Load and Perennial Native Vegetation on Natural Enemies

Descriptive data analysis for the overall numbers of arthropods captured in pitfall traps showed clear differences among the four fields with differing pesticide regimes. Numbers of these ground-active predators were uniformly low across the extent of transects in the field with highest toxicity score (**Figure 4**). Numbers in the field with the next most intense pesticide regime (toxicity score 15) were marginally higher but relatively uniform across the field. Arthropod numbers in the two least intensively sprayed fields were markedly higher (**Table 2**) and, in both cases, showed a pronounced elevation in proximity to the perennial native vegetation. This spatial trend was especially marked in field 3 which had the lowest toxicity score. Here, high predator densities were present in areas of the crop adjacent to perennial woody vegetation. Densities decayed over the range of 120 m from this margin, reaching levels consistent with the most heavily sprayed two fields.

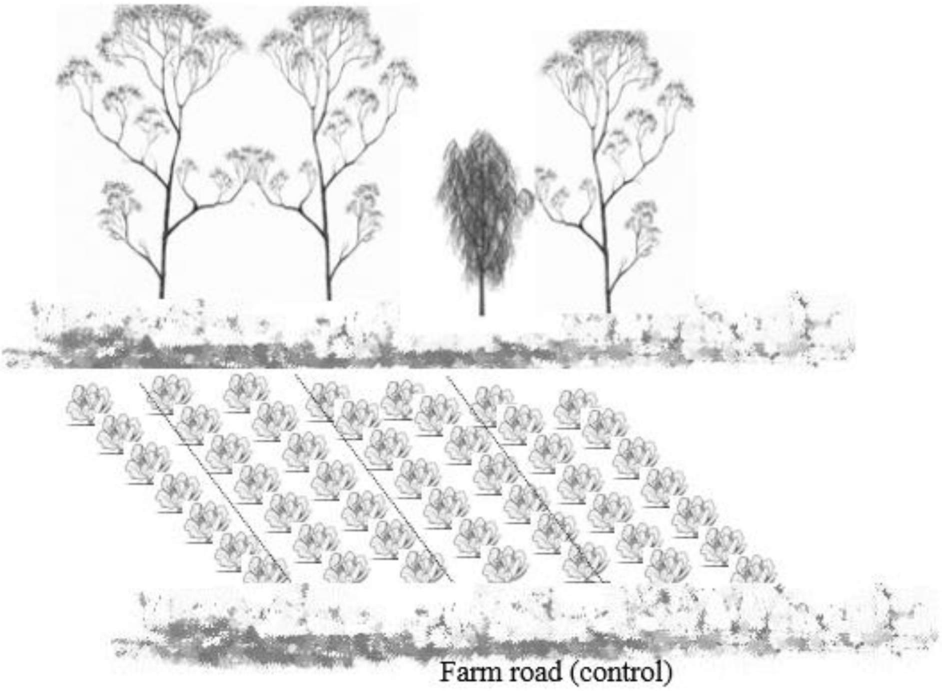

FIGURE 2 | Schematic diagram of each field of study: The perennial native vegetation is considered as a "treatment" for attracting natural enemy insects for the benefit of growing brassica crops and farm road considered as control. The dotted line in the brassica field are transects were data collection was done in 11 distance points (1, 5, 10, 20, and 50 m from each edge and the field center).

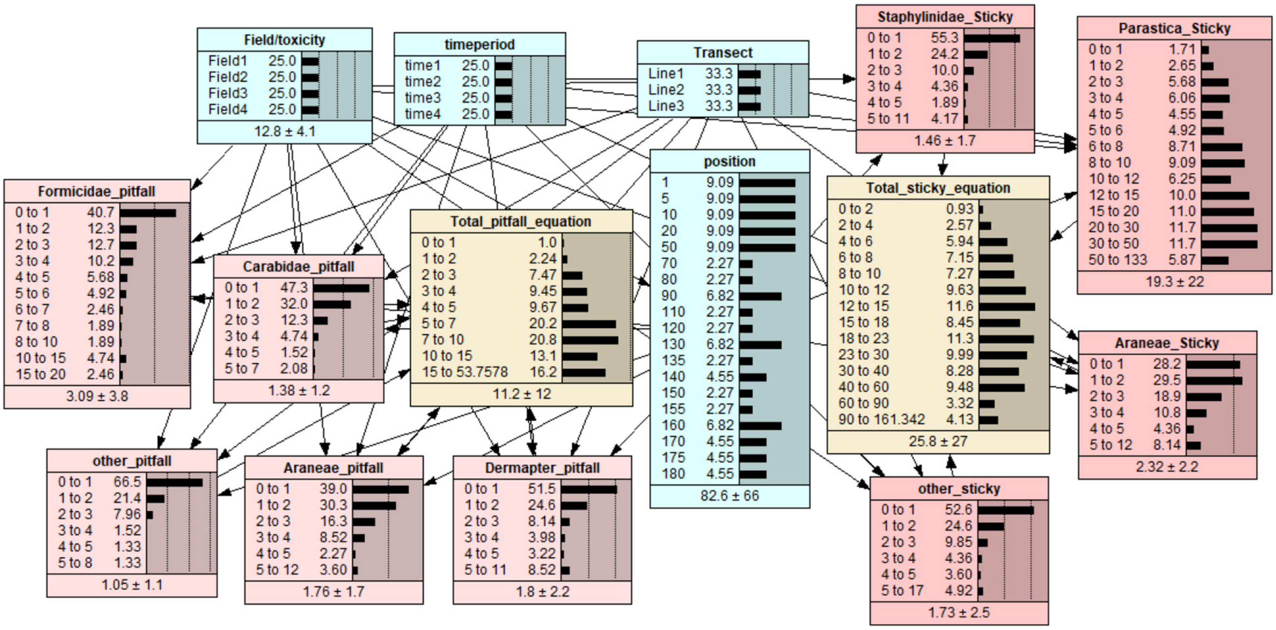

FIGURE 3 | A predictive Bayesian Network model for the overall data set which represents the interrelationships between the pesticide toxicity scores calculated for four brassica vegetable fields, sample position effects resulting from perennial woody vegetation at one field edge, transect number (3 per field) and sample date on the abundance of various natural enemy groups sampled by pitfall and sticky traps.

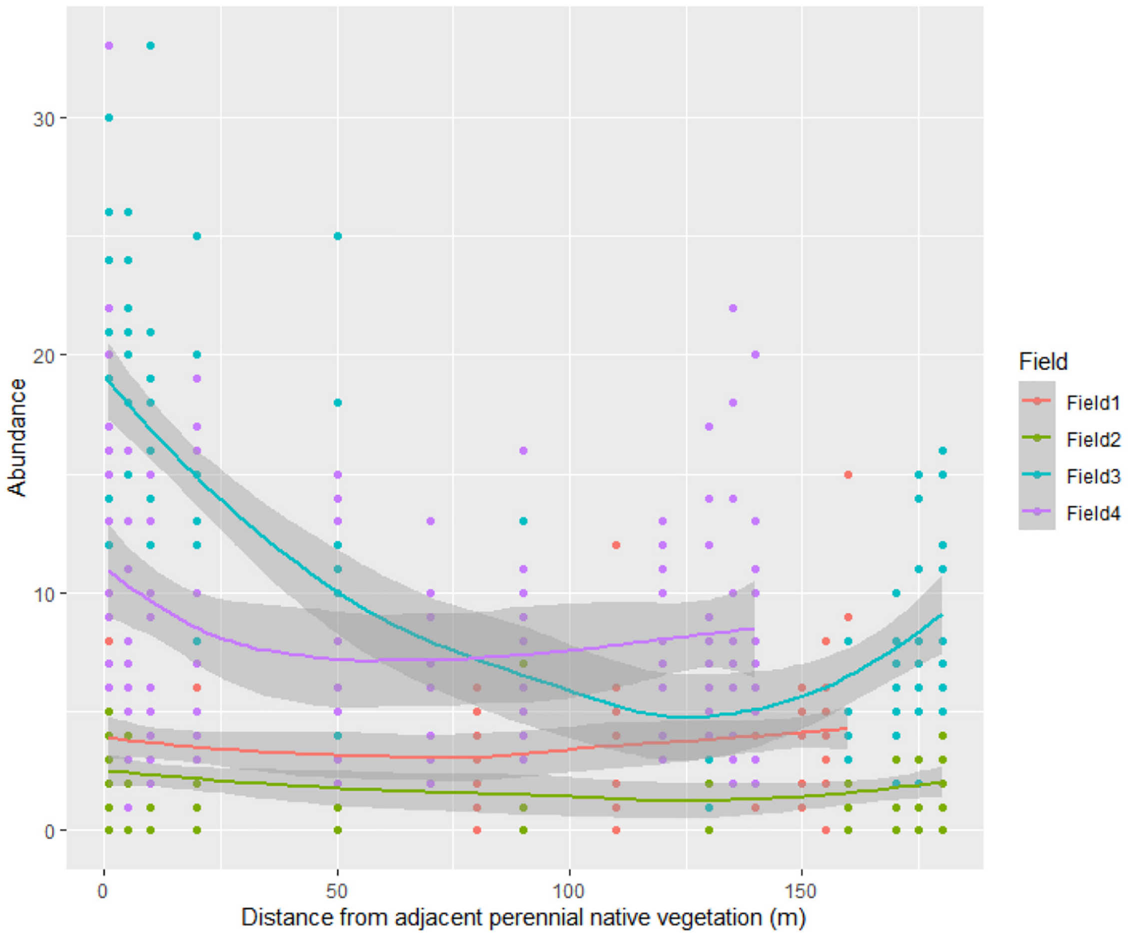

FIGURE 4 | Spatial distribution of the ground active predators (pitfall trap catches) in fields with perennial woody vegetation adjacent to one margin (the zero position) but with contrasting levels of pesticide use quantified as a toxicity score (pale blue = low; purple = moderate; red = high; green = very high).

BN sensitivity analyses run for each of the natural enemy taxa and guilds allowed a quantitative comparison of the relative strength of the pesticide toxicity effect vs. the refuge effect. Overall, the effect of pesticide toxicity was dominant compared with the adjacent perennial native vegetation's refuge effect. This was the case for all taxa and groups except for Staphilinidae, for which the two effects were equivalent (**Table 3**). Dermaptera, parasitoids, ground active Araneae and Formicidae were most strongly influenced by the toxicity effect and there was a strong effect for overall ground active arthropods. Less strong effects were evident for ground active Carabidae and aerial Aranae and Staphilinidae, all taxa within which some species are capable of flight or "ballooning" dispersal on silken threads. For all taxa, the refuge effect was never greater than 57% of that taxon's toxicity effect, and often substantially smaller. In the case of Dermaptera for example, the refuge effect was 7.2 and the toxicity effect 41.7. This is despite the fact that Dermaptera, along with ground active Araneae and Formicidae, were the taxa that exhibited the strongest refuge effect. The taxa with weakest refuge effects were Carabidae and aerial Aranae and Staphilinidae, the same taxa that were least affected by pesticide toxicity. Collectively, this series of BN sensitivity analyses provides a clear indication that for a wide range of natural enemy taxa and guilds (e.g., ground active vs. aerial), the pesticide regime of a crop field is a stronger driver of population size than any potential effect of nearby donor habitat.

The potential strength of benefit to in-crop densities of many natural enemies from perennial native vegetation was clearly apparent from descriptive data analysis of the least intensively sprayed field. For ground active taxa (**Figure 5A**), there were elevated numbers of individuals in areas of the crop closest to the woody vegetation consistent with it serving as donor habitat, especially for the more common taxa of Dermaptera, and Formicidae. Numbers of these insects were strongly elevated close to the native vegetation, an effect that was apparent for around 100 m. Weaker levels of elevation that tended to decay more rapidly were apparent for scarcer ground-active taxa: Araneae, Carabidae, and the category "other" (i.e., not among the afore-named taxa). For aerial arthropod arthropods, parasitic Hymenoptera were the most numerous overall and exhibited a very strong refuge effect that, like the ground active Dermaptera, and Formicidae, was apparent for 100 m into the field

TABLE 2 | Variables explaining the abundance of ground active natural enemies and their finer taxonomic group.

Response variable	Estimated mean	Lower 95% CI	Upper 95% CI
Total ground dwelling natural enemies			
Field 4	14.6	2.59	49.7
Field 3	18.4	2.48	51.2
Field 2	4.81	1.25	10.1
Field 1	6.78	1.82	14.5
Araneaae			
Field 4	3.14	0.330	10.6
Field 3	1.63	0.0717	3.97
Field 2	0.750	0.0324	1.99
Field 1	1.53	0.0688	4.23
Carabidae			
Field 4	1.63	0.0623	5.90
Field 3	1.68	0.0673	4.94
Field 2	0.864	0.0375	1.99
Field 1	1.36	0.0550	4.35
Dermaptera			
Field 4	1.14	0.0500	2.98
Field 3	4.23	0.254	10.6
Field 2	0.591	0.0268	1.82
Field 1	1.22	0.0471	3.96
Formicidae			
Field 4	4.45	0.118	15.9
Field 3	5.03	0.0825	18.2
Field 2	1.42	0.0371	4.96
Field 1	1.43	0.0569	3.78

Shown are estimated means and 95% confidence intervals (CI) of the BN predictive model.

TABLE 3 | Relative strength of effect from pesticide regime used in the field ("toxicity effect") and from the presence of woody vegetation adjacent to one margin of field ("refuge effect") on various natural enemy groups derived from Bayesian network sensitivity analysis.

Response variable	Toxicity effect (%)	Refuge effect (%)
Predator		
Dermaptera (pitfall trap)	41.7	7.2
Araneae (pitfall trap)	24.9	9.6
Formicidae (pitfall trap)	19.4	8.4
Carabidae (pitfall trap)	7.4	4.7
Other (pitfall trap)	9.0	4.7
Total (pitfall trap)	23.2	7.0
Araneae (sticky trap)	6.8	4.4
Staphalinidae (sticky trap)	5.4	5.6
Parasitoid (sticky trap)	29.7	16.9

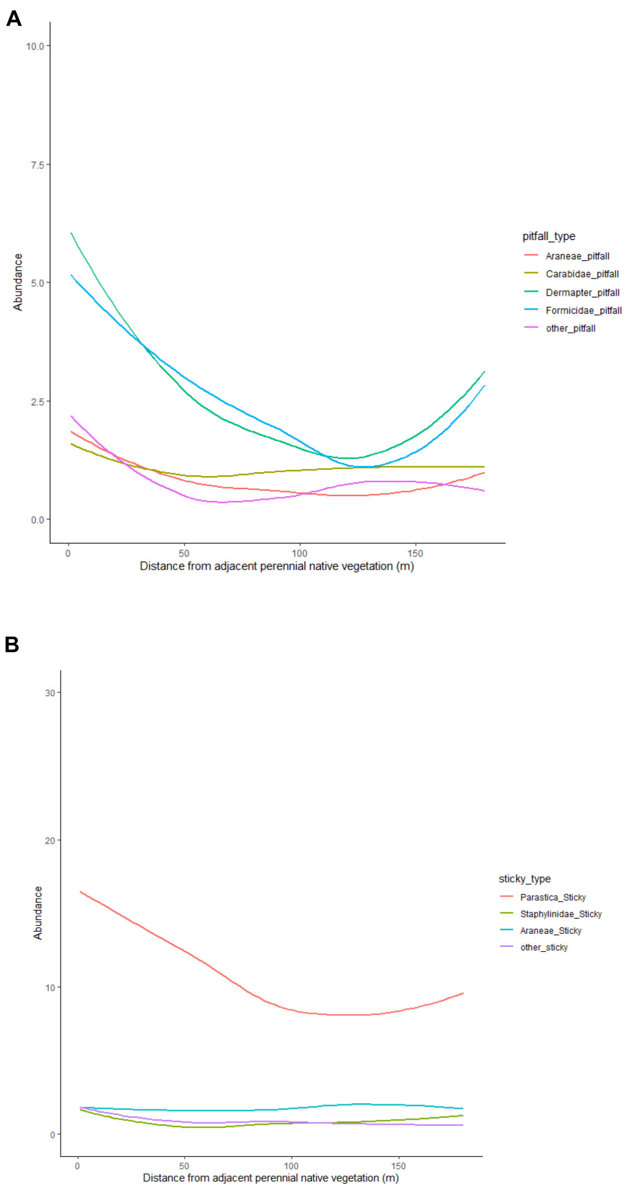

FIGURE 5 | Effect on natural enemy groups of perennial woody vegetation adjacent to one margin of a brassica vegetable field with a pesticide regime calculated to have low toxicity. **(A)** Ground active predators (pitfall trap catches) and **(B)** aerial groups, including ballooning spiders (sticky trap catches).

(**Figure 5B**). No refuge effect was apparent for other aerial natural enemies.

DISCUSSION

We found the abundance of ground active predators and constituent taxa were highly reduced in fields with high pesticide toxicity scores. In fields with lower toxicity scores, the presence of adjacent perennial native vegetation influenced in-field spatial distribution of ground active predators and constituent taxa. The abundance of natural enemies was higher adjacent to perennial native vegetation in the field with lower toxicity score whereas in the fields with relatively higher toxicity score no variation in numbers were observed according to the distance from perennial native vegetation. Importantly, however, intense insecticide use appeared to over-ride the benefits of adjacent woody vegetation in the fields with relatively higher toxicity score to the extent that in-crop abundance of these natural enemies close to the woody vegetation were as low as in remote parts of the field.

Generally, the presence of non-crop vegetation close to crops is widely observed in agroecosystems to offer potential support to natural enemies and biological pest control (Alignier et al., 2014; Schellhorn et al., 2014; Blaauw and Isaacs, 2015; Woodcock et al., 2016; Heimoana et al., 2017). However, inconsistent effects have also been reported in other studies (Blitzer et al., 2012; Jonsson et al., 2012; Parry et al., 2015) and a recent meta-analysis confirmed inconsistency in the response of natural enemy abundance as well as pest suppression and crop yield (Karp et al., 2018). Field management practices such as use of chemical pesticides have been proposed as one of the factors likely to account for the range of observed responses (Tscharntke et al., 2016; Begg et al., 2017) and the present study provides support for this effect to the extent that it overcame the effect of nearby woody vegetation which is likely to have provided resources (e.g., shelter, alternative foods) and served as donor habitat for enemies.

There are few studies of the role of non-crop vegetation on natural enemies in fields with pesticide sprayed crops (Lee et al., 2001; Zhu et al., 2017). The former of those studies suggested that refuge habitats can buffer the negative effects of insecticide use on carabid beetles in a temperate system but indicated that insecticide impact should be minimized during the period of carabid emergence to increase their activity in crops. The later study found, in sub-tropical rice, that the strong benefits to natural enemies of strips of flowering sesame were negated by insecticide application to the rice crop. This high level of disruption is likely to be accounted for by spatial scale. The crop margin flower strips in Zhu et al. (2017) were narrow and immediately adjacent to the sprayed crop so as not effective as a complete, spray-drift-free refuge for natural enemies. In the present study, the perennial woody vegetation present on one edge of all fields extended for at least 30 m from the crop margin. This, together with the height and structural complexity of the woody vegetation, would have greatly reduced the extent to which crop spraying penetrated and impacted natural enemies in the woodland, allowing it to serve more effectively as a refuge for, and source of, natural enemies. However, the adjacent crop fields in this study presented a hostile environment for natural enemies due to the use of broad-spectrum insecticides such as organophosphates, carbamates, and pyrethroids. Earlier studies have demonstrated impacts, including residual effects of these broad spectrum insecticides on predators such as beetles (O'Neal et al., 2005), spiders (Maloney et al., 2003), ladybugs and other predators (Roubos et al., 2014). Consistent with this, studies have concluded that pesticide toxicity is a major factor in disruption to biological control in crop fields despite the potential support of natural vegetation (Geiger et al., 2010; Jonsson et al., 2012; Gagic et al., 2019). The present study supports that general conclusion but suggests that natural enemy taxon and guild (e.g., dispersal capacity and life history omnivory) can strongly influence outcomes.

In contrast to several taxa of ground active predators captured in pitfall traps, sticky trap catches of Staphilinidae and Araneae were less strongly affected by toxicity scores of the contrasting fields. Many species of Staphilinidae fly and the spiders caught on sticky traps can be assumed to have been captured whilst undergoing ballooning dispersal. Among the predators captured in pitfall traps, Carabidae had the least strong toxicity effect in the BN sensitivity analysis and this family includes species capable of flight. Accordingly, the observed effects are likely to reflect their dispersal mode, an important factor in driving how natural enemies are affected by crop management practices and non-crop vegetation (Sorribas et al., 2016; Gagic et al., 2019). In this study, the vagility of the named taxa would allow them to recolonize a sprayed field more rapidly than taxa such as Dermaptera and ground-active spiders that are more likely dependent on walking. The same effects of dispersal capacity can explain the relative strength of refuge effects among the natural enemy taxa: those capable of flight or ballooning being less dependent on nearby donor habitats because they can reach all areas of a crop field equally well from even relatively remote refuges. Hymenopteran parasitoids, however, exhibited a strong toxicity effect and refuge effect despite flight capacity being virtual ubiquitous in this group. This apparent anomaly can be explained by the fact that the vast majority of the parasitoids captured were small or minute species that have relatively weak flight capacity. Additionally, adult parasitoids (including species of importance in brassica crops) exhibit life history omnivory, carnivores as immatures and nectar feeders as adults, sostrongly benefit from access to appropriate nectar plants (Pandey et al., 2018; Pandey and Gurr, 2019; Gardarin et al., 2021). Accordingly, the perennial native vegetation areas in the present study, that also had herbaceous plants in the groundcover vegetation, will have provided nectar in addition to physical shelter making these areas of double value to parasitoids. This contrasts with taxa such as Caribidae, Staphilinidae, and Araneae that do not feed on nectar and for which a shelter effect is more important.

In conclusion, perennial native vegetation has a good potential as a donor habitat for natural enemies in the studied crops, however, this can be negated by pesticide use. The abundance of ground active predators and parasitoids was markedly decreased in fields with higher levels pesticide use and the benefits provided by adjacent vegetation were evident only under conditions of low pesticide toxicity score. For the more vagile canopy dwelling predators, pesticide regime had a weaker effect on abundance and these were less likely to be reliant on nearby donor habitat. These results highlight the need for a nuanced approach to providing recommendations for conservation biological control, and possibly for arthropod conservation in farm landscapes more generally. Whilst providing donor habitats and moderating insecticide regimes are useful first approximations for promoting natural enemies in crop fields, there is a strong need to consider key ecological traits—especially those relating to dispersal capacity and life history omnivory of key natural enemies—when planning conservation biological control interventions.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

SP and GG conceived the study. SP collected and identified samples. SP, GG, and GX analyzed the data. All authors wrote the manuscript.

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Prairie Strips and Lower Land Use Intensity Increase Biodiversity and Ecosystem Services

Lindsey R. Kemmerling[1,2]*, Corinn E. Rutkoski[1,3], Sarah E. Evans[1,2], Jackson A. Helms IV[4], Esbeiry S. Cordova-Ortiz[1], Jamie D. Smith[1], Jorge A. Vázquez Custodio[5], Carmella Vizza[1,6] and Nick M. Haddad[1,2]

[1] W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI, United States, [2] Ecology, Evolution, and Behavior Program, Michigan State University, East Lansing, MI, United States, [3] Environmental Science and Policy Program, Michigan State University, East Lansing, MI, United States, [4] United States Department of Agriculture-Agricultural Research Service (USDA–ARS) Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, United States, [5] Department of Biology, University of Puerto Rico, Arecibo, PR, United States, [6] College of Natural and Computational Sciences, Hawai'i Pacific University, Kaneohe, HI, United States

Agricultural landscapes can be managed to protect biodiversity and maintain ecosystem services. One approach to achieve this is to restore native perennial vegetation within croplands. Where rowcrops have displaced prairie, as in the US Midwest, restoration of native perennial vegetation can align with crops in so called "prairie strips." We tested the effect of prairie strips in addition to other management practices on a variety of taxa and on a suite of ecosystem services. To do so, we worked within a 33-year-old experiment that included treatments that varied methods of agricultural management across a gradient of land use intensity. In the two lowest intensity crop management treatments, we introduced prairie strips that occupied 5% of crop area. We addressed three questions: (1) What are the effects of newly established prairie strips on the spillover of biodiversity and ecosystem services into cropland? (2) How does time since prairie strip establishment affect biodiversity and ecosystem services? (3) What are the tradeoffs and synergies among biodiversity conservation, non-provisioning ecosystem services, and provisioning ecosystem services (crop yield) across a land use intensity gradient (which includes prairie strips)? Within prairie strip treatments, where sampling effort occurred within and at increasing distance from strips, dung beetle abundance, spider abundance and richness, active carbon, decomposition, and pollination decreased with distance from prairie strips, and this effect increased between the first and second year. Across the entire land use intensity gradient, treatments with prairie strips and reduced chemical inputs had higher butterfly abundance, spider abundance, and pollination services. In addition, soil organic carbon, butterfly richness, and spider richness increased with a decrease in land use intensity. Crop yield in one treatment with prairie strips was equal to that of the highest intensity management, even while including the area taken out of production. We found no effects of strips on ant biodiversity and greenhouse gas emissions (N_2O and CH_4). Our results show that, even in early establishment, prairie strips and lower land use intensity can contribute to the conservation of biodiversity and ecosystem services without a disproportionate loss of crop yield.

Keywords: prairie strips, conservation, US Midwest, agriculture, spillover, soil, arthropod

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James Moran,
Galway-Mayo Institute of Technology, Ireland

Reviewed by:
Gabor L. Lovei,
Aarhus University, Denmark
Marco Ferrante,
University of the Azores, Portugal

***Correspondence:**
Lindsey R. Kemmerling
kemmerL4@msu.edu

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INTRODUCTION

Two global challenges of our time are supporting a growing human population and preventing the loss of biodiversity and ecosystem services (Kremen and Merenlender, 2018; Intergovernmental Platform on Biodiversity and Ecosystem Services [IPBES], 2019). The capacity to address these challenges depends largely on management of agricultural lands that dominate the landscape globally (Campbell et al., 2017; Raven and Wagner, 2021). In the US Midwest, for example, 38% of the landscape is planted in principal row crops (USDA National Agricultural Statistics Service, 2019). These agroecosystems were designed to maximize the production of food, fuel, and fiber, and they contribute to greenhouse gas emissions, pollution, and the loss of natural ecosystems and biodiversity (Tilman and Clark, 2015). To prevent further ecological harm and to sustain food, fuel, and fiber production for future generations, agricultural landscapes must be managed for multifunctionality and biodiversity (Asbjornsen et al., 2013; Intergovernmental Platform on Biodiversity and Ecosystem Services [IPBES], 2019; Mitchell et al., 2021). Yet, there are few assessments of the tradeoffs and synergies of biodiversity and ecosystem service responses across crop management and conservation practices required to address the two grand challenges (Wittwer et al., 2021).

Diversifying agricultural landscapes can promote biodiversity and non-provisioning ecosystem services without compromising crop yield (Tamburini et al., 2020; Tscharntke et al., 2021). Landscape diversification, an approach to land management rooted in indigenous knowledge, has been experimentally studied by the scientific community for applications in input-intensive cropping systems (Intergovernmental Platform on Biodiversity and Ecosystem Services [IPBES], 2016; Nkuba et al., 2020). One method of diversifying agricultural landscapes, as is done in the US Midwest, is to establish prairie strips on row crop farms. This conservation practice consists of retiring areas of farmland and actively restoring them by seeding native perennial vegetation. Supported by the United States Department of Agriculture (USDA) Conservation Reserve Program (CRP), prairie strips are one of many "edge of field" practices–including riparian buffers, hedgerows, and wildflower strips–aimed at incorporating native and diverse habitat into agricultural landscapes (The Nature Conservancy [TNC], 2021).

Once established, prairie strips in contoured farm landscapes can reduce soil erosion, improve water quality, and support biodiversity. This can provide benefits to the farm and farmer at disproportionately higher levels than the amount of farmland removed from production (Schulte et al., 2017). Prairie strips also have the potential to provide resources and habitat for beneficial insects and increase their spillover into the farm, where they can provide ecosystem services such as biocontrol and pollination (Blitzer et al., 2012; Morandin and Kremen, 2013; Kordbacheh et al., 2020). While prairie strips are known to benefit biodiversity and ecosystem services once they have been established for multiple years, these impacts have not been studied during their initial years of establishment. Measuring prairie strips' conservation potential during their early establishment period can address this gap and increase the precision of farmers' expectations of prairie strips.

The conservation potential of prairie strips relative to other agricultural conservation practices such as no tillage is also unknown. Biodiversity and ecosystem services in agroecosystems are driven not only by the presence of natural habitat on or near farms, but also by the agricultural management practices used in row crop areas. Crop rotations and cover crops generally increase biodiversity and enhance nutrient availability (Rusch et al., 2013; de Pedro et al., 2020; Bowers et al., 2021), whereas the use of tillage, pesticides, and fertilizer generally decrease biodiversity and increase greenhouse gas emissions (Syswerda and Robertson, 2014; Bowles et al., 2016; Raven and Wagner, 2021). But when implemented jointly, landscape diversification and crop management practices can interact to produce unique impacts on on-farm biodiversity and ecosystem services (Schmidt et al., 2005; Tscharntke et al., 2005; Landis, 2017). There is some evidence that conventionally managed farm fields with prairie strips increase ecosystem services compared to conventionally managed farm fields without strips, and research has focused on the reduction of nutrient runoff and erosion in contoured agricultural landscapes (Schulte et al., 2017). By directly comparing responses in fields with prairie strips with responses in fields managed with other practices, we can better identify combinations of prairie strips and crop management that may optimize crop yield, biodiversity, and ecosystem services.

We address how targeted conservation can promote multifunctionality including biodiversity, agricultural production, and other ecosystem services. Working in a 33-year-old experiment, we tested the effects of prairie strips and a gradient of crop management strategies across a suite of invertebrate biodiversity metrics and ecosystem services during the first 2 years of prairie strip establishment. First, we asked: what are the effects of newly established prairie strips on the spillover of biodiversity and ecosystem services into cropland? To test this, we measured how services changed with distance from a prairie strip. Second, we asked: how does time since prairie strip establishment affect biodiversity and ecosystem services in agricultural plots? Third, we asked: what are the tradeoffs and synergies among biodiversity conservation, non-provisioning ecosystem services, and crop yield across a gradient of land use intensity? To answer our third question, we examined all services across a land use gradient (including treatments with prairie strips) in relation to yield changes. Our study includes biodiversity measurements of ants, butterflies, dung beetles, and spiders, all of which can provide ecosystem services to farms. Our study also includes ecosystem service measurements of microbial activity, decomposition, greenhouse gas emissions, pollination, soil carbon, and crop yield.

MATERIALS AND METHODS

Study Sites and Sampling Locations

We conducted our study at the Kellogg Biological Station Long-Term Ecological Research (KBS LTER) site in Hickory Corners, Michigan, United States (occupied Anishinaabe land) that was established in 1987. The KBS LTER is located in a temperate climate with a mean temperature of 10.1°C and mean annual precipitation of 100.5 cm (1981–2011 means) with increasing trends in temperature over the past few decades (Robertson and Hamilton, 2015; Liang and Robertson, 2021). Surface soils are 17% clay/43% sand Alfisol loams developed on glacial till and outwash (Robertson and Hamilton, 2015).

This study occurred in 2019–2020, the first 2 years after prairie strip planting. We worked in five treatments of KBS-LTER's Main Cropping System Experiment (MCSE): conventionally managed row crops, no till row crops, reduced input row crops, biologically based (organic) row crops, and conservation land [details of crop management treatments are compared in **Figure 1** and in Robertson and Hamilton (2015)]. The experiment consisted of six replicated plots of each treatment (six experimental blocks; **Supplementary Figure 1a**), with each plot having an area of one hectare (87 m × 105 m; **Supplementary Figure 1b**). *Conventional* and *no till* treatments received levels of chemical inputs that follow Generally Accepted Agricultural and Management Practices (GAAMP) in Michigan, United States and are typical for the US Midwest (details on the dates and quantities of fertilizer application, pesticide application, weed management, and soil preparation can be found at https://aglog.kbs.msu.edu). The conventional treatment was tilled with a chisel plow, and the no till treatment was managed as the conventional treatment but was left unplowed. The *reduced input* treatment received lower levels of inputs (nitrogen at planting and pesticides) than conventional and no till and had a legume cover crop in the winter. The *biologically based* treatment did not receive any chemical inputs, compost, or manure, and it had a legume cover crop and was rotary hoed five times after planting in 2020 to control weeds. *Conservation land* (referred to as *early successional* in site maps and earlier publications from this experiment) was unmanaged other than yearly burning in the spring to suppress woody vegetation. This treatment was a grassland with the dominant bloom period in the fall when goldenrods and asters flower. All treatments except conservation land were on a 3-year maize (*Zea mays* L.)—winter wheat (*Triticum aestivum* L.)—soybean (*Glycine max* L.) rotation. In the years of our study, wheat was planted in 2019 and maize in 2020.

Prairie strips were introduced in the reduced input and biologically based treatments in April 2019. In five percent of each plot, configured as a strip parallel to row crops down the middle of each plot, we sowed a native prairie plants species mix. The mix consisted of 4 grass species and 18 forb species (**Supplementary Table 1**) purchased from Native Connections, Kalamazoo, Michigan, United States. The mix was chosen to have species bloom throughout the growing season (Isaacs et al., 2009). The mixes sown in each plot contained the same weight and proportion of each species. The first year's plant community was dominated by agricultural weeds, but with some seeded species flowering. The second year's plant community contained more seeded species, and we expect more seeded species to establish as the prairie strips mature. Prairie strips were mowed three times during the 2019 season to reduce weeds and support establishment of native seeds.

We compared a variety of biodiversity and ecosystem service measures by sampling three sets of sampling locations within the plots (**Supplementary Figure 1b**). First, to compare among plot-level treatments, each plot had five sampling locations distributed throughout, all located outside of prairie strips. These sampling locations are referred to as Standard Sampling Stations. Second, also to compare among plot-level treatments, each plot had six sampling stations at the northeast corner for destructive sampling that could not occur at the Standard Sampling Stations. These sampling locations are referred to as Subplot Sampling Stations. Third, reduced input and biologically based treatments had an additional three transects perpendicular to the prairie strips with sampling locations at distances of 0, 1, 5, and 20 m from the prairie strip (the station at 0 m was located within the prairie strip). These sampling stations were used to measure biodiversity and ecosystem services at different distances from the prairie strips and are referred to as Strip Sampling Stations.

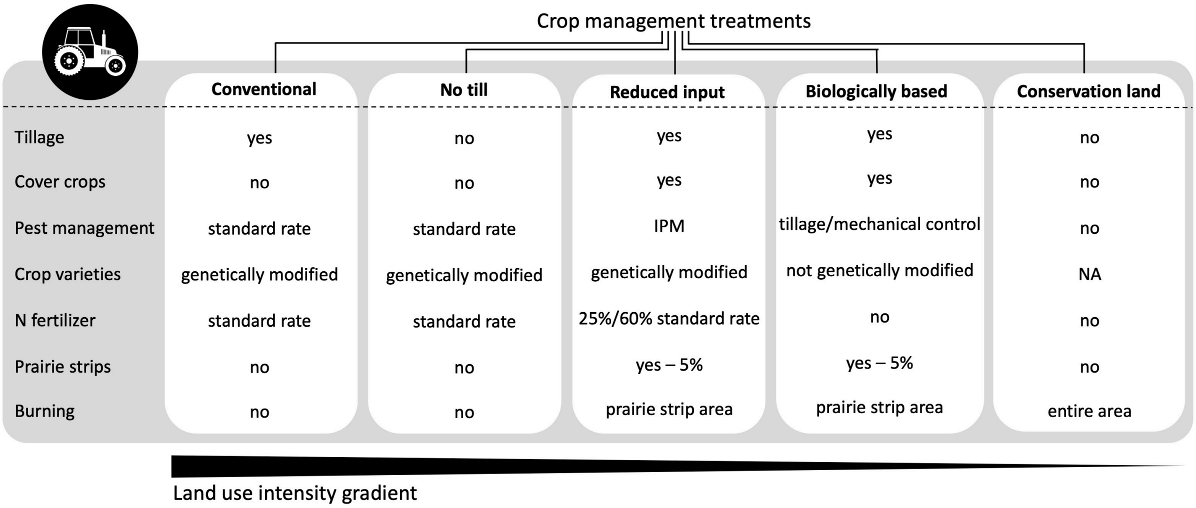
Because prairie strips were implemented in all replicates of the reduced input and biologically based treatments, we do not have a fully factorial experiment. Therefore, we cannot isolate the effect of prairie strips from effects of crop management and year on measures of biodiversity and ecosystem services across all crop management treatments. However, we can (i) make conclusion about how treatments with prairie strips, in the context of their associated management strategies (including historical data on these treatments), compare to other crop management strategies without prairie strips, (ii) examine trends of prairie strips with time since establishment, where increases from year to year would suggest prairie strips play a role for processes that stabilized with the background management treatments (though are confounded by crop rotation), and (iii) attribute significant effects of distance from prairie strips on response variables to the presence of the strip, though benefits of prairie strips are not isolated to spillover effects.

Dung Beetles

Soil dwelling macroarthropods contribute to decomposition in agricultural landscapes by fragmenting litter, altering soil structure, and feeding on other soil dwelling fauna. Dung beetles break apart manure, mobilize nutrients in the soil (Coleman et al., 2018a), and suppress human and livestock pathogens (Nichols et al., 2008; Jones et al., 2016; Sands and Wall, 2017). Dung beetle communities are vulnerable to the effects of crop management and are negatively affected by agricultural intensification (Barbero et al., 1999; Hutton and Giller, 2003).

Across Management Treatments

To compare dung beetle diversity (as well as ants and spiders which are described next) across crop management treatments, we installed pitfall traps at Subplot Sampling Stations within each plot. Pitfall traps consisted of plastic containers (5.1 cm diameter, 120 mL) buried so the container's rim was flush with the soil surface. We partially filled containers with 95% ethanol mixed with a few drops of detergent to break surface tension. To protect the traps from rain and flooding, we mounted clear Plexiglass rain covers (15 × 15 cm) 10 cm above the ground over each trap. We baited traps with approximately 10 g of cow



Crop management treatments

	Conventional	No till	Reduced input	Biologically based	Conservation land
Tillage	yes	no	yes	yes	no
Cover crops	no	no	yes	yes	no
Pest management	standard rate	standard rate	IPM	tillage/mechanical control	no
Crop varieties	genetically modified	genetically modified	genetically modified	not genetically modified	NA
N fertilizer	standard rate	standard rate	25%/60% standard rate	no	no
Prairie strips	no	no	yes – 5%	yes – 5%	no
Burning	no	no	prairie strip area	prairie strip area	entire area

FIGURE 1 | Characteristics of experimental crop management treatments. Standard rate refers to Generally Accepted Agricultural and Management Practices. IPM (Integrated Pest Management) in the reduced input treatment refers to a combination of extra tillage (maize years), narrow row spacing (soy years), and reduced herbicide use, mostly related to the number of residual herbicides. Fungicides and pesticides are applied as problems arise and severity is assessed. Also regarding reduced input, N fertilizer is applied at 25% of the standard rate for maize, and at 60% of the standard rate for wheat.

manure per trap that was collected from the Kellogg Pasture Dairy Farm (located approximately 0.4 km from experimental sites) and homogenized by stirring in a bucket. Cows were treated with an ingested larvicide for fly control (unpublished data shows this had no effect on abundance and richness of dung beetles collected or manure decomposition). We deployed pitfall traps at all Subplot Sampling Stations three times during the growing season in 2019, once each in June, July, and August. For each sampling event, traps were collected after 48 h in the field and the samples collected from the traps were stored in ethanol at −20°C. Dung beetles (Coleoptera: Scarabaeidae of the subfamily Scarabaeinae) were identified to species using a regional guide (Nemes and Price, 2015).

Distances From Prairie Strips

To measure dung beetle richness at distances from prairie strips, baited pitfall traps were deployed at Strip Sampling Stations for each of three sampling rounds each year occurring in June, July, and August of 2019 and 2020.

Ants

Ants comprise half of global insect biomass and perform many ecosystem services (Hölldobler and Wilson, 1990; Folgarait, 1998; Wills and Landis, 2018). Ants are the major predators of agricultural pests at our study site and elsewhere in the US Midwest (Grieshop et al., 2012; Wills et al., 2019; Helms et al., 2020). They also disperse plant material, seeds, and nitrifying bacteria and pool nutrients in the soil (Mueller et al., 2005; Hölldobler and Wilson, 2009; Benckiser, 2010). Ants are sensitive to harvesting and management practices that can reduce ant activity (Peck et al., 1998; Agosti et al., 2000; Wodika et al., 2014; Helms et al., 2021; Hussain et al., 2021).

Across Management Treatments

We collected ants across crop management treatments using baited pitfall traps at Subplot Sampling Stations as described for dung beetles. Captured ants were identified using regional guides (Coovert, 2005; Ellison et al., 2012) and vouchers were stored in the senior author's reference collection.

To test effects of treatments on species richness, we first combined species occurrences from all repeated pitfall traps (maximum of 3 traps per each of 6 sampling stations, 16–18 total pitfall traps per plot). The occurrence of workers of a given species at least once at any of the 6 sampling stations was conservatively treated as indicating the presence of a single colony of that species (abundance = 1) within a plot during the study year, regardless of how many or how frequently workers were captured (Ellison et al., 2007; Gotelli et al., 2011). In this way, we derived one species list for each of the 30 plots (6 plots per each of 5 treatments).

Distances From Prairie Strips

To measure ant richness at different distances from prairie strips, ants were collected with non-baited pitfall traps at the Strip Sampling Stations (reduced input and biologically based treatments). Non-baited traps were sampled on a rolling weekly basis (3 weeks on, one week off during which baited traps were deployed) from May to September with a total of five sampling rounds per station in 2019 and four in 2020. 2019 prairie strip ant data are modified from those used in Helms et al. (2021).

Spiders

Spiders are generalist predators that can contribute to pest control in agricultural landscapes. Spider communities generally respond positively to agricultural conservation practices, such as cover crops and reduced tillage (Sunderland and Samu, 2000;

de Pedro et al., 2020). Increased natural habitat in an agricultural landscape can increase spider abundance and richness, but there is little evidence of spillover of spiders from natural habitat into cropland (Sunderland and Samu, 2000; Schmidt et al., 2005, but see Hussain et al., 2021).

Across Management Treatments

We collected spiders across crop management treatments as described for ants. Spiders were identified to family with a key to spiders of North America (Ubick et al., 2017).

Distances From Prairie Strips

Pitfall traps were used to collect spiders at distances from the prairie strips as described for ants.

Butterflies

Butterflies are diverse pollinators, herbivores, and indicators of insect response to habitat change, and they hold cultural value (Ghazanfar et al., 2016). Butterflies are declining in abundance at a rate of 2% per year in the US Midwest with agriculture as a main reason for this decline due to habitat loss, pesticides, and fertilizers, which are sources of direct mortality and destroy host plants and food resources (Wepprich et al., 2019; van Klink et al., 2020). Reducing the use of pesticides and fertilizer and restoring habitat on farms helps mitigate the loss of butterfly biodiversity in agricultural landscapes (Reeder et al., 2005; Rundlöf et al., 2008).

Across Management Treatments

Unlike samples for all the other species and services, we sampled butterfly species richness and abundance using transect counts, modified from Pollard (1977). We conducted surveys along a 12-min one-way walking transect through each plot. Observers recorded butterflies within 5 m on both sides and above the transect in front of the observer. Transects were surveyed between 10:00 a.m. and 4:00 p.m. weekly from June 2019 to September 2019 and May 2020 to September 2020. Butterflies were identified to species using a regional guide (Nielsen, 1999) and supplementary sources as needed.

Active Carbon

Biologically available soil carbon, also termed "active carbon," reflects a fraction of total soil carbon that is readily mineralized by soil microorganisms and serves as an early indicator of longer-term soil carbon accrual (Culman et al., 2012; Coleman et al., 2018b). Conversion of agricultural fields to perennial vegetation has been shown to increase soil active carbon compared to conventionally managed agricultural soils by increasing the production of fine root biomass (Sprunger et al., 2017; Sprunger and Robertson, 2018). We expect prairie strips to increase levels of active carbon and for active carbon to spill over from prairie strips into cropland at short distances from prairie strips if roots from perennials extend into cropland, if nitrogen from farming doesn't reach to exactly the edge of the prairie strip, or if litter from prairie strips spills over into cropland.

Across Management Treatments

Active carbon was determined *via* a 24-h assay based on Franzluebbers et al. (2000) that measures CO_2 respired from soils rewetted to a common water holding capacity. We collected soil cores at the Standard Sampling Stations in June, July, and August of 2019. Samples were analyzed individually for active carbon, then data were pooled across June, July and September to form a single dataset for each year in each treatment. We collected field soil with a soil push probe at 0–10 cm depth then sieved to 2 mm. Soil water holding capacity (WHC) and gravimetric soil moisture were determined from fresh sieved soil. We added 5 g of air-dried soil and sterile ultrapure water to a 125 mL Wheaton serum bottle to achieve 70% WHC. Bottles were sealed and incubated at room temperature for 24 h. We collected gas samples from bottle headspace at two time points following the incubation period (0 and 24 h). CO_2 samples were collected in overpressurized 6 mL glass vials (Exetainers, Labco Ltd., Lampeter, Wales) flushed with N_2. We analyzed samples with a gas chromatograph (Agilent 7890A) coupled to an autosampler (Gerstel MPS2XL) as described in Shcherbak and Robertson (2019).

We calculated short-term mineralizable C as the difference between 0 and 24-h CO_2 measurements. We report active carbon in micrograms (μg) of CO_2 per day per g of dry soil.

Distances From Prairie Strips

Soil cores were collected at Strip Sampling Stations and processed as described above in both 2019 and 2020.

Decomposition

Decomposition is essential for suppressing pathogens, cycling nutrients, and creating soil organic matter (Barrios, 2007; Coleman et al., 2018c). Diversified landscapes can increase decomposition by increasing the abundance and richness of beneficial soil fauna (Landis et al., 2000; Karp et al., 2016; Jones et al., 2019). To quantify decomposition services, we measured mass loss of manure over time.

Across Management Treatments

We placed one patty of fresh cow manure (20 g) at each of the Subplot Sampling Stations (Jones et al., 2019). Manure (fresh) was weighed in the lab and separated into individual packets prior to deployment. We left manure under a rain cover (same rain cover as described for ants) for 7 days immediately following pitfall trap collection of dung beetles for all sampling rounds. We then collected manure in an envelope, placed the envelope in a drying oven until moisture evaporated, and then weighed it. The dry weight after deployment was divided by the dry weight of the manure (20 g of fresh manure was equivalent to 6 g dried manure). We defined decomposition as the proportion of manure removed.

Distances From Prairie Strips

We placed sentinel cow manure patties (20 g) at the Strip Sampling Stations and processed samples as described above.

Global Warming Impact

Agriculture produces 10–14% of global anthropogenic greenhouse gas emissions (Barker et al., 2007; Smith et al., 2007). Prairie strips, no till management, and cover crops are among management practices that have the potential to sequester carbon in cropping systems (Robertson et al., 2000; Gelfand and Robertson, 2015). We use 100-year global warming impact (GWI) as a measure to convert greenhouse gas emissions (N_2O and CH_4) to units of CO_2 equivalent emissions.

Across Management Treatments

We sampled greenhouse gas fluxes per Kahmark et al. (2020) approximately biweekly May–September and monthly October–April in both 2019 and 2020 using a stainless-steel gas chamber (14.3 cm radius, 22.8 cm height) with a plastic lid. After placing the lid on the chamber, a needle was inserted into the chamber lid septum to relieve any induced pressure changes. We inserted another individual needle into the septum of a 5.9-mL exetainer sample vial to act as a vent. Then we mixed the chamber headspace three times with a 10-mL sampling syringe. After mixing, we withdrew 10-mL and injected the air into the sample vial with the vent needle in place. After flushing the vial three times, we removed the vent needle, drew a 10-mL sample from the chamber, and injected it into the flushed sample vial (so that it was overpressurized). We collected a sample of ambient air at the same time in each sampling round and also a duplicate chamber sample using the same gas sampling procedure described above. We also recorded soil temperature and moisture next to the gas chamber during the sampling period. We collected four gas samples at 15-min intervals over each sampling period. Post gas sampling and flux calculations were conducted following the protocol of Holland et al. (1999).

Pollination

Pollinators are necessary for the function of natural and managed ecosystems. Pollinators have experienced a steep decline in abundance and richness, and prairie strips could restore pollinators and their services to agricultural landscapes (Intergovernmental Platform on Biodiversity and Ecosystem Services [IPBES], 2016; Wepprich et al., 2019; van Klink et al., 2020; Kordbacheh et al., 2020).

Across Management Treatments

Pollination was measured with sentinel plants placed at the Standard Sampling Stations in the conventional, no till, and conservation land treatments, as well as at the Strip Sampling Stations in the reduced input and biologically based treatments. We used Black-eyed Susans (*Rudbeckia hirta*) as our sentinel plants, as it is native to southwest Michigan and was also included in the prairie strip seed mix. We propagated plants from seed (purchased from the same location as the prairie strip seed mix) in a greenhouse. Seedlings were transplanted into 16.5 cm pots with a low dose of 12-12-12 N-P-K controlled release organic fertilizer. To avoid pollination prior to receiving experimental treatment, we marked and covered two flower heads on each plant with pollinator exclusion bags just before they started producing pollen. The following day, we deployed plants with exclusion bags on both flowers into the field. Upon placement in the field, we removed one bag to be exposed to pollinators (called open flowers). One bag remained over the flower through the duration in the field (called closed flowers). Closed flowers acted as a measure of potential self-pollination. We deployed plants in experimental treatments for 14 days during each of the three sampling rounds starting on June 18, 2019, August 7, 2019, and July 8, 2020. We bagged all experimental flower heads prior to removal from experimental stations. For analysis of pollination across treatments, we used all plants from Standard Sampling Stations in conventional, no till, and conservation land, and we randomly selected five plants from the Strip Sampling Stations in the reduced input and biologically based treatments to compare consistently among all treatments.

Following experimental deployment, we returned plants to a greenhouse where they senesced and set seed. Seeds were then harvested and stored in a refrigerator from September to January each year. We randomly selected 30 seeds from each flower head for a germination trial. We placed these seeds in petri dishes in a greenhouse, watered them regularly over a 14-day period, and counted the number of individuals that germinated. We calculated seedset as the ratio of not-germinated:germinated seeds for each seed head (two measures per plant). We measured pollination services as the difference between seedset of the open flower and seedset of the closed flower for each plant (one measure per plant).

Distances From Prairie Strips

The sentinel plants from the Strip Sampling locations were used to measure pollination services at distances from the prairie strips.

Soil Organic Carbon

Soil organic carbon (SOC) is a measure of total carbon in soil organic matter. Agricultural management practices influence SOC accrual and loss. SOC accrual can be stimulated by the addition of high-quality organic inputs like cover crops (Syswerda et al., 2011), as well as the establishment of perennial vegetation (Kravchenko et al., 2019). On the other hand, practices that involve physical soil disturbance, such as tillage, generally reduce SOC by disrupting soil aggregates and releasing organic matter for decomposition (Paul et al., 2015). Whereas active carbon responds quickly to land management changes, SOC generally responds on the order of years to decades, as it is a measure of total carbon across both labile and recalcitrant soil organic matter pools (Culman et al., 2012).

Across Management Treatments

We collected one soil core with a soil push probe at each Standard Sampling Station in April 2019 and May 2020 at a depth of 0–25 cm. Soil cores within each plot (5 stations per plot) were combined into a pooled sample. We air dried soil samples and then pulverized them to a powder using a Shatterbox grinding mill. We then weighed soil samples (15–20 mg) and packed them into tins. Samples were analyzed for total carbon in triplicate (three soil tins for each sample) on a Costech Elemental Combustion System 4010. Because these soils did not contain carbonates, we express these data as percent SOC.

Crop Yield

Across Management Treatments

Crops were harvested from the entire crop area of each plot across all agronomic treatments. Prairie strips were not harvested, but we area-scaled yields in the reduced input and biologically based treatments by reducing yields 5% to account for area in strips. Wheat was harvested from conventional and no till plots on July 24, 2019 and from reduced input and biologically based plots on July 25, 2019. Maize was harvested from all treatments and plots on October 29, 2020. Crops were harvested with a harvest combine, and yield for the entire crop area of each plot was measured with a weigh wagon. We report yield as kg/ha at crop harvest at standard moisture content (13% for wheat, 15.5% for maize). We did not compare yield with the conservation land treatment, although perennial grasslands have potential to be harvested for bioenergy (Robertson et al., 2017).

In addition to measuring crop yields for 2019 and 2020, we separately compared historical crop yields in the same plots prior to the sowing of prairie strips. We used yield measurements from 2013 to 2018—two cycles of the crop rotation prior to prairie strips.

Statistical Analyses

Across Management Treatments

We aggregated the individuals of our measures of invertebrate biodiversity (ants, butterflies, dung beetles, and spiders) surveyed over each year within each plot of each treatment (six plots for each of five treatments) by summing. For all measures of biodiversity and ecosystem services, we calculated effect sizes of no till, reduced input, biologically based, and conservation land treatments relative to the conventional treatment. We measured the Hedge's *g* effect size and 95% confidence intervals using the "compute.es" package in R (Del Re, 2013). The conventional treatment served as the baseline, which does not include a confidence interval.

To determine the differences in arthropod richness (ants, butterflies, dung beetles, and spiders) across treatments, we used generalized linear mixed effects models with normal distributions. All model assumptions were met. Richness was used as the response variable; main effects were treatment and year (except for ants which were sampled in only one year), and the random effect was experimental block. We calculated the estimated species richness of butterflies within each replicate of each treatment per year using the R package "iNext" with Chao1 abundance-based rarefaction (Chao et al., 2014; Hsieh et al., 2016). We used measures of raw richness for ants, dung beetles, and spiders; we recognize that abundance affects richness for these measures, but because our abundances were low, rarefaction was not possible.

To determine the differences in arthropod abundances across treatments, we used generalized linear mixed effects models constructed similarly but with negative binomial distributions (except for ants for which we used a normal distribution to meet model assumptions). R package "lme4" was used to construct the models (Bates et al., 2015). An ANOVA followed by a Tukey test was used for *post hoc* analyses for all models using R packages "car" (Fox and Weisberg, 2019) and "multcomp," respectively (Hothorn et al., 2008). The same method was used for all measured ecosystem services including crop yield (except GWI), but with the measure of the service as the response variable and sampling round included as a fixed effect when multiple sampling rounds occurred within a year (decomposition and pollination). We also modeled crop yield independently for each year using the same method to measure relative yields among treatments for each crop. For GWI, we constructed a generalized linear mixed effects model with log transformed CO_2 as the response variable, treatment, year, sampling round, and temperature as fixed effects, and experimental block as the random effect.

Distances From Prairie Strips

We aggregated the individuals of our measures of invertebrate biodiversity (ants, dung beetles, and spiders) collected over each year within each distance of each plot of each treatment (four distances for each of six plots for each of two treatments) by summing. To determine the effect of distance from prairie strip and year on measures of arthropod richness (ants, dung beetles, and spiders), we constructed a generalized linear mixed effects model with a normal distribution. Richness was the response variable, crop management treatment, distance from prairie strip, and year were fixed effects with an interaction between distance and year, and experimental block was a random effect. We followed this with an ANOVA. The same method was used for measures of abundance, but with a negative binominal distribution (except ants for which we used a normal distribution). We constructed similar models with normal distributions for measures of ecosystem services but included sampling round as a fixed effect when relevant (decomposition and pollination). Distance was treated as a continuous variable. To test if trends were occurring across distances from the prairie strips, or if they were driven solely by high values within the prairie strips, we also ran analyses for all measures with datapoints at 0 m removed.

RESULTS

Dung Beetles

Across Management Treatments

We collected a total of 553 dung beetles in Standard Sampling Plots (**Supplementary Table 2**). The effect sizes of dung beetle richness across all treatments did not differ from baseline (**Figure 2**). Species richness did not differ among treatments, however, dung beetle abundance was 128–992% higher in conservation land than in all other treatments (**Figure 3** and **Table 1**).

Distances From Prairie Strips

We collected a total of 284 dung beetles at Strip Sampling Stations (**Supplementary Table 2**). There was no relationship between distance and dung beetle richness, but dung beetle richness was higher in 2020 than in 2019 (**Figure 4** and **Table 2**). There was an interaction between year and distance from prairie strip for dung beetle abundance, with 2019 having no relationship with distance

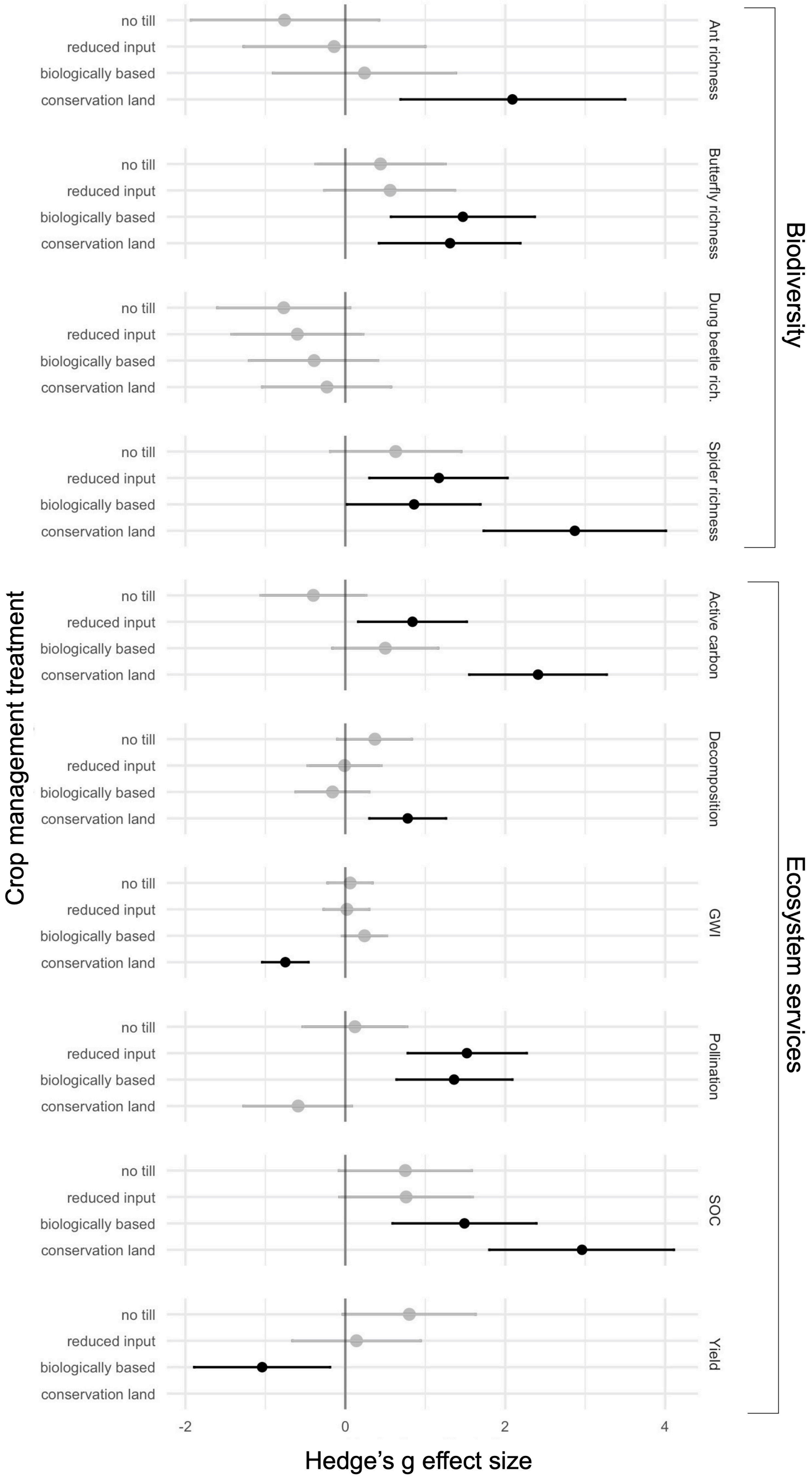


FIGURE 2 | Hedge's *g* effect size (black and gray dots) with 95% confidence interval. The baseline is the conventional management treatment. Negative values are effect sizes lower than that of the baseline, and positive values are effect sizes higher than the baseline. Values that cross zero are shaded gray. Note that lower levels of global warming impact (GWI) would be a more positive ecosystem service. Conservation land was not harvested, therefore crop yield is not applicable for that treatment.

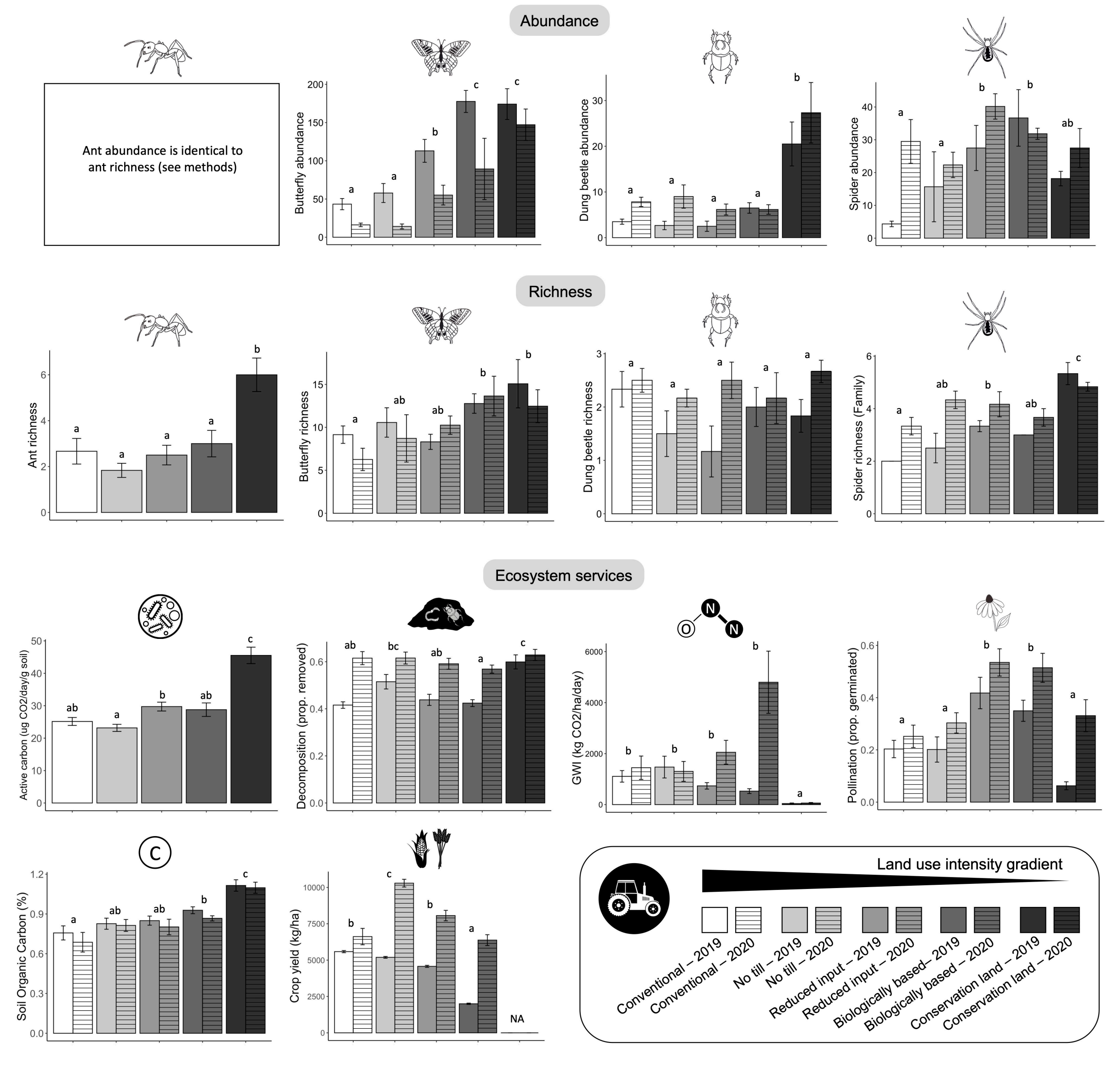


FIGURE 3 | Effects of crop management treatment on measures of biodiversity and ecosystem services between years 2019 and 2020. For measures of biodiversity, bars depict means over an entire year; for measures of ecosystem services, bars depict means for each sampling round. Letters denote statistical differences among treatments. Error bars show standard errors. Ants and active carbon were only measured in 2019.

from strip and 2020 abundance decreasing with distance from strip (**Figure 4** and **Table 2**). When 0 m samples were removed, there was no effect of distance or year on dung beetle abundance (distance: $\chi^2 = 0.6$, $df = 1$, $p = 0.4$; year: $\chi^2 = 0.5$, $df = 1$, $p = 0.8$) or richness (distance: $\chi^2 = 0.4$, $df = 1$, $p = 0.53$; year: $\chi^2 = 1.7$, $df = 1$, $p = 0.2$), meaning the linear trend of abundance was driven by high dung beetle abundance in the prairie strips.

Ants

Across Management Treatments

We collected a total of 1821 worker ants from the Subplot Sampling Stations (**Supplementary Table 3**). The Hedge's g effect sizes of ant richness in conservation land were higher than the baseline (**Figure 2**). Species richness was higher in conservation land treatments than in all row crop treatments (**Figure 3** and **Table 1**). Ant abundance was the same as ant richness because abundance of any particular species could only be 0 or 1 at a single sampling station, and sampling stations were summed per plot per year (see methods).

Distances From Prairie Strips

We collected a total of 3218 ants from the Strip Sampling Stations (**Supplementary Table 3**). Ant species richness did not vary by year nor with distance from the prairie strip (**Figure 4** and **Table 2**). Ant abundance did not differ by distance from prairie strip, but differed by year, decreasing from 2019 to 2020 (**Figure 4** and **Table 2**).

TABLE 1 | Effects of crop management treatments on measures of biodiversity and ecosystem services treatments.

		Treatment			Year		
Measure	**Unit**	χ^2	*df*	*p*	χ^2	*df*	*p*
Ant abundance	Number of individuals	42.6	4	**<0.01**	NA	NA	NA
Ant richness	Number of species	42.6	4	**<0.01**	NA	NA	NA
Butterfly abundance	Number of individuals	223.9	4	**<0.01**	67.3	1	**<0.01**
Butterfly richness	Species richness (chao1)	19.3	4	**<0.01**	0.8	1	0.4
Dung beetle abundance	Number of individuals	95.9	4	**<0.01**	15.2	1	**<0.01**
Dung beetle richness	Number of species	6.4	4	0.2	12.2	1	**<0.01**
Spider abundance	Number of individuals	26.9	4	**<0.01**	17.1	1	**<0.01**
Spider family richness	Number of families	48.0	4	**<0.01**	13.1	1	**<0.01**
Active carbon	μg CO_2/day/g	144.4	4	**<0.01**	NA	NA	NA
Decomposition	Proportion removed	40.8	4	**<0.01**	86.6	1	**<0.01**
GWI	kg CO_2/ha/day	117.5	4	**<0.01**	3.7	1	0.05
Pollination	Proportion germinated	70.2	4	**<0.01**	32.0	1	**<0.01**
SOC	Soil C weight%	102.9	4	**<0.01**	2.7	1	0.1
Crop yield	kg/ha	68.3	3	**<0.01**	130.7	1	**<0.01**

Bolded p-values indicate statistically significant measures.

Spiders

Across Management Treatments

We collected a total of 1522 spiders from Subplot Sampling Stations (**Supplementary Table 4**). The effect size of spider richness for treatments with prairie strips and the conservation land treatment were higher than baseline; no till did not differ from the baseline (**Figure 2**). Spider richness increased from conventional to no till and biologically based, to reduced input, to conservation land (**Figure 3** and **Table 1**). Spider abundance was highest in the treatments with prairie strips followed by conservation land and no till, and lowest in conventional (**Figure 3** and **Table 1**).

Distances From Prairie Strips

We collected a total of 3626 spiders from Strips Sampling Stations (**Supplementary Table 4**). There was an interaction between distance from prairie strip and year to explain spider richness and abundance, with 2019 having no change in richness and abundance with increasing distance and 2020 having a decrease in richness with increasing distance (**Figure 4** and **Table 2**).

Butterflies

Across Management Treatments

We visually identified 5329 butterflies during transect counts (**Supplementary Table 5**). The effect sizes of butterfly richness were higher than the baseline of conventional in the biologically based and conservation land treatments; no till and reduced input did not differ from baseline (**Figure 2**). Butterfly richness was the highest in the conservation land and biologically based treatments followed by reduced input and no till treatments, and lowest in conventional (**Figure 3** and **Table 1**). Giant Swallowtails, Checkered Skippers, and Red Spotted Purples were species identified in treatments with prairie strips that were never observed in conventional or no till treatments. Butterfly abundance was 134–349% higher in treatments with prairie strips than crop treatments without prairie strips (conventional and no till; **Figure 3**).

Active Carbon

Across Management Treatments

The effect sizes of active carbon in the reduced input and the conservation land treatments were higher than the baseline; no till and biologically based did not differ from the baseline (**Figure 2**). Active carbon was highest in the conservation land treatment, lowest in the no till treatment, and intermediate in the reduced input, biologically based, and conventional (**Figure 3** and **Table 1**).

Distances From Prairie Strips

Distance from prairie strip and year had significant effects on active carbon, with 2019 having higher active carbon than 2020, and 2019 having a decrease in active carbon with distance from prairie strip (**Figure 4** and **Table 2**). In the crop area alone (0 m datapoints removed from analyses), there was a significant interaction between distance and year with a decrease in active carbon with increasing distance from prairie strips in 2019 and no change with distance from prairie strip in 2020 ($\chi^2 = 4.9$, $df = 1$, $p < 0.05$).

Decomposition

Across Management Treatments

The effect size of decomposition in conservation land was higher than the baseline; no other treatment differed from the baseline (**Figure 2**). Conservation land and no till treatments had the highest rates of decomposition, followed by conventional and reduced input, and then by biologically based (**Figure 3** and **Table 1**).

Distances From Prairie Strips

There was an interaction between year and distance from prairie strip, with 2019 having a lower rate of decomposition and no

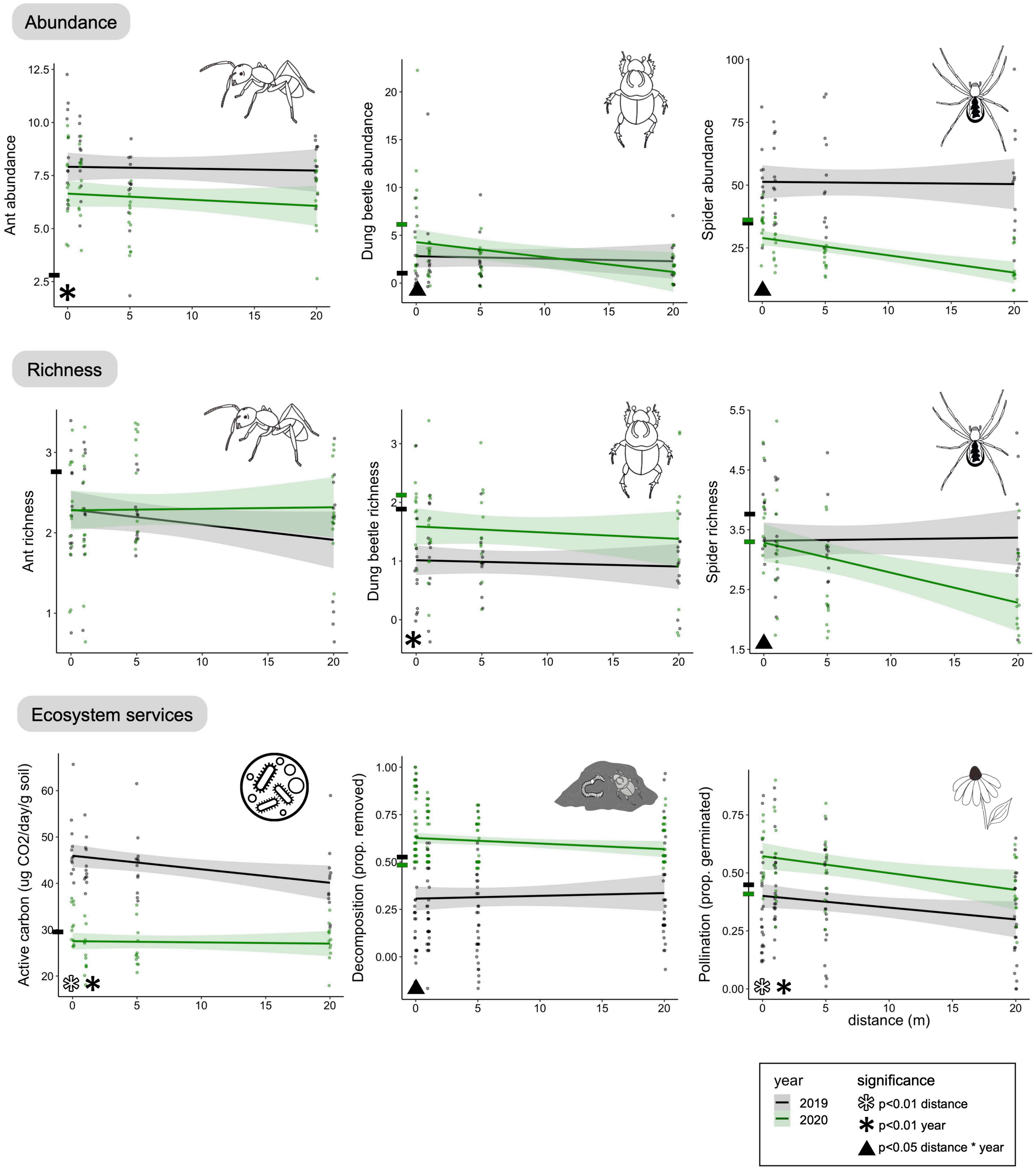


FIGURE 4 | Effects of distance from prairie strips on measures of biodiversity and ecosystem services in both reduced input and biologically based treatments. We considered linear effects of distance from prairie strips. The interaction with year is presented, with 2019 being the first year of prairie strip implementation and a wheat year, and 2020 being the second year and a maize year. Distance 0 m is within the prairie strip. Black dashes on the y-axis represent the mean of the plot level values for reduced input and biologically based for that measure in 2019; green dashes represent 2020. Ant data from 2019 is modified from Helms et al. (2021).

change with distance from prairie strip and 2020 having a higher rate of decomposition with a decreasing trend with increasing distance from prairie strip (**Figure 4** and **Table 2**).

Global Warming Impact

Across Management Treatments

The effect size of GWI in conservation land was lower than the baseline; no other treatments differed from the baseline (**Figure 2**). GWI in the conservation land treatment was at least 25× lower than all other treatments (**Figure 3** and **Table 1**). Methane tended to be consumed in the soils rather than emitted to the atmosphere across all land use types. Fluxes in conservation land across 2019 and 2020 were lowest at -2.68 ± 2.46 (mean ± SD) g CH_4-C ha^{-1} day^{-1}, while those in the biologically based treatment were highest at -0.79 ± 1.78 g CH_4-C ha^{-1} day^{-1}. Similarly, N_2O fluxes were lowest in conservation land and highest in the biologically based

TABLE 2 | Effects of crop management treatments on measures of biodiversity and ecosystem services treatments.

	Distance			Year			Distance × Year		
Measure	χ^2	*df*	*p*	χ^2	*df*	*p*	χ^2	*df*	*p*
Ant abundance	0.09	1	0.9	8.4	1	**<0.01**	0.2	1	0.6
Ant richness	2.8	1	0.1	0.004	1	0.9	1.7	1	0.2
Dung beetle abundance	0.3	1	0.6	5.9	1	**<0.01**	4.6	1	**<0.05**
Dung beetle richness	0.2	1	0.7	8.8	1	**<0.01**	0.08	1	0.8
Spider abundance	0.06	1	0.8	48.7	1	**<0.01**	13.5	1	**<0.01**
Spider family richness	0.03	1	0.86	0.02	1	0.9	6.2	1	**<0.01**
Active carbon	8.2	1	**<0.01**	155.1	1	**<0.01**	3.4	1	0.06
Decomposition services	1.3	1	0.2	33.3	1	**<0.01**	6.1	1	**<0.01**
Pollination services	6.0	1	**<0.01**	40.9	1	**<0.01**	0.4	1	0.5

Bolded p-values indicate statistically significant measures.

treatment at 0.34 ± 0.51 and 7.27 ± 17.13 g N_2O-N ha^{-1} day^{-1}, respectively.

Pollination

Across Management Treatments

The effect size of pollination was higher in reduced input and biologically based treatments than the baseline (**Figure 2**). Pollination rates were 72–222% higher in the treatments with prairie strips than all other treatments (**Figure 3** and **Table 1**).

Distances From Prairie Strips

Pollination services decreased with distance from prairie strip, and pollination services increased from 2019 to 2020 (**Figure 4** and **Table 2**).

Soil Organic Carbon

Across Management Treatments

Soil organic carbon was higher than baseline in biologically based and conservation land treatments (**Figure 2**). SOC was lowest in conventional, intermediate in the no till, reduced input, and biologically based treatments, and highest in conservation land treatment (**Figure 3** and **Table 1**).

Crop Yield

Across Management Treatments

The effect size was lower than baseline in the biologically based treatment but did not differ from the baseline for no till and reduced input treatments (**Figure 2**). When including both wheat and maize in analyses, crop yield was highest in the no till treatment, intermediate in the conventional and reduced input treatments, and lowest in the biologically based treatment (**Figure 3** and **Table 1**). Crop yield was scaled to include the land area of prairie strips in analyses (kg/ha measurements include area of the prairie strips). These relative crop yields across treatments were consistent with the previous two crop rotations where, across all the whole crop rotation, crop yields were highest in no till, intermediate in conventional and reduced input (although conventional and no till were not significantly different), and lowest in the biologically based treatment.

When considering the wheat and maize years independently, the pattern among treatments changed from the previous two crop rotations. When measuring just the wheat year (2019), crop yield was lowest in the biologically based, followed by the reduced input treatment, then no till, then conventional (χ^2 = 1921.2, *df* = 3, $p \leq 0.01$). Historically (2013–2017), however, wheat yield was lowest in biologically based but did not differ among other crop treatments. When measuring just the maize year (2020), crop yield was lowest in the biologically based and conventional, intermediate in the reduced input, and highest in no till (χ^2 = 70.3, *df* = 3, $p < 0.01$). This also differs from the previous two crop rotations, where maize yield was lowest in biologically based, intermediate in reduced input and conventional, and highest in no till. Therefore, maize yields in the conventional treatment in 2020 were low compared to the previous two crop rotations. The third crop in the rotation, soy, historically was lowest in conventional and biologically based and highest in no till and reduced input.

DISCUSSION

We show that prairie strips, even early in their establishment, combined with lower land use intensity can promote biodiversity and ecosystem services without compromising crop yield. Within reduced input and biologically based treatments, where sampling effort occurred within prairie strips and at increasing distance from strips, biodiversity and ecosystem services spilled over into agronomic areas for five out of six measures. Among all treatments, using data from the entire plot area (not sampling within prairie strips for treatments with strips), pollination services and the abundance of butterflies and spiders were higher in plots with prairie strips. In addition, soil organic carbon, butterfly richness, and spider richness increased with a decrease in land use intensity. Crop yield in the reduced input treatment was equal to that of conventional management, even while including the area taken out of production. These effects were evident early in strip establishment, during which prairie strip plant communities changed from mostly weeds to a diversity of planted species. We expect the effects of strips to grow over time as native plants establish and become more abundant, and as lagged effects of historic agronomic disturbances abate.

The benefits of prairie strips decreased with distance into cropland for spider abundance and richness, dung beetle abundance, active carbon, decomposition, and pollination (**Figure 4**). Each of these responses decreased with distance from the prairie strip, but did so by different mechanisms. For spiders, the spillover into cropland was delayed one year after the prairie strips were sown. Prairie strips provided new habitat and sources of prey for spiders (Hussain et al., 2021), such that spiders could move into cropland to capture additional prey. The impact on dung beetles and decomposition are likely due to the prairie strips harboring dung beetles in the year after restoration, which in turn caused a higher rate of manure removal near the prairie strips and a decrease in dung beetle abundance and manure removal with increasing distance from the strips (Manning and Cutler, 2018). For pollination, prairie strips increased floral resources which attracted pollinators and then exported them into the surrounding habitat (Garibaldi et al., 2011). We were surprised that prairie strips increased pollination as our plot sizes are a fraction of pollinator foraging range (Ricketts et al., 2008), and we suspect that pollinators were attracted to the high concentration of resources that contrasted strongly with crops. For active carbon, the effect of distance from prairie strip may have been driven by high prairie litter inputs. Mowing prairie strips in 2019 may have caused a spillover of prairie litter inputs into cropland that did not occur in 2020, leading to higher levels of active carbon in crop soils immediately adjacent to prairie strips. While the increase of active carbon in nearby sites was subtle, it still highlights the biogeochemical benefits of adjacent prairies for agricultural lands, which are generally underappreciated (Pérez-Suárez et al., 2014).

Our study shows that spillover effects from prairie strips extend across measures of biodiversity and ecosystem services. Pollinators and pollination services have been studied at distances from restorations and habitat edges, often with higher numbers of pollinators and rates of pollination near non-crop habitat (Ricketts, 2004; Carvalheiro et al., 2010; Kordbacheh et al., 2020); by also demonstrating this phenomenon in dung beetles, spiders, active carbon, and decomposition, we show that these spatial effects apply to a broader array of organisms and ecosystem services. These results, with detailed attention to mechanism in our controlled experiment, strengthen evidence that suggests that strategic placement and amount of natural habitat in agricultural landscapes can add both conservation value and ecosystem services to an agricultural landscape (Basso et al., 2019; Mitchell et al., 2021). Our study focused on ecosystem services, and we did not measure potential ecosystem disservices from prairie strips that could impact yield, such as herbivory, however, such disservices could be addressed in future studies.

Prairie strips require several years after planting to resemble a restored prairie community, over which time diversity and ecosystem services have been shown to accrue (Kurtz, 2013; Griffin et al., 2017). Supporting this, we found that year since prairie strip establishment affected all responses that we measured at distances from prairie strips except ant richness (**Figure 4**). Lack of response of ant species richness is consistent with other grassland restoration projects where it takes several years for ant communities to turnover (Dauber and Wolters, 2005; Menke et al., 2015; Hussain et al., 2021; Scharnhorst et al., 2021). With the exception of active carbon, variables displayed a general progression of the first year having small to no effect of distance from prairie strip, to the second year showing a stronger negative effect of distance. It is important to note that year differences in our experiment are confounded by crop type (wheat or maize). These effects will become easier to separate from effects of prairie strips as measurements are repeated over the next 4 years (after two full crop rotations) and then in seven years (one full rotation after strip maturation). We expect that as more plant species establish, prairie strips will increase biodiversity even further.

Prairie strips are also likely to continue to increase the provision of soil services; for example, we found higher levels of active carbon and SOC in the prairie strips in this study, with potential for these benefits to extend into the cropland at short distances. Over time, prairie strips could thus be a significant carbon sink, which could provide benefits to agricultural landscapes and may come with economic reward with future carbon pricing. Quantifying the aggregated potential of this sequestration should be a priority, and continued measurement of these variables in our study after the early establishment phase of prairie strips will provide insight to their long-term potential for conservation and impact on crop yield, and more comprehensive opportunities for synergies.

Across all crop management treatments, there was a variable effect of land use intensity on measures of biodiversity (**Figure 3**). Conservation land consistently had the highest level of richness, and often had the highest level of ecosystem services. We found that lower land use intensity treatments with prairie strips increased butterfly abundance and pollination services compared to other crop management schemes. This may be the consequence of pollinators and butterflies being mobile agents with large ranges. They can therefore find and utilize the resources of prairie strips early in strip establishment (Cant et al., 2005; Pasquet et al., 2008). In addition to the prairie strips, reduced input and biologically based treatments have reduced pesticides which likely contributes to increased butterfly richness and pollination services; but the decreasing pollination services with distance from the prairie strips suggests that pollinators are attracted to the strips. Butterflies were most diverse in the conservation land treatment due to the increased floral and habitat diversity throughout the plot (Menéndez et al., 2007). We attribute the low level of pollination services in the conservation land treatment, especially in 2019, to the forager dilution effect, in which pollination services are diluted in an area of mass flowering (Holzschuh et al., 2011). While butterflies and pollination services do not improve the yield of wheat or maize crops, they may improve yield in soy crops (Cunningham-Minnick et al., 2019) or other crops in the landscapes that benefit from pollinators, and the potential of these services remain among the most important to surveyed farmers (Arbuckle, 2019; Hevia et al., 2021).

Our hypotheses of increasing biodiversity with a decrease in land use intensity were not supported uniformly. For ground dwelling arthropods, conventional management had surprisingly high species richness. This finding is not without precedent; despite previous findings that organic farms support more biodiversity than conventional farms (Bengtsson et al., 2005; Tuck et al., 2014), ants and dung beetles show mixed results (Hutton and Giller, 2003; Jones et al., 2019; Piccini et al., 2019; Helms et al., 2021). In addition, while diversifying farms generally increases spider diversity (Schmidt et al., 2005), prairie strips in our study have not increased spider diversity at the plot level, but have increased spider abundance.

Soil-related ecosystem services (active carbon, decomposition, GWI, and SOC) across the land use intensity gradient were highest in conservation lands, with variable differences among cropping treatments. Decomposition was highest in untilled treatments (no till and conservation land), possibly due to the higher microbial activity and soil moisture maintained by soil aggregates with greater physical protection (Paul et al., 2015), or due to mesofaunal differences that we did not measure, such as earthworm abundance (Smith et al., 2008). As strips develop, we expect decomposition to increase with dung beetle abundance and diversity in prairie strip treatments (Hosler et al., 2021). Conservation land had increased active carbon likely due to the fine root production of diverse perennial vegetation, which we also expect to increase in and near prairie strips as they mature (Sprunger et al., 2017; Sprunger and Robertson, 2018). The increased SOC along the land use intensity gradient was likely due to carbon from perennial plants (Syswerda et al., 2011; Mosier et al., 2021). GWI was almost entirely driven by N_2O in our study. The reduced GWI in the conservation land treatment was likely due to reduced fertilizer inputs. Although there was no difference in overall GWI across row crop management treatments, as Gelfand et al. (2016) has also found for N_2O emissions, there was higher GWI in the wheat year, compared to maize. This was likely due to several days of tillage early in the growing season for biologically based maize, management that is different from other treatments that receive alternative methods of weed management. We note that GWI only represents soil emissions and is not a full life cycle analysis.

The potential for prairie strips to enhance biodiversity and ecosystem services at large scales will be most powerful if they do not sacrifice agricultural yield. Historically in our experiment, the no till treatment has the highest yield, followed by the conventional and reduced input treatments with intermediate yields, and the biologically based treatment with the lowest yield. We show that converting 5% of crop area to prairie strips (and using yield measures that include the area taken out of production) does not change differences in yield across treatments beyond differences already induced by existing management. Reduced input management with prairie strips maintained a high yield, equivalent to conventional, while having high levels of pollination, spider abundance, and butterfly richness; biologically based crop management with prairie strips similarly maximized these services, but at the cost of a large cut in yields. This reduction was likely due to limitation of inorganic nitrogen (Robertson et al., 2015), unrelated to prairie strips. While no single method of crop management performed highest or lowest across all measures (e.g., no till treatments had the highest yield but did not have the highest levels of biodiversity or other ecosystem services), there was a synergy among crop yield, biodiversity, and non-provisioning ecosystem services in the reduced input treatment. Prairie strips are an effective conservation practice that can be combined with other techniques—reduced fertilizer and pesticides—to conserve biodiversity and ecosystem services without compromising crop yield.

When considering both 2019 and 2020 yields together, yield in reduced input treatments may have remained equivalent to yield in conventional treatments for at least three reasons. First, converting 5% of cropland to prairie was not enough area to result in significant changes among treatments. Second, ecosystem services generated by strips could increase yield in the remainder of the plot. Several of the responses we measured were higher at the plot scale outside of prairie strips. For example, relative to conventional treatments, active carbon was higher in reduced input treatments, and SOC trended toward higher (**Figure 2**). Third, climate or other environmental conditions during the time of our study could have had stronger negative impact in conventional treatments. As the strips mature, and with results through multiple rotations, the mechanism of yield response will become clearer.

Prairie strips were implemented on land that had previously been in crop production in this experiment, but prairie strips could also be implemented on the landscape in a way that does not reduce farm profitability. For instance, prairie strips can be strategically placed on marginal land—land that has consistent low yields relative to nutrient and greenhouse gas inputs. Marginal land occupies 26% of annual cropland land in the US Midwest, resulting in excessive pollution and wasted monetary and nutrient resources (Basso et al., 2019). Converting marginal cropland to prairie strips would reduce inputs without sacrificing crop yield. In addition, prairie strips could be harvested as perennial biofuel for added profitability, and we suggest future studies address how this would impact biodiversity and ecosystem services. Future studies might also examine how the optimal benefits of prairie strips could be achieved with strategic location, particularly cropland that is consistently underperforming and therefore not as profitable, on slopes to prevent soil erosion, or in locations that could increase habitat connectivity in the broader landscape (Basso, 2021).

We expect that as prairie strips mature their effects on biodiversity and ecosystem services will grow. We converted just 5% of cropland to prairie, however, optimal benefit may be achieved by even larger strips, such as the 10% conversion of cropland to prairie as recommended by Schulte et al. (2017). Our study supports that prairie strips are an effective strategy for conserving biodiversity, and can in some cases be created without impacting crop yield in the US Midwest. More broadly, diversifying agricultural landscapes can help mitigate the loss of biodiversity and ecosystem services while supporting the growing human population.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article are available at: doi: 10.5281/zenodo.6497814. Any further requests should be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LK, NH, SE, JH, and CR designed research. LK, CR, JH, JS, and EC-O conducted research and collected data in addition to other researchers. LK, CR, JH, CV, and JVC analyzed data and results. LK led the synthesis and writing. All authors contributed to manuscript drafts, contributed to the article, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.833170/full#supplementary-material

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Fertilization Rapidly Alters the Feeding Activity of Grassland Soil Mesofauna Independent of Management History

Klaus Birkhofer[1]*, Dennis Baulechner[2], Tim Diekötter[3], Andrey Zaitsev[2] and Volkmar Wolters[2]*

[1] Department of Ecology, Brandenburg University of Technology Cottbus–Senftenberg, Cottbus, Germany, [2] Institute of Animal Ecology, Justus Liebig University Giessen, Giessen, Germany, [3] Department of Landscape Ecology, Institute for Natural Resource Conservation, Kiel University, Kiel, Germany

Edited by:
Christopher Williams,
Liverpool John Moores University,
United Kingdom

Reviewed by:
Katalin Szlavecz,
Johns Hopkins University,
United States
Andreea Nita,
University of Bucharest, Romania

***Correspondence:**
Klaus Birkhofer
Klaus.Birkhofer@b-tu.de
Volkmar Wolters
volkmar.wolters@allzool.bio.uni-giessen.de

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Nitrogen fertilization of permanent grasslands affects soil fauna communities by modifying their taxonomic composition, population dynamics and feeding activity. However, it is not well understood if the edaphic fauna adapts to these external inputs so that the immediate response to fertilizer application depends on the long-term nutrient management strategy. We performed a field experiment in permanent grasslands under agricultural management in three regions across Germany. We used experimental fertilization with an organic plant-sourced fertilizer along a long-term nutrient management gradient to study the immediate and long-term effects of fertilization and their interdependence on the taxonomic composition and feeding activity of the soil mesofauna (Nematoda, Oribatida, and Collembola). Sampling season, soil properties, vegetation structure, and geographic location were considered as additional predictor variables to reflect heterogeneity in environmental conditions. The taxonomic composition, richness and total abundance of soil mesofauna communities were significantly affected by long-term nutrient management, but not by experimental fertilization. However, N pulses rapidly (within days) reduced the feeding activity estimated with bait-lamina strips independent of long-term nutrient management strategies. Experimental addition of organic plant-sourced fertilizer may have led to a rapid build-up of microbial biomass, providing alternative food sources for the soil mesofauna and causing a shift away from the bait-lamina substrate. Our study indicates that community changes associated with the long-term nutrient management regime in permanent grasslands do not alter the strong functional response of the soil mesofauna to N pulses. There is an urgent need to develop nutrient management strategies for permanent grasslands that take into account both the conservation of the edaphic faunal community and changes of ecosystem functions caused by rapid responses of the soil mesofauna to fertilizer inputs.

Keywords: bait-lamina, ecosystem function, land-use intensity, nitrogen input, permanent grassland, Acari (mites), Collembola (springtails), Nematoda

INTRODUCTION

Permanent agricultural grasslands provide important habitats to many plant and animal species (Veen et al., 2009). Thus, they are a key conservation measure at the national and EU scale (Hristov et al., 2020). Yet, EU regulation allows for a broad range of nutrient management intensities, including the quantity of fertilizer and livestock densities. Decades of research have shown that the structure and richness of soil invertebrate communities can be altered by long-term land use (Birkhofer et al., 2008) and short-term anthropogenic stressors (Zaitsev et al., 2006), with effects being modulated by factors such as landscape characteristics (Zaitsev et al., 2013). It is thus very likely that grassland management severely affects soil biodiversity (e.g., Brussaard, 1997; Bardgett and Van Der Putten, 2014). However, a comprehensive understanding of the functional consequences associated with these changes is still lacking (Eisenhauer et al., 2017; Delgado-Baquerizo et al., 2020).

The edaphic mesofauna is unable to overcome the physical barriers of the soil, as soil animals such as Collembola or Acari cannot actively construct their own passages and pore spaces in soil (Potapov et al., 2022). Most functional effects of this group are thus related to its feeding activity, with changes in these activities being primarily caused by changes in both available food sources and community composition (Wolters and Joergensen, 1991). Fertilizer application has previously been shown to reduce the soil fauna feeding activity in tropical oil palm plantations (for chemical fertilizers: Tao et al., 2016) or to increase feeding activity in annual crop fields (for organic fertilizers: Pfotzer and Schüler, 1997). Understanding the effect of fertilizer application on the feeding activity of belowground communities is essential for evaluating the functional consequences of management strategies. The long-term application of synthetic fertilizers may lead to soil acidification and affect soil fauna negatively (Birkhofer et al., 2008). Alternatively, organic fertilizers, either animal (e.g., manure or slurry) or plant-sourced (e.g., green manure or biofertilizers) materials, may promote soil fauna abundances and activity due to provision of dead organic matter as food resource for detritivores or due to enhanced microbial growth and provision of food resources to microbivorous and fungivorous soil fauna (Birkhofer et al., 2012). In the study presented here, we focused on whether the immediate trophic response of soil communities of permanent agricultural grasslands to fertilizer application occurs independently of taxonomic composition. Alternatively, it could be modulated by changes in taxonomic composition associated with long-term nutrient management strategies. In contrast to forests and annual crop fields (Geissen and Brümmer, 1999; Birkhofer et al., 2008; Ding et al., 2019; Shaw et al., 2019), effects of long-term fertilization (e.g., fertilizer application, livestock density) on soil mesofauna communities of permanent grasslands are not well studied (Forge et al., 2005). Huhta et al. (1986) suggested that soil invertebrate communities are less sensitive to moderate fertilizer pulses compared to soil microbes and that fertilizer application affects soil fauna primarily indirectly through changes in vegetation and soil properties (see also Birkhofer et al., 2011; Zhu et al., 2016). However, the short-term alteration of soil microbial and fungal communities by fertilization (Lazcano et al., 2013) likely has an indirect effect of the activity of soil mesofauna. Nutrient availability changes the feeding behavior of soil mesofauna (Tao et al., 2016) which can influence soil functioning through altered nutrient cycles. Livestock grazing has similar effects, as nutrients are deposited in pastures (Haynes and Williams, 1993; Chaneton et al., 1996).

Here we address the questions (a) if experimental fertilization with an organic plant-sourced fertilizer rapidly (over a few days) affects the taxonomic composition or feeding activity of the soil mesofauna compared to the long-term strategy of nutrient management over 3 years and (b) if effects of N-pulses through experimental fertilization are independent of the long-term strategy of nutrient management. To answer these questions, we studied effects of experimental fertilization on the taxonomic composition and feeding activity of the soil mesofauna (Collembola, Oribatida, and Nematoda) immediately after application in permanent grasslands along a gradient of long-term nutrient management strategies and in three regions across Germany [German Research Foundation (DFG) Biodiversity Exploratories, Fischer et al., 2010]. We hypothesize that (H1) feeding activity of soil invertebrates increases rapidly in subplots after the experimental application of an organic fertilizer and (H2) taxonomic composition of the soil mesofauna primarily responds to long-term nutrient management strategies, but not rapidly to experimental fertilization. Finally, we hypothesize that feeding activity in grasslands with very low long-term fertilization levels or livestock densities respond more strongly to experimental fertilization than those with higher nutrient inputs (H3).

MATERIALS AND METHODS

Sampling Sites and Experimental Design

Sampling was conducted in spring and autumn 2009 on 12 permanent agricultural grasslands in each of the three regions of the DFG Biodiversity Exploratories in Germany (Fischer et al., 2010): Schwäbische Alb (AEG), Hainich-Dün (HEG), and Schorfheide-Chorin (SEG) (**Table 1**). The sampling sites were selected along a gradient of land-use intensities based on a standardized annual questionnaire for farmers including information about the mowing frequency, livestock density and the amount of N fertilization (Vogt et al., 2019). In our study, we used the available 3-year averages (2006–2008) of the livestock density (livestock unit days of grazing ha^{-1} year^{-1}) and N fertilizer amount (kg nitrogen ha^{-1} year^{-1}) (**Table 1**) standardized across the three study regions as predictor variables for the long-term nutrient management strategy of each grassland (Blüthgen et al., 2012). Sampling season, soil properties, vegetation structure and geographic location were recorded as additional predictors to reflect environmental heterogeneity (**Table 2** and section "Soil Fauna, Vegetation, and Soil Properties" for details). At each grassland site, two subplots with a size of 5 m × 5 m were established with a minimum distance from the grassland edge of 20 and 2 m distance from each other. We randomly selected one subplot

TABLE 1 | (A) Dates of experimental fertilization, placement and collection of bait-lamina strips and soil fauna sampling in spring and autumn 2009, **(B)** 3-year averages (2006–2008) of N fertilization and livestock density, maximum average vegetation height, vegetation surface coverage, soil pH and soil organic carbon content (means ± SD and ranges) for the 12 grasslands sites in each of the three study regions.

(A) Dates	**Schwäbische Alb AEG**	**Hainich-Dün HEG**	**Schorfheide-Chorin SEG**
Fertilization and bait-lamina placement spring 2009	04.05–06.05	27.04–29.04	08.05 –10.05
Sampling spring 2009	18.05–19.05	11.05–13.05	22.05–24.05
Fertilization and bait-lamina placement autumn 2009	21.09–23.09	02.10–04.10	14.09–16.09
Sampling autumn 2009	05.10–07.10	16.10–18.10	28.09–30.09

(B) Predictors	**Schwäbische Alb AEG**	**Hainich-Dün HEG**	**Schorfheide-Chorin SEG**
Fertilization (kg N ha^{-1} $year^{-1}$)	24.5 ± 33.3 (0.0–100.0)	35.0 ± 38.5 (0.0–103.3)	40.8 ± 45.6 (0.0–112.7)
Livestock density (livestock unit days of grazing ha^{-1} $year^{-1}$)	118.0 ± 177.73 (0.0–596.3)	122.4 ± 122.0 (0.0–395.9)	100.4 ± 45.6 (0.0–450.2)
Vegetation coverage (%)	94.4 ± 9.1 (51.7–100.0)	92.3 ± 7.8 (68.3–100.0)	89.8 ± 12.9 (52.3–100.0)
Maximum average vegetation height (cm)	16.6 ± 6.1 (6.2–29.1)	22.1 ± 8.6 (10.8–49.4)	24.6 ± 13.5 (3.8–58.0)
Soil pH	6.3 ± 0.5 (5.4–7.3)	6.9 ± 0.4 (5.6–7.5)	6.1 ± 0.9 (4.7–7.8)
Soil organic carbon content (g 100 g^{-1} soil)	0.63 ± 0.07 (0.50–0.81)	0.45 ± 0.09 (0.28–0.65)	0.46 ± 0.36 (0.12–1.33)

TABLE 2 | Indicator groups and the individual variables in each indicator group as used in all distance-based linear models (DistLM, McArdle and Anderson, 2001).

Indicator group	**Variable type**	**Variables**
Location	Continuous	Latitude and Longitude
Vegetation	Continuous	Surface cover (%) and Maximum average height (cm)
pH values	Continuous	Unitless
Soil organic carbon	Continuous	SOC content (g 100 g^{-1} soil)
Experimental fertilization	Binary	Experimentally fertilized (1 = yes or 0 = no)
Long-term nutrient management	Continuous	3-year average livestock density (LU $ha^{-1}a^{-1}$) and fertilizer amount (kg $ha^{-1}a^{-1}$)
Season	Binary	Spring (1 = yes, 0 = no, autumn)

as control and the other as experimental fertilizer treatment subplot. The latter was fertilized with 2,000 kg ha^{-1} of an organic plant-sourced commercial fertilizer [$N = 6.67\%$ (=133.4 kg N ha^{-1}), $P_2O_2 = 0.85\%$, $K_2O = 0.86\%$; AGROBIOSOL®, SW-Düngesysteme, Wolfenbüttel, Germany] in spring and autumn 2009 (for dates see **Table 1**). This plant-sourced fertilizer is certified for organic agriculture in the EU and is produced by fermenting agricultural by-products (e.g., soy flour or sugars), fungal biomass growth and subsequent drying of the material. The control subplots were not experimentally fertilized.

Feeding Activity

The bait-lamina test provides a standardized method to estimate the feeding activity of soil mesofauna (Törne, 1990; Kratz, 1998). In spring and autumn 2009 feeding activity of soil fauna was investigated by using the bait-lamina test following the schedule in **Table 1**. Bait-lamina strips with a size of 6 mm × 160 mm had 16 holes of 1.5 mm diameter arranged in a single vertical row with 5 mm intervals. The bait substrate consisted of cellulose, agar-agar, bentonite, and bran flakes following Eisenbeis et al. (1996). Consumption of this bait substrate over time provides an estimate of the feeding activity of soil macro- and mesofauna under different environmental conditions (Birkhofer et al., 2011) or agricultural management practices (Birkhofer et al., 2021). To avoid displacement of the bait, the bait-lamina strips were inserted into the soil in a preformed vertical slit using a stainless-steel metal knife. The top hole was inserted to surface level and the bottom hole reached 7 cm depth. At each date, each subplot received 8 bait-lamina strips in a 2 × 4 arrangement with 10 cm spacing between strips. Bait-lamina strips were collected after 14 days and the feeding activity was assessed by counting the number of completely empty holes (Vorobeichik and Bergman, 2021).

Soil Fauna, Vegetation, and Soil Properties

In each subplot, four intact soil cores with a diameter of 8 cm and a depth of 5 cm were sampled, resulting in 288 soil cores per sampling season (36 grassland sites × 2 subplots × 4 soil cores). Soil fauna was extracted using a modified Kempson extraction method (Kempson et al., 1963). Heat extraction was performed over 8 days with stepwise (5°C) increasing temperatures every day from 20 to 60°C. All adult Oribatida and Collembola from these samples were identified to species level. To sample Nematoda communities in each subplot, nine samples were collected as intact soil cores with a diameter of 3 cm and a depth of 10 cm. We extracted nematodes from 100 g bulk soil per subplot following a modified Oostenbrink elutriator-cottonwool filter method. An aliquot containing about 100 nematode individuals was randomly taken from each extracted sample for identification. The specimens were then preserved in 4% formalin and identified to genus level. Nematoda abundance was not analyzed, as only a predefined number of individuals was identified to genus level and counted.

Vegetation cover and maximum average height were measured in three 1 m × 1 m quadrats per subplot at each sampling date. Surface cover was visually estimated on a percentage scale and maximum average height was measured for the highest plant in each corner of the 1 m × 1 m quadrat. Soil pH was measured for each subplot in a solution of $CaCl_2$ (10 g bulk soil in 25 ml $CaCl_2$) using an electrical pH sensor (with a resolution of two decimals). Soil organic carbon content was quantified for each subplot with the loss-on-ignition (LOI) method for bulk soil quantities between 2.89 and 3.57 g. Bulk soil samples for pH value and soil organic carbon content measurements consisted of three samples of the upper 5 cm in each subplot.

Statistical Analyses

To account for the large number of potentially relevant predictors in this study (**Table 2**) and the multivariate nature of taxonomic community composition data, we used distance-based linear models (DistLM) as an appropriate method for multivariate, multiple regression analyses of community data (McArdle and Anderson, 2001). These regression models allow for the analyses of uni- and multivariate dependent data based on the selection of an appropriate resemblance measure to create resemblance matrices for all pairs of samples. For a general flowchart of the DistLM analyses refer to **Supplementary Figure 1**. Community composition data were analyzed separately for the three taxonomic groups (Collembola, Oribatida, and Nematoda) based on log $(x + 1)$ transformed abundance data of all species (Collembola, Oribatida) or genera (Nematoda) using the Bray-Curtis similarity measure. Transformation of abundances was performed to weight down the importance of very abundant compared to less abundant taxa (Anderson et al., 2008). The univariate variables feeding activity, Collembola, Oribatida and Nematoda taxonomic richness and total abundance were individually analyzed based on untransformed data and Euclidean distances. Model selection in all DistLMs was based on the AIC selection criterion and a stepwise selection procedure (combining forward and backward selection) using the indicator groups listed in **Table 2**. Location, as individual plot coordinates, was fitted prior to all model selection procedures to account for geographic distribution. In case of significant relationships between indicator groups and univariate dependent variables, Pearson correlations were calculated between the dependent variable and each individual independent variable in the respective indicator group (**Table 2**). In case of a significant relationship between a categorical indicator group (coded binary) and a univariate dependent variable, means and 95% confidence intervals were compared between levels of the respective indicator variable(s). Effects of indicators selected in DistLMs and the respective individual predictor variables on multivariate community data are shown in distance-based RDA ordinations. Distance-based linear models test for sequential, additive effects under hypotheses H1 and H2, but not for interdependencies between predictors (H3). We therefore used additional permutational analysis of variance (PERMANOVA, Anderson, 2014) models to explicitly test if effects of experimental fertilization statistically depend on the long-term nutrient management strategy of each grassland to address H3. In PERMANOVA models, we used experimental fertilization as categorical fixed factor and the 3-year average of fertilizer amount as covariable including the interaction between both model terms. All PERMANOVA models were based on the same resemblance matrices used for DistLMs and were calculated with type III sums of squares and 9999 permutations of residuals under a reduced model (Anderson, 2014). All statistical analyses were performed in PRIMER 7.0.21 with the PERMANOVA + 1 add-on (PRIMER-e).

RESULTS

Soil Fauna Feeding Activity

Experimental fertilization significantly affected soil fauna feeding activity estimated with bait lamina strips (**Table 3A**), with feeding activity being 1.6 times lower in experimentally fertilized subplots (**Figure 1**). Long-term nutrient management and soil pH were selected as additional predictors in the final model, but they had no significant effect on soil fauna feeding activity and only explained very low proportion in the variation of feeding activity (**Table 3A**). The effect of experimental fertilization did not depend significantly on the long-term fertilization strategy (PERMANOVA: Pseudo-$F_{1,68} = 0.30$, $P = 0.583$).

Taxonomic Richness and Abundance

The long-term nutrient management strategy had a significant effect on the taxonomic richness of all soil faunal groups studied (**Tables 4A–C**), while experimental fertilization did not affect taxonomic richness. Nematoda (Pearson correlation $R = -0.166$, $P = 0.047$; **Supplementary Figure 2A**) and Oribatida ($R = -0.327$, $P < 0.001$; **Supplementary Figure 2A**) richness and Oribatida abundance ($R = -0.210$, $P = 0.012$; **Supplementary Figure 2B**) were negatively correlated to the long-term average fertilizer amount. Collembola richness was positively correlated to the livestock density ($R = 0.169$, $P = 0.043$; **Supplementary Figure 3**). Seasonal differences were significant in Nematoda and Collembola richness, and Nematoda richness was further negatively affected by soil organic carbon content (**Table 4A**). Nematoda communities on average had two (AEG, SEG) or three (HEG) genera fewer in autumn than in spring. Oribatida communities on average had two more species in spring in AEG, one fewer in spring in SEG, with no differences observed for HEG. Collembola abundance was only significantly affected by location (Pseudo-$F_{141,3} = 28.65$, Prop. = 0.29, $P < 0.001$) and Oribatida abundance was significantly and negatively affected by long-term nutrient management (Pseudo-$F_{139,5} = 35.25$, Prop. = 0.08, $P = 0.003$), but not by experimental fertilization.

Community Composition

Nematode communities differed markedly between locations and between spring and autumn samples (**Figure 2**). Nematode communities in HEG were characterized by higher abundances of *Helicotylenchus* and *Amplimerlinius*.

TABLE 3 | Results of distance-based linear models (DistLMs) testing for effects of indicator groups (**Table 2**) as predictors for **(A)** soil fauna feeding activity, **(B)** Nematoda, **(C)** Oribatida, and **(D)** Collembola community composition.

Indicator group	Pseudo-*F*	*P*-value	Proportion	Cumulative	Res. df	Regr. df
(A) Feeding activity						
1. Pulse	18.21	<0.001	0.10	0.20	140	4
2. Soil pH	2.98	0.085	0.02	0.22	139	5
3. Management	2.62	0.076	0.03	0.25	137	7
(B) Nematoda						
1. C_{org}	7.55	<0.001	0.04	0.23	140	4
2. Season	5.79	<0.001	0.03	0.26	139	5
3. Management	3.33	<0.001	0.03	0.30	137	7
(C) Oribatida						
1. C_{org}	11.45	<0.001	0.06	0.22	140	4
2. Season	12.21	<0.001	0.06	0.28	139	5
3. Management	4.39	<0.001	0.04	0.33	137	7
4. Soil pH	3.17	0.001	0.02	0.34	136	8
(D) Collembola						
1. Soil pH	9.59	<0.001	0.05	0.21	140	4
2. Management	4.60	<0.001	0.05	0.26	138	6
3. C_{org}	3.77	0.001	0.02	0.28	137	7
4. Vegetation	2.09	0.018	0.02	0.30	135	9

Indicator groups are given in the order of model selection with experimental fertilization treatment (pulse), soil pH values, long-term nutrient management, soil organic carbon content (C_{org}), season and vegetation properties. Pseudo-F and P-values are provided together with the individual and cumulative proportion of explained variation in the sequential model and residual and regression degrees of freedom. Note that coordinates of study plots (location) were fitted first in all models to account for geographic distribution.

SEG plots had higher abundances of *Tylenchorhynchidae* and *Eucephalobus*, while representatives of *Prodorylaimus* were more abundant in grassland soils in AEG and HEG. Soil organic carbon content and long-term nutrient management were selected in the final model (**Table 3B**), with higher abundances of *Tylenchorhynchidae* and *Eucephalobus* with increasing long-term fertilization (**Figure 2**). Experimental fertilization neither significantly affected Nematoda community composition in the final model nor in marginal tests for individual predictors.

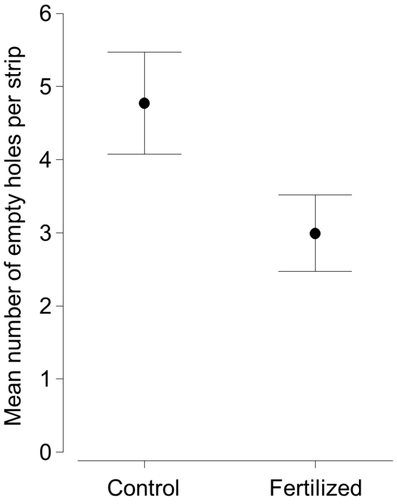

FIGURE 1 | Mean feeding activity as the mean number of empty holes per bait-lamina strip with 95% confidence intervals for the number of empty holes per bait strip in subplots without (Control; *N* = 72) and with (Fertilized, *N* = 72) experimental fertilization (Pseudo-$F_{140,4}$ = 18.21, $P < 0.001$).

Communities of oribatid mites differed between locations and between spring and autumn samples (**Figure 3**). Oribatid communities in autumn were characterized by higher abundances of *Malaconothrus monodactylus*. Communities in AEG and some communities in SEG had high abundances of *Eupelops acromios* and *Achipteria coleoptrata*, whereas communities in HEG and some communities in SEG were characterized by high abundances of *Tectocepheus velatus*. Subplots with higher soil organic carbon content and pH values, but lower long-term fertilization had higher abundances of *A. nitens*, *A. coleoptrata,* and *E. acromios* and lower abundances of *T. velatus*. Experimental fertilization neither affected Oribatida community composition significantly in the final model nor in marginal tests for individual predictors (**Table 3C**).

Unlike nematode and oribatid communities, the composition of Collembola communities differed between locations, but not between spring and autumn sample dates (**Figure 4**). *Folsomia quadrioculata*, *Lepidocyrtus lanuginosus,* and *Parisotoma notabilis* were more abundant in AEG and HEG than in SEG. The abundances of *L. lanuginosus* and *P. notabilis* increased with soil pH values, *F. quadrioculata* and *L. lanuginosus* were positively related to livestock density (**Table 3D**). Experimental fertilization neither affected Collembola community composition significantly in the final model nor in marginal tests for individual predictors.

TABLE 4 | Results of distance-based linear models (DistLMs) testing for effects of indicator groups (**Table 2**) as predictors for **(A)** Nematoda genus, **(B)** Oribatida species, and **(C)** Collembola species richness.

Indicator group	Pseudo-*F*	*P*-value	Proportion	Cumulative	Res. df	Regr. df
(A) Nematoda						
1. Season	11.89	<0.001	0.06	0.26	140	4
2. C_{org}	9.64	0.002	0.05	0.31	139	5
3. Management	6.89	0.002	0.06	0.37	137	7
4. Vegetation	2.61	0.083	0.02	0.39	135	9
(B) Oribatida						
1. Management	18.29	<0.001	0.14	0.45	139	5
(C) Collembola						
1. Season	4.83	0.027	0.03	0.23	140	4
2. Management	3.43	0.037	0.04	0.26	138	6
3. Pulse	2.58	0.110	0.01	0.28	137	7
4. Soil pH	2.05	0.155	0.01	0.29	136	8

Indicator predictor groups are given in the order of model selection with experimental fertilization treatment (pulse), soil organic carbon content (C_{org}), soil pH values, long-term nutrient management, season and vegetation properties. Pseudo-F values and P-values are provided together with the individual and cumulative proportion of explained variation in the sequential model and residual and regression degrees of freedom. Note that coordinates of study plots (location) were fitted first in all models to account for geographic location. Res. df, residual degrees of freedom; Regr. df, regression degrees of freedom.

DISCUSSION

Soil fauna feeding activity responded to experimental fertilization with a plant-sourced organic fertilizer rapidly, within 14 days after application. This response was independent of the changes in taxonomic community composition, richness and total abundance associated with long-term nutrient management. Thus, in terms of immediate response, edaphic mesofauna communities do not appear to adapt to external inputs. Considering the impact of nitrogen availability on priming effects in the root system (Dijkstra et al., 2013), this may have serious consequences, e.g., for the carbon storage in grassland ecosystems and should therefore caution relevant actors (e.g., Manolache et al., 2020) involved in the development of future grassland management strategies.

The almost immediate reduction of the feeding activity does not confirm results from previous studies with other organic fertilizers (Pfotzer and Schüler, 1997) nor our first hypothesis. It might be explained by an increase in soil microbial biomass from experimental fertilizer pulses (Ali et al., 2021) and the manufacturer of the AGROBIOSOL® organic fertilizer claims that the application "activates" soil life. Microbes provide an alternative protein-rich food source for soil invertebrates and their increased availability may have drastically reduced the attractiveness of protein-poor bait lamina substrates (Li et al., 2020). The rapid response of the mesofauna reported here is consistent with the fast negative effect of chemical fertilizer application on soil fauna feeding activity in tropical oil palm agroecosystems compared to the application of plant residues (Tao et al., 2016). The generality and permanence of organic and chemical fertilizer effects on soil mesofauna in grassland soils nevertheless needs to be investigated in the future. Wahyuningsih et al. (2019), for example, even reported an initial positive response of feeding activity to the application of chemical nitrogen fertilizer within a few days after application. Alternatively, the application of organic fertilizer may have rapidly induced a certain level of feeding inactivity in soil fauna communities, but this explanation does not seem very probable based on our existing knowledge.

Soil invertebrate species composition, taxonomic richness and overall abundance were significantly altered by long-term nutrient management for all taxa. This confirms our second hypothesis and proves that the management regime strongly influences the structure of belowground communities (see also Degtyarev et al., 2019; Korobushkin et al., 2019; Saifutdinov et al., 2020). Given the duration of the meso- and microfauna life cycles (Hopkin, 1997; Khan and Kim, 2007) and the comparatively small-scale fertilizer application (see section "Materials and Methods"), immediate community changes due to demographic responses or to lethal and repulsive effects are highly unlikely. However, our study suggests that rapid changes in feeding activity must not be accompanied by structural changes of the soil community. The evaluation of management effects on grassland soils must thus consider both short-term and long-term responses of edaphic biota. The fact that the species richness of Oribatida was only affected by long-term nutrient management confirms that this taxon is less sensitive to pulse stress (sensu Bengtsson, 2002) and rather reflect conditions over longer temporal scales (Zaitsev et al., 2013). Land-use intensity is known to vary between years in the studied grasslands (Allan et al., 2014) and soil taxa such as Oribatida may rather respond to this inter-annual variation.

Our results indicate that the response to fertilization and to local variation of soil properties is dominated by a relatively small number of species in each of the taxonomic groups. The remaining species form a species pool that varies locally from region to region and, to a lesser extent, from season to season. As in other studies (Bongers and Bongers, 1998; Yeates, 1999), organic fertilization increased the relative density of some bacterivorous nematodes (e.g., *Eucephalobus* in **Figure 2**). This suggests that the experimental fertilization

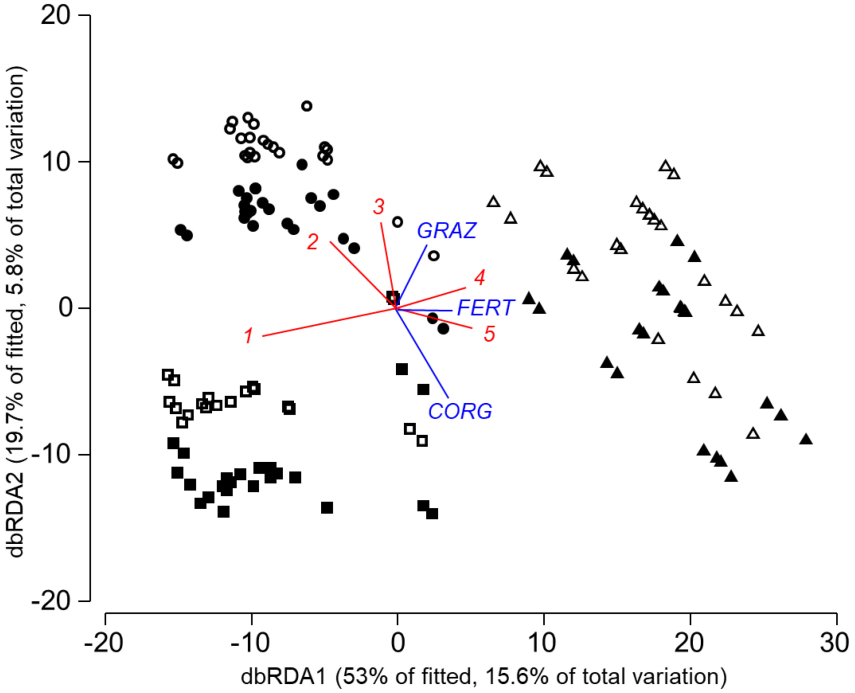

FIGURE 2 | Distance-based RDA plot of study plots based on Nematode community data and the model in **Table 3B** from AEG (■), HEG (●) and SEG (▲) with samples from spring (solid) and autumn (open) 2008. Individual predictor variables from selected indicator groups of the model in **Table 3B** are superimposed (CORG, soil organic carbon content; GRAZ, average livestock density 2006–2008; FERT, average fertilizer amount 2006–2008). Nematoda genera with correlation coefficients >0.3 between axis scores of sites and abundances are superimposed with (1) *Prodorylaimus*, (2) *Helicotylenchus*, (3) *Amplimerlinius*, (4) *Tylenchorhynchidae,* and (5) *Eucephalobus*.

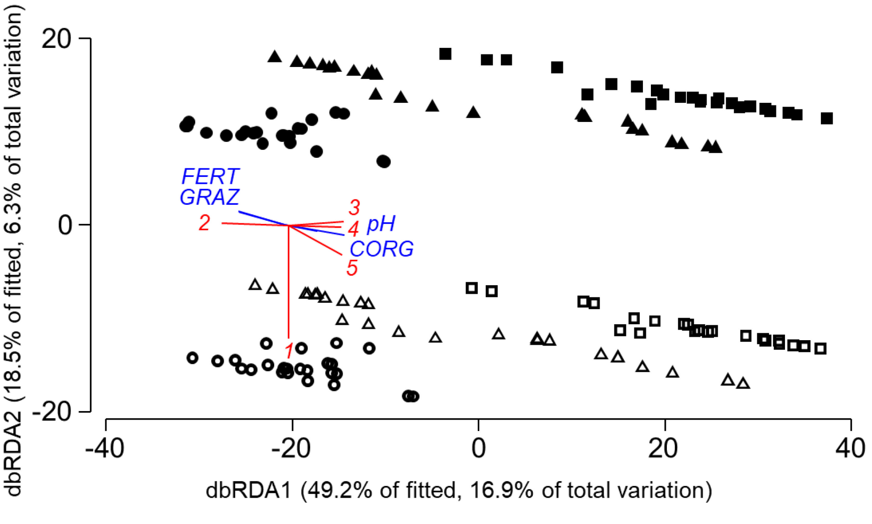

FIGURE 3 | Distance-based RDA plot of study plots based on oribatid mite community data and the model in **Table 3C** from AEG (■), HEG (●) and SEG (▲) with samples from spring (solid) and autumn (open) 2009. Individual predictor variables from selected indicator groups of the model in **Table 3C** are superimposed (CORG, soil organic carbon content; pH, soil pH value; GRAZ, average livestock density 2006–2008; FERT, average fertilizer amount 2006–2008). Oribatida species with correlation coefficients >0.3 between axis scores of sites and abundances are also superimposed with (1) *Malaconothrus monodactylus*, (2) *Tectocepheus velatus,* (3) *Eupelops acromios,* (4) *Achipteria coleoptrata,* and (5) *A. nitens*.

not only promoted microbial growth, but also shifted the soil fungal to bacteria ratio toward bacteria (see also De Vries et al., 2006). Interestingly, as previously indicated also for forests (Zaitsev et al., 2013), only Oribatida species with rapid generation time like the non-specialized *T. velatus* responded to experimental fertilization pulses. Larger sexually

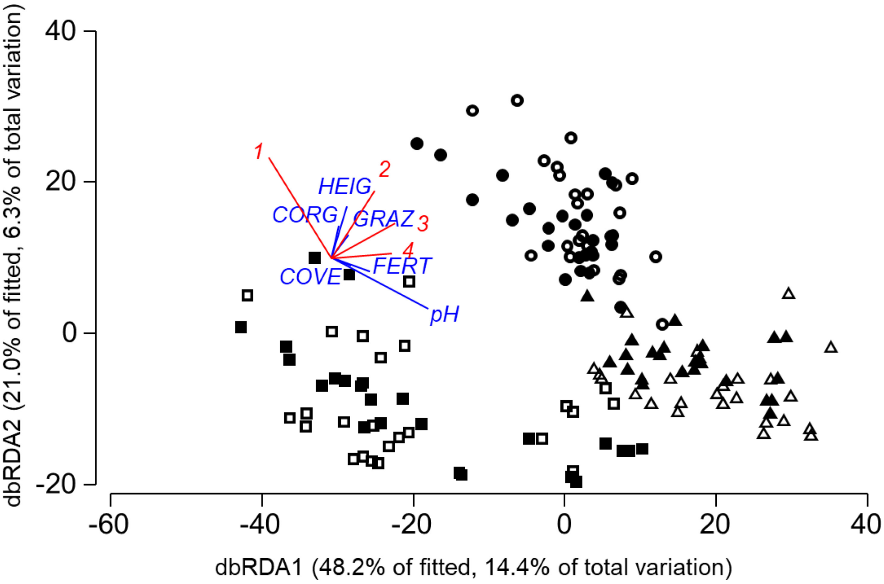

FIGURE 4 | Distance-based RDA plot of study plots based on Collembola community data and the model in **Table 3D** from AEG (■), HEG (●) and SEG (▲) with samples from spring (solid) and autumn (open) 2009. Individual predictor variables from selected indicator groups of the model in **Table 3D** are superimposed (CORG, soil organic carbon content; pH, soil pH value; GRAZ, average livestock density 2006–2008; FERT, average fertilizer amount 2006–2008; COVE, vegetation cover; HEIG, vegetation height). Collembola species with correlation coefficients >0.3 between axis scores of sites and abundances are also superimposed with (1) *Isotoma viridis,* (2) *Folsomia quadrioculata,* (3) *Lepidocyrtus lanuginosus,* and (4) *Parisotoma notabilis*.

reproducing, fungivorous and herbifungivorous species in the genus *Achipteria* rather responded to soil conditions which generally reflect land-use history. Compared, to Oribatida, the weaker response of Collembola species to experimental fertilization may be best explained by the higher vertical and horizontal mobility in comparison to the other soil taxa in this study (Hopkin, 1997; Meyer et al., 2021). Very strong seasonal variation of Oribatida communities highlights the need for multiple sampling campaigns over the year to realistically determine soil microarthropod diversity and community composition.

CONCLUSION

The rapid change in feeding activity proves the sensitivity of meso- and microfauna in permanent grassland soils to organic fertilizer application. This response is probably due to the stimulation of microbial growth, especially that of bacteria, in organically fertilized soils. Several studies based on food web analysis support this assumption by showing a shift from the fungal and to the bacterial channel in fertilized grassland soils (Bardgett et al., 1996; De Vries et al., 2006; Denef et al., 2009). However, detecting changes in nutrient or energy fluxes through soil food web analysis requires massive structural changes in the community, as these methods derive estimates of matter fluxes based on abundance or biomass changes. Therefore, these approaches cannot detect immediate changes in feeding activity that are not accompanied by changes in community structure, as was the case in our study. We hypothesize that food web analyses underestimate the ecological consequences of intensive management because they do not account for animal behavior. Since short-term and small-scale processes play a key role in the rhizosphere (Philippot et al., 2013), there is an urgent need to develop nutrient management strategies for permanent grasslands that take into account both the conservation of the edaphic community and changes of ecosystem functions caused by rapid responses of the soil mesofauna to fertilizer inputs. These considerations are fundamentally important as a more sustainable management of agricultural permanent grasslands is a key component of ongoing discussions about further developing the common agricultural policy in the EU (Peeters et al., 2020; Montanarella and Panagos, 2021).

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because datasets are stored as non-public data in the Information System of the Biodiversity Exploratories (BEXIS id's 31171, 31174, 31176, and 31181), but are available upon request. Requests to access the datasets should be directed to KB, Klaus.Birkhofer@b-tu.de.

AUTHOR CONTRIBUTIONS

KB, TD, and VW contributed to the conception and design of the study. KB and TD performed the field work. AZ coordinated the identification of Acari and Collembola samples. KB and DB performed the statistical analysis. KB wrote the first draft of the manuscript. DB, TD, AZ, and VW wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.864470/full#supplementary-material

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REVIEW
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The Value of Hazard Quotients in Honey Bee (*Apis mellifera*) Ecotoxicology: A Review

Emily A. Carlson[1,2]****, Andony Melathopoulos***[1] ***and Ramesh Sagili***[2]

[1] Pollinator Health Lab, Department of Horticulture, Oregon State University, Corvallis, OR, United States, [2] Honey Bee Lab, Department of Horticulture, Oregon State University, Corvallis, OR, United States

Edited by:
Christopher Williams,
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United States
Chuleui Jung,
Andong National University,
South Korea

***Correspondence:**
Emily A. Carlson
Emily.Carlson@oregonstate.edu

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Estimates of pesticide application hazards have grown to be one of the most common methodologies for evaluating the impact of pest management practices on honey bees. Typically, hazards are estimated by calculating a Hazard Quotient (HQ), which is based on acute toxicity data for different pesticides and the quantity of those pesticides applied to a field or detected on bees and matrices associated with their hive (honey, wax, pollen, and/or bee bread). Although use of HQ is widespread, there have been few reviews of this methodology, particularly with focus on how effective this method is at predicting effects of pesticides on hives. We evaluated 36 relevant papers, containing calculations of HQ to estimate hazards to honey bees. We observed that HQ was primarily calculated using two different approaches: (1) from the concentration of pesticides in the food, hive, or tissues of honey bees or (2) using the field application rate of the active ingredient as the estimation of pesticide hazard. Within and between HQ calculation methods, thresholds vary widely with some HQ thresholds set below 1 and others set at 10,000. Based on our review we identify key weakness with current HQ methodology and how studies relate HQ to honey bee health endpoints. First, HQ thresholds from studies of pesticides in hives are not based on the same pesticide consumption models from the EPA, potentially overestimating the risk of impacts to colonies. Conversely, HQ estimates calculated from field application rates are not based on eco-toxicological estimates of field exposure, resulting in an overestimation of pesticide reaching colonies. We suggest it is for these reasons that there is poor correspondence between HQ and field-level honey bee health endpoints. Considering these challenges, HQ calculations should be used cautiously in future studies and more research should be dedicated to field level exposure models.

Keywords: honey bee, hazard quotient, HQ, ecotoxicology, *Apis mellifera*, pollen hazard quotient

INTRODUCTION

Environmental hazards and risks are key concepts in quantifying how dangerous pesticides are to honey bees. These concepts are frequently confused, particularly, as we demonstrate in this paper, across the growing number of studies looking to quantify the effects of field applied pesticides on honey bees.

Environmental risk assessment is the process of determining the consequences of pesticide applications for environmental quality, including non-target organisms like honey bees. The risk of a pesticide to honey bees represents the likelihood that that the colony will be negatively impacted by the treatment when applied to a given crop at a given rate. Case in point is a recent risk assessment framework developed jointly by the United States Environmental Protection Agency (EPA), California Department of Pesticide Regulation (CDPR) and the Canadian Pest Management Regulatory Agency (PMRA) (US EPA, 2012; EPA, 2014). The framework allows for the estimation of risk as the *likelihood* that a bee will visit the treated crop and collect sufficient pesticide that it will be harmful to the colony. The estimate relies on a combination of exposure models, laboratory tests, and if necessary, tests that represent an increasingly realistic exposure a colony would experience if situated adjacent to a treated crop. EPA risk assessment involves tiers, beginning with the most simplistic and conservative estimates which generate expected environmental concentrations and toxicity estimates from lab studies on individual bees (tier one). Higher tiers (two and three) refine both the expected environmental concentration and estimate effects on the colony level. The goal of each tier is to be conservative with risk estimations that maximize potential environmental concentrations and to use higher tiers to refine exposure estimates (US EPA, 2012; EPA, 2014). Notably, this framework replaced an earlier method of evaluation that estimated the hazards of a pesticide, defined as the *potential* for harm. Unlike risk assessment, estimates of the hazard of a pesticide to honey bees is based on the laboratory toxicity of the product alone, and does not incorporate information about the likelihood of exposure and how exposure translates into harm of the colony.

In parallel to the regulatory shift in assessing the risks of pesticides to honey bees, there has been intense interest in quantifying the effects of pesticides in terms of a hazard quotient (HQ). HQ quantifies the total hazards associated with actual or expected concentrations of pesticides in the environment or bee matrices. These amounts are then related to the LD_{50} values of the pesticides detected (Thompson, 2021). The widespread use of this method in honey bee toxicology followed Stoner and Eitzer (2013) who calculated HQ for pollen trapped from honey bee hives. In order to discern which levels of pesticides were of concern, they assigned threshold values, above which harm to honey bees would be expected. Thompson (2021), however, noted that the threshold values from HQ studies frequently do not align with levels deemed of concern using the risk assessment framework from regulators. Thompson concluded that HQ thresholds likely overestimate the risk of pesticides to honey bees, casting doubt on the validity of HQ.

Although the purpose of this review is not to compare the EPA's BeeREX Risk Quotient (RQ) with the use of HQ in the literature (see Thompson, 2021 for this analysis), it is important to note key similarities and differences between RQ and HQ. Both HQ and RQ are assessments intended to trigger more investigation if a particular threshold is exceeded. However, unlike HQ, RQ estimates exposure from a dietary consumption model which incorporates the expected levels of pesticide in bee diets with the chronic and acute toxicity and feeding rates of each bee caste (EPA, 2014). HQ uses thresholds derived, in most cases, from a 10-day nursing period of adult bees and only adult acute toxicity data. The insights of Thompson (2021) point to deeper issues associated with the widespread use of HQ in the ecotoxicological literature. While Thompson identified the failure of literature which focuses on pesticide contaminated hive matrices to account for actual consumption patterns, we believe there are further problems associated with the current interpretation of HQ estimations.

This review will provide additional information on the role of HQ within the literature and the challenges associated with using HQ to understand how management practices are linked to changes in pesticide risk. As indicated by Thompson (2021), RQ may be a more appropriate method of estimating pesticide risk to bees from detections in bee food resources. However, while RQ provides an understanding of the dietary impacts of single chemicals on honey bee health, HQ can be used to understand factors which RQ does not consider (i.e., wax and bee bodies), including additive hazard from multiple chemical residues. Contextualizing HQ calculations and providing insight into the limitations of HQ as it related to hive health and landscape use patterns can help future authors refine questions around HQ. Our analysis reviews each paper for how HQ was calculated, the way HQ calculations incorporated landscape level honey bee foraging patterns and interpreted the impacts of specific HQ levels on honey bee colony health.

REVIEW PROCESS AND METHODS

This review assesses the use of HQ in understanding pesticide risk to the European honey bee, *Apis mellifera*. A literature search was performed with three search engines; the resulting publications were filtered for inclusion.

First, papers were retrieved using searches for "Hazard Quotient," "Apis mellifera," "Hazard Ratio," and "honey bee" in PubMed, Web of Science, and Google Scholar. Literature referencing Stoner and Eitzer (2013) was included in the review by searching for papers which cited this paper. In total, this process produced 306 papers.

Next, criteria for inclusion in this review were developed. First, all papers included in this review were interested in pesticide hazard to *Apis mellifera*. Each paper included in the review calculated HQ and provided adequate information on how HQ was calculated. This reduced the number to about 150 papers. Second, all papers included in this review were published in peer reviewed journals. Those in industry publications, reports, or meeting notes were excluded. Duplicates were removed at this stage, resulting in 44 papers. Next, papers which did not provide enough information about HQ calculations in relation to study design were excluded from this review, narrowing the pool to 36 total papers included in this review.

Each HQ calculation within this review is considered distinct. This choice was made because many papers included HQ calculations for multiple bee matrixes or scenarios. Separating these calculations allowed individual analysis of HQ calculations and threshold values within each matrix analyzed.

HOW HAZARD QUOTIENT WAS CALCULATED

Hazard Quotient (HQ) was calculated across all papers using two parameters: quantity of pesticide in the bee environment and the toxicity of the pesticide (in micrograms per bee).

HQ = (Actual or expected concentration)/(Toxicity)

The actual concentration of pesticide is most commonly parts per billion of pesticide within bee matrices. The expected concentration of pesticide is most commonly the field application rate (grams of active ingredient/hectare). However, as noted in Thompson (2021), HQ is considered a unitless value. Toxicity was estimated either as oral or contact acute toxicity, represented as the dose required to kill 50% of bees in laboratory assays (LD_{50}; **Table 1**). Although HQ was occasionally estimated for single pesticides, most studies calculated HQ across multiple pesticides by summing together the HQ for each pesticide (HQ_{sum}).

$$HQ_{sum} = HQ_1 + HQ_2 + HQ_3 + \ldots + HQ_n$$

Thresholds were commonly used to indicate the hazards that would likely have negative impacts to honey bee health (**Tables 1**, **2**).

HAZARD QUOTIENT CALCULATED FROM PESTICIDE DETECTIONS IN BEE MATRICES

The most common way HQ was calculated across the studies was by measuring pesticide residue(s) within a bee matrix (i.e., wax, pollen, honey). This type of calculation was performed in 28 studies that were reviewed (**Table 1**). The pesticide residue was calculated from concentrations of pesticide found either in the locations that bees are likely to visit (i.e., nectar and pollen collected from flowers in Hrynko et al., 2019; foliage in Humann-Guilleminot et al., 2019) or from bee matrices.

Four matrices are commonly focused in the literature were pollen (33% of studies), bee bread (17%), wax (17%), and live or dead bee bodies (15%) (**Table 1**). Approximately one third of papers in this review calculate HQ in more than one bee matrix. For each of these matrices pesticide hazard is estimated by taking the concentration of pesticide in the matrix and relating that to the adult LD_{50} of the pesticide; this is a measurement of acute toxicity. There are also differences in the use of an oral or contact LD_{50} of a pesticide to calculate HQ. Contact LD_{50} values have historically been more readily available and were therefore used in the 23% of HQ studies. Some studies chose to use the lower, more conservative LD_{50} value for a pesticide when available, regardless of the likelihood of oral or contact exposure (Traynor et al., 2016). Finally, it is important to note that studies which test bee matrices for pesticides are primarily observational studies which monitor for pesticides in the bee environment, which have recognized limitations and biases; primarily that inferences are weaker in observational studies and replication is challenging to achieve (Eberhardt and Thomas, 1991).

Thresholds differ significantly in the literature for HQ calculated from honey bee matrices (**Table 1**). Many studies, approximately 65%, set no threshold for at least one level and at least one matrix (e.g., Böhme et al., 2018); sets a relevant threshold of 50 for pollen detections of HQ but no elevated threshold. Papers frequently set different thresholds for each matrix calculated for HQ. The most common thresholds set are HQ 50 as a relevant threshold (37%) and HQ 1,000 as an elevated threshold (37%). The heterogeneity of threshold values seen in this review is indicative of the lack of mechanistic understanding of how a HQ value moves from a pesticide detection to a potential impact on hive health.

BIASES ASSOCIATED WITH DIFFERENT BEE MATRICES

Two different terms around threshold HQ values were introduced by Traynor et al. (2016) to provide more nuanced understanding of HQ; relevant and elevated threshold values. The authors consider HQ value at or below the relevant threshold is considered harmlessness. HQ values above the relevant threshold are considered potentially harmful. The authors designated elevated thresholds to indicate unacceptable levels of risk. These thresholds demonstrate one way in which HQ studies attempt to estimate risk; thresholds implicitly make a connection between a pesticide detection level and the likelihood that a given pesticide is likely to cause harm.

The thresholds set for HQ at the hive are based on the percent of the LD_{50} reached in an approximated bee diet, as discussed in Stoner and Eitzer (2013). Stoner and Eitzer (2013) assumed the following: a honey bee adult consumes 9.5 mg of pollen per day throughout her 10-day nursing period; if the bee consumes a pollen diet of HQ 50, a bee will consume 0.05% of her LD_{50} each day; Kasiotis et al. (2018) built on this, noting this consumption would result an in an accumulated risk of death of 0.5% over her nursing period (Kasiotis et al., 2018). It is concerning to use acute LD_{50} values to understand metrics of chronic exposure as there is a mismatch in the toxicity metric of an acute LD_{50} and a threshold based on 10-day period. As discussed in Thompson (2021), these calculations and thresholds do not align with exposure models based on average pollen consumption rates, such as EPA's BeeREX model and are instead based on other metrics of individual or colony health. While LD_{50} equivalents used in HQ thresholds are based on similar feeding models for adult nurse bees used in the Bee-REX model, Thompson (2021) found that using the same detections in bee matrices, HQ overestimated hazard when compared to EPA standards. Justifications for thresholds are based in percentages of LD_{50} equivalents (Calatayud-Vernich et al., 2019) or an expectation of imminent colony death due to high worker mortality (Drummond et al., 2018).

TABLE 1 | A table displaying the contact or oral LD50 value, thresholds associated, and justifications for the thresholds, if provided.

Matrix	References	Contact LD50	Oral LD50	Both LD50	Not listed	Threshold(s)	Justification
Bee bread	Calatayud-Vernich et al., 2018			x (oral if available, contact if not)		Relevant 50 elevated 1,000	10% of LD_{50} during nursing period
	Calatayud-Vernich et al., 2019				x	Relevant 50 elevated 1,000	Percentage of LD_{50} equivalents
	El Agrebi et al., 2020b		x			1,000	10% of the LD_{50} consumed during nursing period
	McArt et al., 2017				x	0.4 0.2	US EPA Level of concern for acute contact exposure European food safety authority acute contact exposure
	Traynor et al., 2016				x	50 relevant 1,000 elevated	0.5% LD_{50} over nursing phase 10% of LD_{50} over nursing phase
	Traynor et al., 2021a				x	50 little risk 1,000 high risk	
Bee bread	Urbanowicz et al., 2019	x				None	
Composite samples (flowers, trapped pollen, bees)*	Frazier et al., 2015				x	10,000	One LD_{50} equivalent
Corbicula-trapped pollen	Böhme et al., 2018		x			Relevant: 50 500	PHQ of 50 would correspond to 0.05% of the LD_{50} consumed in 1 day (resulting in 0.5% of the LD_{50} in an average 10-day nursing period)
	Colwell et al., 2017		x			None	
	Drummond et al., 2018			x		1	1,000 ng per μg/mean bee weight Called "Risk Quotient"
	Favaro et al., 2019			x (whichever is lowest)		Relevant: 50 elevated: 1,000	Böhme et al., 2018
	Friedle et al., 2021		x			Relevant: 50 100	HQ of 50 (Böhme et al., 2018) HQ of 100 is 1% of the LD_{50} per day
	Nai et al., 2017	X				50 High risk: 500	
	Ruiz-Toledo et al., 2018		x			1,000	1% median lethal dose per day
	Smart et al., 2016	x				None	
	Stoner and Eitzer, 2013			x		50 500	0.05% of the LD50 per day 0.5% of LD_{50} per day
	Stoner et al., 2019		x			1,000	1% oral LD_{50} per day
	Tosi et al., 2018				x	1,000	Consuming 1% of the median lethal dose (LD_{50}) per day, which adds up to 10% after the 10 day nursing phase
	Urbanowicz et al., 2019	x				None	
Flowers	Hrynko et al., 2019			x		50–100 low risk 1,000 elevated risk	Traynor et al., 2016

(Continued)

TABLE 1 | (Continued)

Matrix	References	Contact LD50	Oral LD50	Both LD50	Not listed	Threshold(s)	Justification
Honey	El Agrebi et al., 2020b		x			1,000	10% of the LD$_{50}$ consumed during nursing period
	Pohorecka et al., 2017	x				None	
	Ruiz-Toledo et al., 2018		x			1,000	1% median lethal dose per day
	Woodcock et al., 2018		x			None	
Live or dead bees	Calatayud-Vernich et al., 2018			x (oral if available, contact if not)		None	
	Calatayud-Vernich et al., 2019				x	Relevant 50 elevated 1,000	Percentage of LD$_{50}$ equivalents
	Kasiotis et al., 2018			x		50	
	Kiljanek et al., 2017				x	50 relevant 1,000 elevated	0.5 and 10% of LD$_{50}$ reached over nursing period
	Pohorecka et al., 2017	x				None	
	Traynor et al., 2016				x		Low residues found in bees, adult bee samples were not further analyzed
Plant or soil	Humann-Guilleminot et al., 2019	x				1	
Wax	Calatayud-Vernich et al., 2018			x (oral if available, contact if not)		Relevant 250 elevated 5,000	Exposure through this matrix is not well understood
	Calatayud-Vernich et al., 2019				x	Relevant 250 elevated 5,000	Only a fraction of pesticide load is exposed to individuals in colony
	El Agrebi et al., 2020b		x			5,000	Contact exposure is poorly understood and residue detections are high in wax
	El Agrebi et al., 2020a	x				Relevant 250 elevated 5,000	Only a fraction of pesticide load is exposed to individuals in colony
	El Agrebi et al., 2019			x		50 considered a risk 5,000 elevated	Traynor et al., 2016
	Pohorecka et al., 2017	x				None	
	Traynor et al., 2016				x	5,000 elevated	Transmission routes are poorly understood and wax residues are higher compared to other matrices

**Composite samples are composed of flowers, trapped pollen, and bees.*

Studies commonly set different thresholds values for different types of matrices. The reason for these differences is rooted in a recognition of potential unequal exposure of bees to pesticides contained in different matrices. For example, relevant and elevated thresholds used for beeswax are commonly set higher than thresholds in other matrix types, owing to the slower release of pesticides to bees in wax compared to pesticides obtained from eating contaminated honey or pollen (Traynor et al., 2016; Pohorecka et al., 2017; Calatayud-Vernich et al., 2018, 2019). Wax relevancy thresholds are commonly set at 1,000 and wax elevated thresholds at 5,000 (Traynor et al., 2016; Calatayud-Vernich et al., 2017, 2018, 2019; Pohorecka et al., 2017). Notably, none of the papers base their threshold values on empirical estimates of the relative or absolute exposure of bees to pesticides in wax compared to other matrices.

The most common way of estimating pesticide hazard in terms of HQ is by trapping pollen from bees as they return to the hive using external pollen trapping equipment. By intercepting pollen before it reaches the hive environment, this matrix may best represent the external pesticide hazard across the bee's foraging environment. Honey bees are generalist foragers known to travel 3 km away from the hive and trapped pollen can be used to sample the landscape for pesticide usage (Couvillon et al., 2014; Richardson et al., 2015). Trapped pollen can be sorted by color, homogenizing pollen species within the color group (Böhme et al., 2018; Stoner et al., 2019) and can be identified through microscopy and acetolysis (Topitzhofer et al., 2019). However, measuring pesticide residues from pollen in this way has limitations; low pollen availability or poor foraging weather can lead to insufficient pollen collected from traps (Topitzhofer et al., 2019). Moreover, as pollen traps or only engaged for short periods of time (typically 24–48 h) they may over or underestimate prolonged exposure to a pesticide, depending on whether traps are engaged when pesticide application is taking place. For example, Drummond et al. (2018) trapped pollen for a week of each 2-month period, which may not be reflective of the pesticide detections throughout the entire season; it is possible to miss pesticide emissions or to capture rare pesticide emissions and generalize these to the entire study period.

Collection of comb-stored pollen (bee bread) provides an alternative method of sampling pollen that estimates of pesticide hazard over a longer period than is possible using pollen trapping. Comb-stored pollen is processed by bees for long-term storage in the hive; it is packed into cells for storage and mixed with a small amount of nectar (Winston, 1987). Comb-stored pollen is most often collected by opening the hive and scraping "fresh" pollen out of the comb (Traynor et al., 2016; Drummond et al., 2018) and extracting the desired quantity. Bee bread becomes the food for the larvae, nurse bees, and the queen within the colony; therefore, using bee bread for estimation of hazard provides an estimate of pesticide load for the bees consuming this matrix; however, HQ detections from bee bread lack a mechanistic model of the inter-hive mechanisms through which the social aspects of honey bee feeding occurs (Sponsler and Johnson, 2017).

Wax is the structural matrix of the hive secreted by bees used to both store food and rear larvae; frames of drawn comb are commonly exchanged between hives in beekeeping practices (Winston, 1987; Calatayud-Vernich et al., 2017). Wax has a higher lipid content than pollen or honey and may be able to accumulate pesticides more readily (Mullin et al., 2010); even if environmental exposure is low, pesticides can accumulate in wax comb. Using wax to understand pesticide hazard provides valuable insights into an exposure pathway that is currently absent in risk assessment models. El Agrebi et al. (2020b) found that brood comb wax had the highest HQ values of all wax types they studied, indicating potential for exposure of developing larvae. Wax may become contaminated in several ways. First, beekeepers routinely apply miticides in the hive to control *Varroa destructor* and these chemicals have been detected at potentially concerning levels (El Agrebi et al., 2019). Understanding how pesticides in wax may become bioavailable to bees is nuanced; *in vitro* studies which examine realistic pesticide exposure in wax have done so through contaminated diet fed to larval honey bees where diet concentrations of pesticides were based off detections in wax and pollen, resulting in reduced survival of larvae and altering gene expression of detoxifying enzymes (Tomé et al., 2020). However, in a study where wax was removed from contaminated colonies and brood development was tracked, no significant impacts on larvae were found (Alkassab et al., 2020). Wax may be contaminated with pesticides *via* food sources (i.e., wax absorbs pesticides from contaminated pollen and nectar). There is evidence that bee bread and honey have higher HQ values after contact with contaminated wax due to the lipophilic nature of wax and the high levels of contamination common in honey bee wax (Calatayud-Vernich et al., 2017). Finally, even new wax secreted by bees has detectable pesticide loads, indicating that bees may be excreting pesticides from their bodies into this matrix (Calatayud-Vernich et al., 2017). Therefore, wax may be a pesticide sink where bees excrete pesticides into their environment and simultaneously wax may be a source of contamination increasing pesticide residues in bee diets.

Bee bodies can be sampled from within the hive or taken from suspected pesticide poisoning events. Both sampling scenarios present significant biases that are recognized in the literature (Traynor et al., 2016; Pohorecka et al., 2017). The amount of pesticide found on bees likely varies by the age of bees, given that older foraging bees are more likely to have direct contact with pesticides than nurse bees, which have never left the hive. Yet, determining the age of bees while sampling is nearly impossible; commonly, bees are sampled from the broodnest in order to standardize these factors (Traynor et al., 2016). Finally, HQ studies based on bee cadavers vs. live bees are expected to yield different results given that the metabolic processes within live bees begins degrading the pesticide rapidly (Magesh et al., 2017) and therefore any residues left over in the bee body could underestimate pesticide exposure.

HAZARD QUOTIENT CALCULATED FROM FIELD APPLICATION RATES

HQ was calculated from field application rates in eight studies that were reviewed (**Table 2**).

TABLE 2 | A table displaying the contact or oral LD50 value, thresholds associated, and justifications for the thresholds, if provided for HQ calculations from the field application rate.

References	Contact LD50	Oral LD50	Both LD50	Threshold	Justification
Abdu-Allah and Pittendrigh (2018)			x	0.01–100% of field rate	
Barmaz et al. (2010)			x	50	EC guidance document on terrestrial ecotoxicology under council directive
Ladurner et al. (2004)			x	50	EPPO
Perry and Moschini (2020)	x			None	
Thompson and Thorbahn (2010)			x (lower)	50	EPPO
Villa et al. (2000)			x	Below 50 harmless to bees 50–250 slight to moderate risk to bees Over 2,500 dangerous to bees	
Laurino et al. (2011)		x		None	

Studies calculating HQ from field application rates used both a combination of oral and contact LD_{50} values (**Table 2**). Thompson and Thorbahn (2010) advocate for the use of whatever LD_{50} is lower (oral or contact) in order to be as conservative as possible.

Thresholds set for HQ calculated from field application rates also vary within the literature (**Table 2**). Elevated thresholds are uncommon in this methodology; only two of the eight studies included in this review set elevated thresholds. In six of the eight studies included, the relevant threshold of HQ was set at 50. HQ values of 50 are rooted in EPPO regulatory guidelines (European and Mediterranean Plant Protection Organization [EPPO], 2010; US EPA, 2012) and Thompson and Thorbahn (2010) which used HQ calculations and poisoning events to validate thresholds in relation to poisoning events. The EPPO regulations outline a threshold below which a product is not deemed in need of risk assessment. It is, at its core, a conservative filter to remove relatively non-toxic or non-attractive products out of the framework for approval.

HAZARD QUOTIENT AND AGRONOMIC MANAGEMENT PRACTICES

In current literature, HQ and landscape analysis are used in combination to address: (1) where is pesticide exposure occurring in the landscape and/or (2) what blooming plant species are primarily associated with pesticide detections. Some papers (n = 17, **Table 3**) used a geo-spatial component in their analysis of HQ. Authors account for the variation in landcover in a foraging landscape by: (1) classifying a site on important characteristic/management technique (e.g., designate a location as “organic” or “conventional” as in Humann-Guilleminot et al., 2019), (2) determining relative composition of land-use categories surrounding apiaries. These are questions of management practices around an apiary and how different land cover classes or crops may contribute pesticides to detections in bee matrices or dilute pesticide detections in bee matrices.

Most studies that considered landscape composition found it was unclear how crop-specific pesticide use patterns were associated with HQ (**Table 3**). In some studies, HQ estimates were so high that pesticide use patterns from crop areas adjacent to apiaries could not be discerned. For example, Tosi et al. (2018) presents a detailed analysis of HQ detections in trapped pollen over 3 years; this study examined different HQ risk at organically and conventionally managed sites. The study demonstrated that pesticide contamination is widespread throughout Italy and that low-impact agricultural practices do not necessarily reduce pesticide risk to pollinators. Similarly, Humann-Guilleminot et al. (2019) found pesticide hazard to pollinators was high, even at organically managed sites and habitat set aside as unsprayed refuge. Drummond et al. (2018) used the percent of different land classes to contextualize HQ detections within foraging radii and found that HQ was significantly correlated with agricultural land cover. Urbanowicz et al. (2019) investigated the relationship between HQ detections and the prevalence of corn within a landscape. The authors addressed this with two different levels of temporal resolution. Maize is a wind pollinated crop, moderately attractive to honey bees, and is treated with neonicotinoids (United States Department of Agriculture [USDA], 2015; Urbanowicz et al., 2019). The authors found that neither percent maize within the foraging radius of the hive, nor percent maize pollen collected by bees in bee bread was significantly correlated with higher HQ detections.

In some instances, management practices have provided insight into how pesticide hazards are distributed through the landscape. Colwell et al. (2017) found that HQ was associated with site type (fallow, blueberry, cranberry, and apple sites), but that HQ was also associated with local floral diversity. Notably, the sites with the highest floral diversity had the lowest HQ values, and metrics were associated with fallow sites, suggesting that diversity of available forage may reduce pesticide hazard. However, in apple pollination systems, McArt et al. (2017) intensively sampled bee bread from thirty orchards to understand how pesticide risk accumulates in fresh bee bread. Over 60% of the pesticide hazard did not come from pesticides known to be used in apple orchards. This suggests that other cropping systems nearby may be disproportionately contributing to pesticide loads.

Taken in aggregate, however, these studies largely show poor correlation between HQ and specific crop pesticide use patterns. HQ is not reliably correlated with landscape designations around the apiary being monitored or the percentage of land surrounding the apiary (within a reasonable foraging distance) (**Table 3**). One explanation for this poor association is that it assumes bees forage uniformly across all habitat types, providing equal sampling of pesticide residues in the surrounding environment. Yet, dance language analysis has demonstrated that this is not the case (Couvillon et al., 2015; Samuelson et al., 2019). In dance analysis, bees are recorded performing waggle dances and the dance is decoded to determine where the bee is recruiting her sisters to forage within the landscape (Couvillon and Ratnieks, 2015). Bees prioritize resources close to the hive and foraging locations change with fluctuations in floral resources; bees will forage farther from the hive in times of floral dearth (Couvillon et al., 2014). In some cases, the change in floral resources result in shifts in foraging behavior which results in bees spending more time in crops with elevated pesticide use, like oilseed rape (Garbuzov et al., 2015), resulting in disproportionate exposure to pesticides relative to the aggregate in the landscape.

HAZARD QUOTIENT AND LAND USE CHANGES

In some situations, analysis of HQ from management of a single field is inadequate and an understanding of an aggregate exposure pattern is needed on a landscape level. In this respect, HQ has been used to understand both validate thresholds and to understand changes in pesticide use patterns over time. HQ calculations from the application rate have also been used to validate current thresholds for regulatory decision making around potential honey bee poisoning events (Mineau et al., 2008; Thompson and Thorbahn, 2010). In these studies, HQ thresholds appear validated; that is, thresholds were exceeded during poisoning events. This indicates that poisoning events are not occurring below relevant thresholds.

Perry and Moschini (2020) used HQ at the emissions point to understand how pesticide risk to bees (and other organisms) changed over time in corn cropping systems. During their study period, 1998 to 2014, authors found that while more pesticide treatments were being applied, the HQ risk to bees from these applications remained relatively consistent while risk to fish, mammals, and birds decreased. Notably, Perry and Moschini (2020) does not consider the attractiveness of corn to honey bees, nor the timing of the application of the pesticide. However, such conclusions are at odds with other studies using of HQ to infer historical trends. Two other papers have examined how hazard calculations may underestimate risk during a similar time period using an estimation of toxicity, Acute Insecticide Toxic Load (AITL), DiBartolomeis et al. (2019) factored in the environmental half-life of insecticides and found a 4 and 48-fold increase in acute insecticide toxic load for contact and oral toxicity due to the use of neonicotinoids in agriculture in the United States. Douglas et al. (2020) found a 9-fold increase in oral toxicity to bees with some regions showing a 121-fold increase in toxicity insecticide load driven by the use of seed treatments in corn and soy. Both HQ and AITL do not account for the actual exposure dynamics of honey bees foraging on contaminated crops or contacting residues lingering in soil and plants. Actual exposure is the result of the combination of foraging dynamics and pesticide applications to bee attractive crops (Sponsler et al., 2019), and simply estimating the hazards within the environment through either HQ or other metrics does not capture this process.

TEMPORAL DIMENSIONS OF HAZARD QUOTIENT

When pesticide hazard is estimated using HQ at the hive, it reflects the potential dietary exposure of bees within a specific environment. Exposure at the hive has detected banned or misused pesticides in bee products (Ruiz-Toledo et al., 2018; Woodcock et al., 2018). In some cases, studies report the

TABLE 3 | A table displaying how landscape context is used in HQ calculated from bee matrices.

	Landscape analysis by site type	Landscape analysis by percent composition of land classes
HQ correlated with landscape	Colwell et al., 2017; Böhme et al., 2018; Calatayud-Vernich et al., 2019	Drummond et al., 2018
HQ not correlated with landscape factor of interest or relationship unclear	Stoner and Eitzer, 2013; Frazier et al., 2015; Nai et al., 2017; Tosi et al., 2018; El Agrebi et al., 2019; Humann-Guilleminot et al., 2019	Smart et al., 2016; McArt et al., 2017; Calatayud-Vernich et al., 2018; Ruiz-Toledo et al., 2018; Stoner et al., 2019 Urbanowicz et al., 2019

Sixteen papers using HQ at the hive to understand landscape context. These papers are divided into two categories: classifications based off percent land class in the foraging radius of the hive, and site-type classifications where only the immediate surroundings were considered.

detection of illegal pesticide use (Woodcock et al., 2017; Tosi et al., 2018). HQ at the hive uses honey bee colonies as ecological sensors which collect and aggregate information from a landscape and report it to scientists (Richardson et al., 2015). These detections are snapshots of exposure at a given time, and sampling at different times of year can produce variation in pesticide residues (Böhme et al., 2018). This specificity in time-bound measurements can demonstrate where pesticide exposures may be taking place. For example, Böhme et al. (2018) sampled pollen every day and stratified within samples to determine the relative pesticide contributions of specific taxa to the HQ value of the sample. This methodology was able to identify that the sub-fraction of grapevine pollen (*Vitis vinifera* L.) was disproportionately contributing pesticide residues to the composite sample. Similarly, Stoner et al. (2019) found that *Spiraea* spp. L. pollen had high concentrations of pesticide relative to the other pollen in their samples. Favaro et al. (2019) examined changes in HQ before and after apple bloom; however, the high variability in HQ resulted in no association between HQ values and timing of trapping. Favaro et al. (2019) also divided pollen into colors and found no associations between HQ and pollen color, which they attributed to the potential contamination of pollen before color sorting occurred or that pesticide contamination was high in both apple orchards and the surrounding environment.

When pesticide hazard is calculated using HQ from the field application rate, estimations are based on application rate of pesticide for a given crop type. Because LD_{50} values and land use information are readily available through public agencies (Douglas et al., 2020), this method of HQ could allow models of past or future pesticide use patterns to understand pesticide hazard. As in Chen et al. (2017), this also allows the potential to make recommendations to land managers interested in reducing pesticide exposure to bees by identifying hazard-risk scenarios and taking mitigating action. This method assumes that bees will contact the full application rate and is potentially useful as a worst-case-scenario estimation of hazard. The difficulty of connecting mitigating measures at a field-level (i.e., avoiding sprays to bee attractive crops or spraying at night when bees are not foraging) to hazard calculations from the application rate, as foraging behavior is not accounted for (Sponsler et al., 2019).

HAZARD QUOTIENT AND HIVE HEALTH ENDPOINTS

A key limitation of HQ estimates is that they attempt to evaluate the likelihood of negative impacts to colony health based on two strongly mediated points of data; laboratory acute and chronic toxicity tests or field application rates. In contrast, risk assessment integrates both types of data into a framework that links exposure and toxicity. This poses considerable challenges. Five studies included in this review link colony health outcomes to HQ values: Traynor et al. (2016) in the eastern United States, Traynor et al. (2021a) in the United States, Lee et al. (2019) in the United States, Smart et al. (2016) in the northern great plains, and El Agrebi et al. (2019) in Belgium. Of these five, only one study found clear association of colony health with HQ and two found a weak association.

Traynor et al. (2016) examined how colony death and queen events were related to HQ detections by collecting matrices from commercial colonies providing migratory pollination services. Colony health and colony loss were associated with a higher number of generally relevant HQ detections (HQ $>$ 50) and HQ values with large contributions from fungicides. These associations were stronger than actual HQ values; the number of pesticides detected within a sample was a stronger predictor of colony death than the total HQ additive value. In a study tracking HQ in bee bread over 7 years and across the entire United States, Traynor et al. (2021a) found no statistically significant associations when tracking how HQ changed over time.

HQ can only be considered additively; HQ cannot be used to understand synergistic pesticide hazard without modification. Although multiple papers assessing risk with HQ note this (e.g., Colwell et al., 2017; Stoner et al., 2019), few adjust HQ. Adjustments may be unnecessary as Belden and Brain (2018) has suggested that testing of tank mixtures of multiple chemicals is not warranted; instead suggesting a focus on the chemical that dominates toxicity. Conversely, Sanchez-Bayo and Goka (2014) suggest addressing these underestimations of risk by including a synergistic factor in the estimation of the LD_{50} of pesticide mixtures. However, determining synergistic factors is time-intensive and must be computed for each combination of chemicals (Sanchez-Bayo and Goka, 2014).

Fungicide and insecticide synergies may be one area where HQ chronically underestimates risk, warranting further exploration of how this has been demonstrated in HQ literature. The use of insecticides and fungicides in almond pollination systems is wide-spread; from 2007 to 2015, acres of almond crop treated with insecticide and fungicide has increased (Wade et al., 2019). Fungicides are generally considered low-toxicity for contact to pollinators and have high LD_{50} values, indicating that bees can be exposed to comparatively large doses of fungicide with little acute toxic effects (Ladurner et al., 2004).

Fungicide and insecticide synergism, while documented at field-realistic exposure levels (Wade et al., 2019), is still concentration dependent and cannot be assumed to occur based on the presence of two pesticides in a sample or a system. In both larval and adult toxicity tests, combinations of fungicide and insecticides increased acute bee mortality compared to controls (Iverson et al., 2019; Wade et al., 2019). Field trials which exposed bees to combinations of insecticides and fungicides have shown negative effects on both larvae and adults, indicating a likelihood that at high concentrations these chemistries could impact colony population size and adult foraging force (Fisher et al., 2021). Even in isolation, fungicide exposure is associated with brood loss, queen events, and reduced hypopharyngeal gland size (Traynor et al., 2021b). These interactions, which are known to increase mortality in bees, would not be captured in a HQ value, as HQ is only capable of capturing additive effects.

Despite its difficulty, understanding synergy in pesticide risk is a critical missing piece of understanding realistic pesticide risk to pollinators. Several classes of insecticides (carbamate, organophosphates, and pyrethroids) and azole fungicides are known to be over represented in synergistic interactions in pesticide mixtures (Cedergreen, 2014); all of these are commonly detected in bee matrices. The mechanism behind this synergy is rooted in the potential for triazole fungicides to inhibit detoxifying enzymes of the honey bee, increasing toxicity of insecticides when these pesticides co-occur (Haas and Nauen, 2021; Haas et al., 2022). Within the studies covered in this review, several pesticide combinations known to synergize co-occurred within a system or sample; although it is beyond the scope of this review to address every instance, these examples demonstrate how common it is to underestimate pesticide risk when relying on HQ alone. Colwell et al. (2017) report that two combinations of pesticides: chlorothalonil and coumaphos, chlorothalonil and fluvalinate, are known to have synergistic effects and at least one of these combinations occur at every site the authors sampled. Frazier et al. (2015) determined that pumpkin pollination systems contain the highest or second highest concentrations of chlorothalonil, coumaphos, and fluvalinate-tau. Despite this, pumpkin had a moderate total HQ compared to other systems but exhibited a steep drop-off in adult bee foragers, indicating colony-level impacts from pesticide stress. This provides evidence of a mismatch between HQ values within the system (low) and potential stress on the honey bee colony due to a decrease in foraging force. As noted in Sponsler and Johnson (2017), less foraging bees can reduce the potential exposure of the colony to pesticides, through reduced incoming contaminated pollen and nectar. Traynor et al. (2016) also noted that increased chlorothalonil HQ values in bee bread were specifically associated with colony death, while HQ detections in general were not. It is possible that the increased colony death associated with this detection may be due to the likelihood of chlorothalonil to synergize with other pesticides. Traynor et al. (2021b) found that fungicide residues present in bee bread were significantly associated with disease (*Nosema* infection and brood disease) and queen issues. These co-occurrences, while interesting, do not directly indicate synergism is occurring, however they do point to the potential for synergistic toxicity to occur if each pesticide is present in a high enough concentration.

Lee et al. (2019) analyzed the relationship between complete and unbroken brood pattern and patchy brood pattern and found that HQ was not correlated with brood pattern. However, the number of pesticides detected was significantly correlated with brood pattern in at least 1 year. Notably, Lee et al. (2019) found much lower HQ values in wax throughout the study compared to Traynor et al. (2016) which may explain the lack of connection with brood pattern.

The remaining two studies which examined colony health parameters did not find any significant correlation with HQ detections. Smart et al. (2016) examined the percent loss of colonies in six apiaries over 3 years. A strong relationship was found between percent uncultivated forage land and apiary survival; pollen quantity was also found to influence apiary survival more than pollen diversity and did not appear to be related to HQ values at the same sites. This suggests that the forage quantity (and to a lesser degree, quality) had a larger impact on colony survival than HQ detections. Similarly, El Agrebi et al. (2019) did not find any link between HQ detections of flumethrin and apiaries where colony losses exceeded 10%. As this study examined only one pesticide at a county-wide scale, it is possible that other pesticide detections or management practices had stronger impacts on colony health than HQ of a single pesticide.

Other factors beyond HQ values may have a direct impact on the success of the colony, confounding the relationship between HQ detections and hive health. For example, mite levels of *Varroa destructor* and viruses associated with this parasite are well documented to have impacts on honey bee colony health and have been consistently identified as one of the major drivers of annual colony losses. Traynor et al. (2021a) noted that higher HQ scores were associated with both the extreme high and extreme low ends of Varroa levels; that is, HQ values were highest in colonies with very little mite presence or 10 + mites per 100 bees. The authors interpret this as evidence that either Varroa are more fit in environments of high pesticide residues or the adult bee population has been reduced by contamination of the pollen (Traynor et al., 2021a).

SYNTHESIS

Calculating HQ is a growing practice among researchers and it is used to make inferences on the risk of specific pesticides to honey bees. We found that HQ is currently being calculated from two points along the path of a pesticide from application to bee, from the amount of pesticide accumulating in bee matrices and dead bees or from the rate of the pesticide applied to a crop. Thresholds are then used to move discussions of hazard into the terminology of risk. Yet, thresholds in HQ calculations are inconsistent across studies and HQ is not consistently associated with hive health measurements.

One concern which has been presented throughout this review is the lack of a full, mechanistic model for understanding pesticide exposure both as it relates to foraging dynamics and pesticide emissions (Sponsler and Johnson, 2017; Sponsler et al., 2019) and transfer of pesticides within the social structure of the hive itself (Sponsler and Johnson, 2017). This is perhaps illuminated when comparing honey bee ecotoxicology with another area—aquatic toxicology. For example, in aquatic toxicology, mechanistic models exist to predict impacts of pesticide applications to organism by integrating key factors associated with the application such as landscape composition, weather, and other abiotic factors (Janney and Jenkins, 2022). These models can, and have, been validated with continuous water sampling, even though "grab" sample detections do not accurately represent the system (Janney and Jenkins, 2022).

Models like these and continuous sampling can be used to answer central questions of risk assessment: for a given application of pesticide, at a given rate, on a particular crop—is this pesticide safe? HQ is not capable of answering that question for several reasons. Most importantly, as mentioned above, we lack an understanding of the dynamic mechanism by which a pesticide makes its way from a pesticide sprayer to a colony. This limitation expands on Thompson (2021) who observed that HQ calculations from contaminated pollen fail to take advantage of known about the consumption rates of developing larvae. Our observation goes further to point out that the process by which a larva becomes exposed to contaminated pollen is but one segment of the larger pathway by which a bee becomes exposed. HQ has considerable difficulty connecting field applied pesticide rates to residues found in colonies, providing descriptive, rather than predictive power. Second, HQ remains rooted in acute, individual bee toxicity rather than chronic hive toxicity, which is a problem when thresholds set in the literature assume accumulation of toxic load over days to weeks. HQ is based off of contact or oral LD_{50} values, however, actual exposure mechanisms are more complex than these toxicity metrics would imply. It is for these reasons that we believe that HQ is not reliably linked to hive health outcomes in short (one season) or long (years) time frames. It is often difficult to use HQ to understand or predict colony health outcomes; in some ways, this is to be expected as HQ is a tier-one assessment tool; however, in its role as a monitoring or observational tool, there are not clear connections between HQ and hive-level health metrics. Risk estimation would benefit greatly from a mechanistic model that could use lab assessments to predict risk in the field. Tools regarding these models may be emerging in the form of predictive, mechanistic models that demonstrate increased likelihood of synergy between compounds (Haas and Nauen, 2021; Haas et al., 2022). However, HQ has been used to link pesticide detections in bee tissues with negative outcomes for colony-level health. Moreover, the disconnection between specific pesticide uses means HQ provides little insights into how a pesticide use could be mitigated to reduce risk (e.g., by changing the application rate or formulation or timing of treatment).

While many of the studies we reviewed use HQ to predict the risk of pesticides to bees, they do so in a way that diverges from how regulatory agencies assess risk, which relies on predicting the quantity of pesticide likely to be collected by and consumed by bees. Where the goal of hazard estimation is to understand dietary risk through consumption of contaminated nectar and pollen, RQ calculated using BeeREX may be the most appropriate model (Thompson, 2021). However, while Thompson (2021) identified the need to incorporate consumptive models of exposure, there are additional issues with HQ calculations identified in this review. As HQ is currently used in the literature, it is difficult to connect hazards to specific pesticide use practices. It is assumed, for example, when HQ is calculated from an application rate that all the pesticide reaches a foraging bee. In contrast, while HQ calculated from hive matrices can aggregate pesticide hazard, it has proven unable to trace these hazards back to specific pesticide uses. What becomes clear from this review is that the use of HQ misses a centerpiece of pesticide eco-toxicology, between point of emission and pesticide accumulation in the hive—field level exposure. Given this limitation, HQ methodologies have proven inadequate to addressing key questions around mitigating hazards, most prominently how hazards might be reduced using modified pesticide use practices (e.g., restricting sprays to the evening, spraying at lower rates, using precision spray technology).

This further highlights a difficulty within HQ literature—the difference between regulatory risk and the consequences of pesticide exposure for an individual hive. In linking HQ to hive health outcomes, researchers may be able to connect health impacts to relevant levels of pesticide in a hive (Traynor et al., 2016). However, this does not illustrate how pesticide use patterns could change to reduce those negative impacts, and therefore reduce risk. It is interesting and compelling to understand that certain levels of pesticide within a hive are associated with queen events or hive death. However, this cannot provide information on how bees are exposed (on what crop, at what time, under what use practices). Therefore, there is considerable need to understand the limitations of using HQ to predict the true risk of specific pesticide use to honey bees. Finally, our review points in the direction of the need to address the missing element in HQ studies, namely a more mechanistic and empirically grounded model of how bees are exposed to specific pesticide under field conditions.

AUTHOR CONTRIBUTIONS

EC, AM, and RS contributed to conception and design of the review. EC organized the review process and wrote the first draft of the manuscript. AM and RS provided feedback and edited the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Historic and Contemporary Land Use Shape Plant-Pollinator Networks and Community Composition

Camila A. Cortina[1]*, *John L. Neff*[2] *and Shalene Jha*[1,3]

[1] Department of Integrative Biology, University of Texas at Austin, Austin, TX, United States, [2] Central Texas Melittological Institute, Austin, TX, United States, [3] Ladybird Johnson Wildflower Center, University of Texas at Austin, Austin, TX, United States

Globally, grasslands represent a critical but shrinking habitat for native plants and pollinators, with declines driven by alterations to landscape-scale habitat cover and local-scale disturbance regimes, among other factors. Specifically, as cities expand in size, an increasing proportion of regional pasture and grassland habitat is being replaced by urban development, and fewer periodic grazing and burning regimes are being supported locally, despite evidence that such regimes promote plant species richness and facilitate their interaction with native pollinators. The quantification of these plant-pollinator networks—through indices such as network connectance, specialization, nestedness, and robustness—can provide a unique opportunity to characterize key structural properties of species interactions and their response to human management and seasonal phenology. While urbanization and local disturbance regimes likely influence plant and pollinator communities and their interactions, past research in this area has primarily been conducted at limited spatial and temporal scales and has not typically quantified the impacts of both local and landscape forces on network properties. In this study, we investigate the effects of contemporary (past 10 years) and historic (prior 90 years) disturbance regimes on plant-pollinator community composition and network structure across more than 200 km of grassland in Central Texas. Our analyses indicate that for plant and pollinator communities, both contemporary and historic land management practices have led to significantly dissimilar community composition. Plant and pollinator richness and network nestedness are negatively correlated with phenological period, while pollinator richness is positively correlated with landscape-scale (2 km) urbanized land cover and is higher in historically grazed land, likely due to greater food and nesting resource availability. In contrast, we show that network connectance is positively correlated with phenological period and negatively correlated with landscape-scale urban cover. Finally, we show that pollinator robustness, a measure of resilience to plant species loss, is positively correlated with landscape-scale urbanization, likely due to greater redundancy provided by common weedy plant species. Overall, our results demonstrate that historic grazing regimes, current urbanization levels, and distinct phenological periods can simultaneously drive plant-pollinator community composition and network dynamics in shrinking but critical grassland ecosystems.

Keywords: grassland, urbanization, prescribed burns, phenology, grazing

Edited by:
Christopher Williams,
Liverpool John Moores University,
United Kingdom

Reviewed by:
Diane Lynn Larson,
United States Geological Survey
(USGS), United States
Wayne C. Zipperer,
Southern Research Station, Forest
Service, United States Forest Service
(USDA), United States

***Correspondence:**
Camila A. Cortina
ccortina@utexas.edu

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INTRODUCTION

In ecology, disturbances are often defined as stochastic processes which remove a substantial amount of biomass from an ecosystem at a given point in time (White and Pickett, 1985) and include processes such as wildfires, large mammal grazing, windstorms, and riverbank erosion (Shea et al., 2004). These disturbance regimes have dramatic impacts on vegetation density, ground cover, and soil substrate availability (reviewed in Mori, 2011), and can alter the diversity and flowering patterns of terrestrial plant communities (Sprugel, 1991; Moranz et al., 2012). Given that disturbance regimes can influence plant diversity and flowering patterns (e.g., Collins, 1987; Grundel et al., 2010; Moranz et al., 2012), they also have the potential to critically impact native insect pollinator communities [Peralta et al., 2017; reviewed in Koltz et al. (2018)], as many pollinators depend on plants for pollen and nectar to fuel their activity and provision thei1r brood (Michener, 2000). Specifically, contemporary land management practices such as prescribed burning, grazing, and mowing, which are employed globally (Bond and Keeley, 2005; Mapiye et al., 2008), have been shown to alter plant community composition (Collins, 1987; Howe, 1994) and ground cover (Gibson, 1988; Fidelis et al., 2012) across grassland systems. Indeed, there is growing evidence that current practices of low-level grazing (Vanbergen et al., 2014), occasional mowing (Weiner et al., 2011, 2014), and intermittent burning (Brown et al., 2017), may increase plant flowering, which could potentially alter pollinator abundance, diversity, and plant-pollinator interactions (e.g., Vanbergen et al., 2014). However, these studies are limited and have typically not considered the critical impact of historical land use when interpreting contemporary disturbance effects on plant and pollinator communities.

An increasingly large body of ecological research has demonstrated that historic land-use can have lasting impacts on contemporary vegetation assemblies (Greenlee and Langenheim, 1990; Floyd et al., 2003; Taverna et al., 2005; Ellis and Coppins, 2007; du Toit et al., 2016) and can even predict contemporary pollinator abundances (Cusser et al., 2015). For example, Taverna et al. (2005) found that current day vegetation patterns within hardwood tree stands were linked to past agricultural use, and Johnson et al. (2015) found that historic land-use, in the form of garden or building sites, differentially drove contemporary plant beta diversity. In a study of early successional forests, abandoned pastures supported very distinct contemporary plant species relative to abandoned crop fields (Benjamin et al., 2005). Likewise, in grassland-dominated bioregions, historic land use, including grazing or farming, has been documented as a significant predictor of pollinator abundances ∼50 years later (Foster et al., 2003), likely due to the fact that overwintering insects, like solitary bees, can have delayed population responses to pulsing floral resources that can be seen for many years after the disturbance event (Cusser et al., 2015). Within woody grasslands, past work has documented multi-decade lags in the impact of urbanization, where dissimilarities in contemporary vegetation composition were best predicted by landscape features ∼20–40 years before the survey period (e.g., road network density, percentage of natural area) (du Toit et al., 2016). In the same study, vegetation species richness of open grasslands was also predicted by recent (landscape features from 1 to 2 years before the survey) urbanization events. These studies have highlighted the importance of evaluating both contemporary and historic land management practices when quantifying drivers of plant and pollinator community composition and species interactions.

Species interaction networks, such as plant-pollinator networks, are excellent tools for quantifying the structure of mutualistic interactions [reviewed in Bascompte et al. (2003), Thébault and Fontaine (2010), and Dehling (2018)] and can also capture the impacts of local management and landscape composition on these critical interactions (e.g., Memmott et al., 2004). For example, networks can characterize the degree of connectedness (proportion of actual links to all possible links, sensu Dunne et al., 2002), specialization (the degree of niche partitioning across species, sensu Blüthgen et al., 2006), and nestedness (the degree to which specialized interactions are bound within more generalized interactions, sensu Bascompte et al., 2003) within a community. Interestingly, past studies have revealed that increases in floral species richness due to grazing can cause decreases in plant-pollinator network nestedness and increases in connectance (Vanbergen et al., 2014), indicating that communities with high biomass turnover rates may have a low buffer against specialized species loss. Indeed regional urbanization has been shown to lead to bird species loss, leaving only those species adapted to urban environments (Schneiberg et al., 2020); this may lead to increases in interaction evenness, where most animals are visiting the same plant species, typically because the remaining animal species tend to be generalist (Schneiberg et al., 2020). In addition, past work has indicated that decreases in local vegetation richness and structure can cause species loss and decreases in nestedness *via* a decline in floral resource availability (Moreira et al., 2015), though it is not known if this pattern persists across phenological periods within human-altered landscapes.

Indeed, because flowering duration for many plant species is short and pollinators are also often short-lived, the phenological stage of a plant and pollinator community is necessary to consider when quantifying interactions (Olesen et al., 2008), as network features such as network specialization can vary with the seasons and across different levels of floral resource availability (Harrison et al., 2018; Souza et al., 2018; Escobedo-Kenefic et al., 2020). Studies that have measured week-to-week variation in plant-pollinator networks demonstrate that interactions can be highly variable in their connectance and robustness, leading to flexibility (changing values between timepoints) in the network structure relative to the cumulative network of all interactions in a season (CaraDonna and Waser, 2020). Connectance is another feature of networks that can vary based on the season, often due to fluctuations in the size of the network; networks tend to have lower connectance when there are more overall interactions occurring (Basilio et al., 2006). On the other hand, nestedness tends to increase with network size (Bascompte et al., 2003) and can be indicative of reduced interspecific competition and increased species coexistence (Bastolla et al., 2009). Despite the importance of phenology in network structure, past work conducted within human-altered landscapes has rarely explored the impacts of phenology alongside contemporary and historic land management practices.

In this study, we use a model grassland system in Central Texas to quantify the impacts of historic and contemporary land management on plant and pollinator community composition and interaction network structure. We hypothesize that similarity in contemporary disturbance regimes drives plant and pollinator community similarity more than historic land use. Specifically, we predict that sites that are currently actively managed (e.g., frequent fire or mowing) will exhibit greater plant and pollinator species richness than passively managed sites and will be more similar in composition than those with the same historic land use. Pollinators, and bees in particular, respond quickly to changes in vegetation and ground cover (Kimoto et al., 2012) thus, we anticipate that areas that are currently actively managed will have more similar plant and pollinator composition. We also hypothesize that landscapes with higher levels of surrounding urban cover will have lower network nestedness, robustness, specialization, and plant and pollinator species richness, due to a lack of colonizers that would otherwise contribute to community stability.

MATERIALS AND METHODS

Study System

Research was conducted within 10 grassland study regions within rapidly urbanizing Central Texas, extending across more than 500 km (SW corner: 30.1015 N, 97.9608 W, NE corner: 33.6494, 95.6987 W) and comprising of state, federal, and non-profit managed grasslands (described in Ritchie et al., 2016). To characterize each study region, five equidistant 50 m × 50 m plots were equally spaced along a 1.2 km linear transect within each region (Jha and Kremen, 2013; Jha et al., 2013). Based on land manager interviews, we classified study regions based on both historic and current land management practices (similar to Gustavsson et al., 2007). Specifically, study regions were classified as either "historically farmed," with hayfield or homestead farming between the late 1900's to the early 1990's, or "historically grazed," with cattle and/or goats in the same time period. Contemporary management was categorized as either "active" or "passive," with active being mowed or burned at least twice in the 10 years prior to sampling, and passive being unburned and unmowed in the 10 years prior to sampling (i.e., unmanaged). Management practices were orthogonal, as each current management category consisted of both historic land use types. Many past studies have grouped mowing and burning together given that they both remove substantial biomass and can have similar effects on plant production (e.g., MacDougall and Turkington, 2007; Dickson, 2019; Vermeire et al., 2020), though it is possible they distinctly impact individual pollinator species, a topic outside the scope of this study. We used the 2012 National Land Cover Database (30 m resolution, Homer et al., 2015) to characterize percent cover at a 2 km radius from the centroid of each study region, by first creating two broad categories, urban (made up of open, low, medium, and high development) (defined at mrlc.gov) and natural habitat (made up of grassland, forest, and shrub) (as per Plascencia and Philpott, 2017); because these categories were highly correlated (Pearson correlation = −0.878) we chose to use urban land cover in further analyses, as per many other studies (e.g., Matteson et al., 2013; Cusser et al., 2015; Plascencia and Philpott, 2017; Sexton and Emery, 2020). The 2 km radius was chosen to include typical pollinator foraging distances (Greenleaf et al., 2007), as in past studies conducted within the region (e.g., Ballare et al., 2019). In our study system, urban land cover was primarily comprised of low-level development in rural areas, including low-density housing in previously natural or agricultural areas (Hansen et al., 2005). This type of development is occasionally referred to as exurban and is one of the fastest types of land conversion in the United States, given substantial human relocation from cities to areas beyond the suburbs (Hansen et al., 2005).

Pollinator and Floral Resource Sampling

In the summers of 2012 and 2013, we surveyed floral communities *via* quadrat surveys, and we sampled native bees and butterflies *via* netting and trapping at each plot during three distinct phenological periods: early bloom (April 18 to May 15), mid-bloom (May 20 to June 16), and late bloom (June 20 to July 14) (Ritchie et al., 2016). Plots were sampled once during each period. Specifically, during each of the three phenological periods, we measured floral species richness and floral density in 30 1 m × 1 m quadrats per site. The quadrats were evenly positioned 4 m apart along three 50 m transects running from North to South that were located at 10 m, 25 m, and 40 m from the NW corner of the plot (Ritchie et al., 2016). Specifically, within each quadrat, the number of forb inflorescences per species were counted. We also quantified ground cover (bare ground, vegetation, rocky, and impervious cover) within each quadrat, and across the study system, and we measured the size of five flowering heads per species to calculate total floral cover. Ground cover metrics were highly correlated (e.g., bare ground and vegetation cor = −0.434, $p < 0.0001$), therefore we focused on bare ground for further analyses (Ballare et al., 2019).

For the pollinator surveys, 2 researchers netted for 30 min by walking slowly back and forth on the east or west side of the plot for 15 min and then switching sides with their partner for the other 15 min, between the hours of 7 am and 12 pm during each phenological period, and only on sunny days. Timers were not stopped while insects were put into kill vials (Ballare et al., 2019). During this 30-min time period, all native bees and butterflies observed foraging on flowers were caught and the flower species was recorded, as in past plant-pollinator network studies (Winfree et al., 2014). We focused on bees and butterflies as in previous studies (Buhk et al., 2018; Librán-Embid et al., 2021) and because these groups are among the most common and effective pollinator taxa in the study region (Sexton and Emery, 2020). Individuals were placed in separate kill jars and all individuals were pinned, labeled, and identified to species. After completing visitation surveys, pan trapping was conducted by placing 30 pan traps (6-oz plastic bowls, SOLO model number: PB9-0099) 1 meter apart and alternating by color between white, blue, and yellow, in an X-formation in the middle of the study plot (LeBuhn et al., 2003) and blue vane trapping was conducted by placing 4 blue vane traps in the center of the plot, hanging one meter off the ground on a wooden stand (Ballare et al., 2019). The pan-traps were filled with 4 oz of a diluted soap water (1 gallon water: 1 tbsp Dawn dishwashing soap) and left in the field for 24 h, after which insects were stored in 90% ethanol, and blue vane traps were left in the field for 5 days before specimens were collected and stored in 90% ethanol (as per Ballare et al., 2019). To comprehensively characterize each study region, the ground cover data, netted pollinator and plant data, and trapped pollinator data from the five plots within each region were combined (Baldock et al., 2015) for each of the phenological periods (similar to Prendergast and Ollerton, 2021), for a total of 3 ground cover, netted pollinator and plant, and trapped pollinator datasets per study region per year ($n = 60$ per dataset).

Network Analysis

Plant-pollinator networks were created from the netted data using the R package bipartite (Dormann et al., 2009). We focused on network-level nestedness (NODF), specialization (H2), connectance, higher (pollinator) and lower (plant) level species richness, and higher- and lower- level robustness. Nestedness is a term used to describe the structure or organization of network interactions, where more generalist species from both the higher and lower orders (animals and plants) interact with more specialist species (Bascompte et al., 2003) and is measured by the overlap and decreasing fill of the plant-pollinator matrix, with values between 0 and 100 where higher values indicate higher nestedness (Almeida-Neto et al., 2008; Ulrich et al., 2009). Past work suggests that greater nestedness is indicative of lower interspecific competition and greater coexistence between species (Bastolla et al., 2009) and thus is a signature of more stable communities (sensu May, 1972) that rebound more quickly to equilibrium following perturbations (Thébault and Fontaine, 2010). Specialization (H2) describes the entire network's level of specialization, or the degree of niche partitioning across species (Blüthgen et al., 2006) compared to the expected interactions given the number of interacting species (Dormann et al., 2009), where a value of 0 represents no specialization and a value of 1 represents a completely specialized network. Connectance is the proportion of all possible links in the network that are connected, where a value of 0 indicates no interactions and a value of 1 indicates that all plant species are interacting with all pollinator species. Higher levels of connectance lead to higher levels of robustness to extinction (Dunne et al., 2002) as well as greater stability (sensu May, 1972). Higher level robustness characterizes the pollinator guild and lower level robustness characterizes the plant guild, where the area below the extinction curve quantifies the robustness of the system to species loss; this is based on the assumption that if a fraction of species from one guild are eliminated, then many species of the other guild will go extinct (Dormann et al., 2008). For nestedness, robustness, and specialization indices, we converted these values into Z-scores by calculating the mean of the network index divided by the standard deviation of 1,000 null models created with the nullmodel function in the package bipartite and subtracting this from the observed network level value (Vázquez and Aizen, 2003). We decided to use the z-scores for this because it allows comparison of the focal network to what is expected if all interactions were random (Gotelli, 2001).

Composition Analyses

We tested for differences in plant and pollinator community composition across historic land-use, current management types, and phenological period using permutational MANOVA using the adonis function in the R package vegan, with year as a controlled stratification factor given that the same regions were sampled 2 years in a row. Specifically, PERMANOVAs were conducted on the raw, $\log(X + 1)$ transformed, and presence absence data to control for compositional differences driven primarily by abundance (Ballare et al., 2019). We used bray-curtis dissimilarity as this metric is commonly used for community studies (Burkle and Alarcón, 2011). We also ran the PERMANOVAs using the morisita-horn metric and found similar results (**Supplementary Table 5**). We used non-metric multidimensional scaling with the metaMDS function in the R package vegan to visualize differences in the communities.

Habitat Indicator Species Analysis

We used the multipatt function in R to perform multi-level pattern analysis in the indicspecies package to quantify indicator species for both contemporary management and historic land use types (package "indicspecies"). Indicator species capture the strength of the relationship between species and the groups of regions where they occur, and indicate which species are the predominant species in that habitat type and not in others (Cáceres and Legendre, 2009).

Regression Analyses

We used regression models to investigate the impact of five predictor variables: historical land use (farmed or grazed), current land management type (active or passive), landscape-level urban habitat cover (2 km radius), phenological period (1, 2, or 3), and local bare ground cover on two response variables from the trapped datasets, pollinator abundance and richness, and seven network response variables from the netted data: higher-level species richness, lower-level species richness, specialization, connectance, nestedness, higher-level robustness, and lower-level robustness. All continuous predictor variables (bare ground and percent grassland habitat cover) were scaled, and the year and study region were used as random effects in all models. We tested for an interaction effect between urban cover and phenological period and found no significant effect in any models, and therefore decided not to include the interaction in the final models. We created generalized linear mixed effect models using the glmer function in the R package lme4 with a Poisson distribution for higher- and lower- level species richness, given these are count data. We created generalized linear mixed models using the glmmTMB function in the R package glmmTMB for specialization and connectance with a beta distribution, because they range from 0 to 1. Nestedness and higher and lower-level robustness were normally distributed so we used linear mixed effects models using the lmer function in the R package lme4.

All models were checked for collinearity by calculating a variance inflation factor (VIF) using the car package in R (Fox and Weisberg, 2019) and all models were below our conservative cut-off of 3. Finally, AICc-based model selection was run for all models using the dredge function in the R package MuMIn, given that AICc is particularly suitable for smaller datasets (Bedrick and Tsai, 1994), and we used a delta AICc of 2 for averaging top models within this bound.

RESULTS

We recorded and identified a total of 223,632 inflorescences in the vegetation surveys, with between 1 and 16 plant species represented in each study region per phenological period (mean 7.82 SE 0.45). Bare ground covered 7.38% of the average surface in each study region. We collected 16,950 insects in the pan and blue vane traps, consisting of 240 different pollinator species, ranging from 10 to 67 species in each study region per phenological period. The three most abundant bee species found in the pan- and blue vane-traps were *Lasioglossum TX. sp.3* (2,839 individuals), *Lasioglossum coactum* (1,674 individuals), and *Lasioglossum bardum* (1,587 individuals); the three most abundant butterfly species were *Lerodea eufala* (284 individuals), *Pyrisitia lisa* (185 individuals), and *Pyrgus communis albescens* (101 individuals).

We observed a total of 2,655 total interactions in the netted surveys, ranging from 1 to 16 plant species and 2–32 pollinator species in each study region per phenological period (mean 11.58, SE 0.43), and a total of 177 pollinator species (bees and butterflies) and 112 plant species overall. The most abundant bee species found in the netted surveys was *Bombus pensylvanicus* (244 interactions), followed by *Xylocopa virginica* (223 interactions) and *Melissodes coreopsis* (141 interactions). The most abundant butterflies caught were *Euristrymon Ontario* (97 interactions), *Euptoieta Claudia* (57 interactions), and *Nathalis iole* (54 interactions).

Regression Analyses

After model selection, we found that plant species richness was significantly negatively affected by phenological period ($z = -4.889$, $p < 0.001$) (**Figure 1A**) and communities were significantly differentiated by historic land-use, with higher richness in historically grazed sites ($z = 2.037$, $p = 0.042$ (**Figure 2A**). Trapped pollinator richness was also significantly higher in historically grazed land ($z = 3.315$, $p = 0.000915$) (**Figure 2C**) and negatively correlated with phenological period ($z = -6.967$, $p < 0.001$) (**Figure 1B**). After model selection, we found that netted pollinator species richness was significantly negatively affected by phenological period ($z = -8.015, p \leq 0.001$) (**Figure 1D**), significantly positively affected by urban cover ($z = 3.111$, $p = 0.00187$) (**Figure 3A**), and was significantly higher within grazed historic land use ($z = 2.937$, $p = 0.00331$) (**Figure 2D** and **Supplementary Table 1**). Netted pollinator abundance was significantly negatively affected by phenological period ($z = 8.701$, $p \leq 0.001$) (**Figure 1E**), significantly positively affected by local bare ground cover ($z = 3.748$, $p = 0.000179$), and significantly positively affected by urban cover ($z = 2.630$, $p = 0.008535$) (**Figure 3B**), while trapped pollinator abundances were significantly higher in historically grazed land ($z = 2.529$, $p = 0.0115$) (**Figure 2B**) and negatively correlated with phenological period ($z = 41.381$, $p \leq 0.001$) (**Figure 1C** and **Supplementary Table 2**).

There were no significant predictors of specialization z-scores. Nestedness z-scores were significantly negatively correlated with the phenological period ($z = -2.233$, $p = 0.026$) (**Figure 1F**). Connectance was significantly positively correlated with phenological period ($z = 3.912$, $p < 0.001$) (**Figure 1G**) and significantly negatively correlated with current management ($z = -1.961$, $p = 0.050$) and urban land cover ($z = -3.011$, $p = 0.003$) (**Figure 3C**). There were no significant predictors of lower-level robustness z-scores while higher level robustness z-scores were significantly higher in current passive management and ($t = 2.52$, $p = 0.011$), and were positively correlated with urban cover ($t = 2.37$, $p = 0.018$) (**Supplementary Table 3** and **Figure 3D**).

Composition Analyses

Our PERMANOVAs showed that the floral communities were significantly different between historic land-use types for the raw ($P = 0.001$), log-transformed ($P = 0.001$), and presence absence data ($P = 0.001$) (raw data visualized, **Figure 4A**) and significantly different between current management groups for the raw ($P = 0.015$), log-transformed ($P = 0.023$), but not the presence absence data ($P = 0.089$) (raw data visualized, **Figure 4B**). Results were nearly identical when using the Morisita-horn method in place of the Bray-Curtis method (**Supplementary Table 4**). Trapped pollinators were also significantly different between historic land-use types for the raw ($P = 0.001$), log-transformed ($P = 0.001$), and presence-absence data ($P = 0.001$) (raw data visualized, **Figure 4C**) and significantly different between current management groups for the raw ($P = 0.002$), log-transformed ($P = 0.001$), and presence-absence data ($P = 0.034$) (raw data visualized, **Figure 4D**). Netted pollinator communities were significantly different between historic land-use types for raw ($P = 0.001$), log-transformed ($P = 0.001$), and presence-absence data ($P = 0.001$) (raw data visualized, **Figure 4E**), while current management was not a significant driver of different netted pollinator communities with the raw ($P = 0.078$), log-transformed ($P = 0.164$), or presence absence data ($P = 0.251$) (raw data visualized, **Figure 4F**). For both communities, netted and trapped pollinators, results were identical when using the Morisita-horn method in place of the Bray-Curtis method (**Supplementary Table 4**).

Floral community composition was significantly different between phenological periods for the raw ($P = 0.001$), log-transformed ($P = 0.001$), and presence absence data ($P = 0.001$) (raw data visualized, **Figure 5A**). The trapped pollinators were significantly different between phenological periods for the raw ($P = 0.001$), log-transformed ($P = 0.001$), and presence-absence data ($P = 0.001$) (raw data visualized, **Figure 5B**). The netted pollinator community composition was also significantly different between phenological periods for the raw ($P = 0.001$),

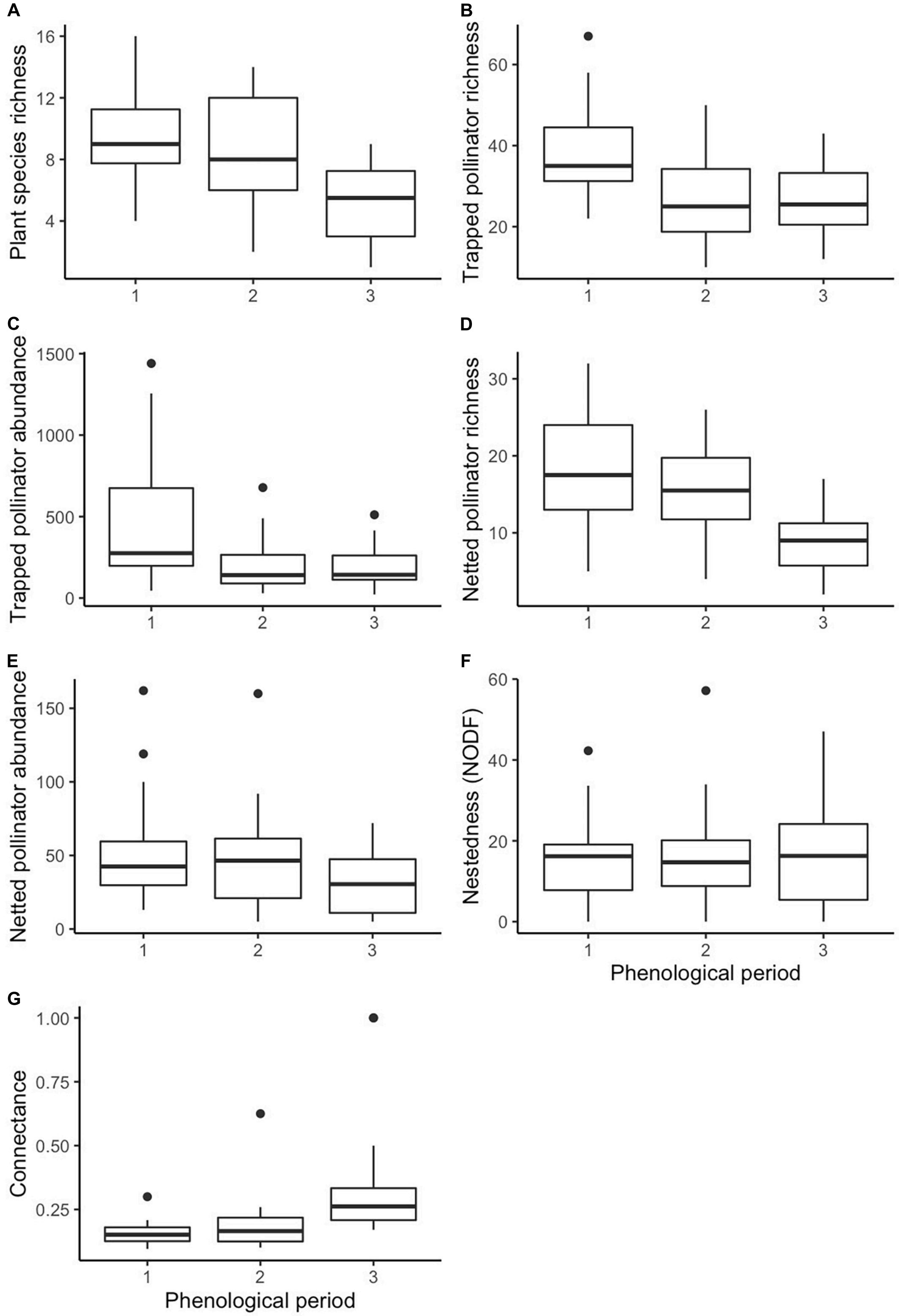

FIGURE 1 | Boxplots displaying phenological period as a significant predictor of plant species richness **(A)**, trapped pollinator richness **(B)**, trapped pollinator abundance **(C)**, netted pollinator richness **(D)**, netted pollinator abundance **(E)**, network NODF **(F)**, and network connectance **(G)**.

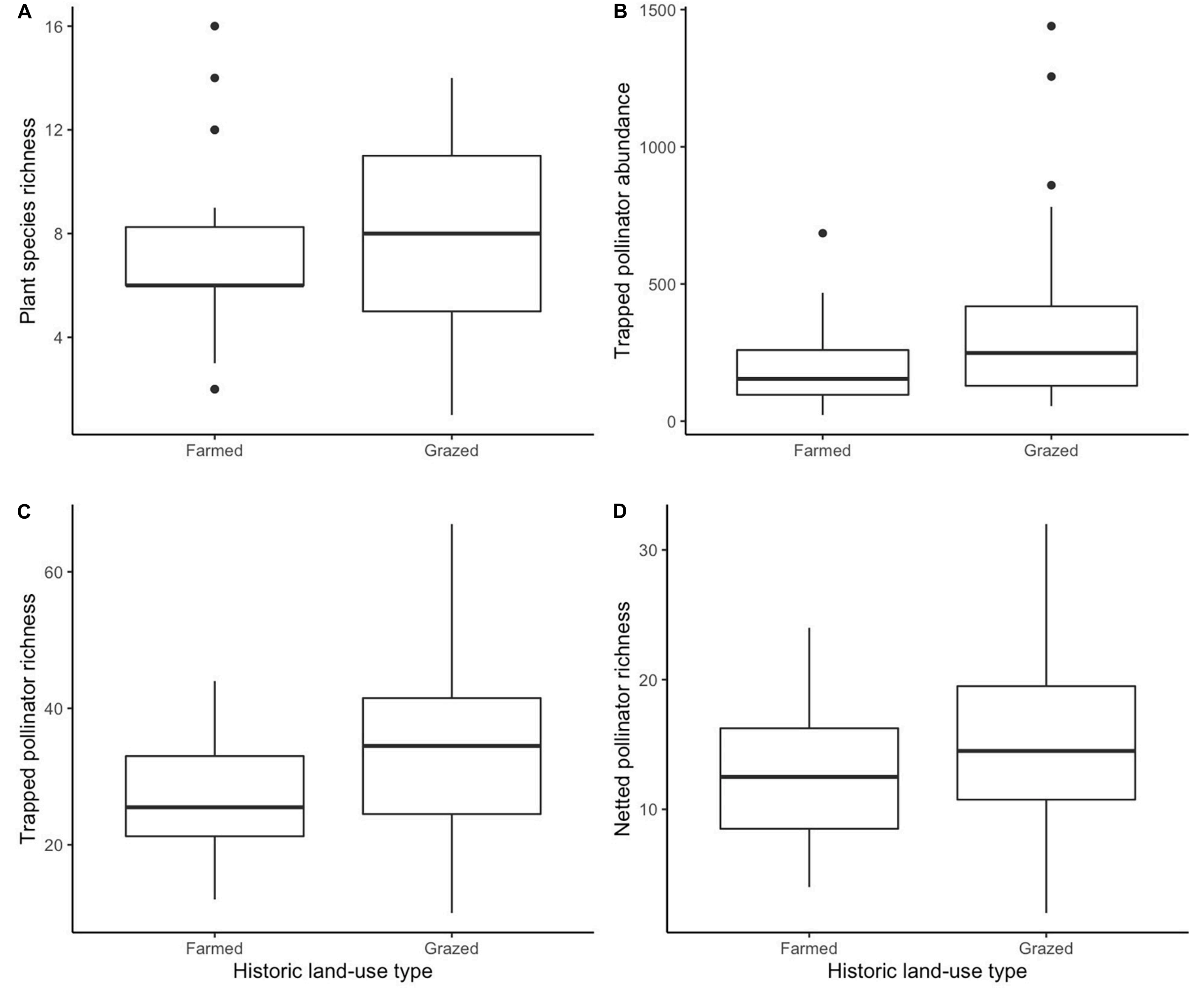

FIGURE 2 | Boxplots displaying historic land-use as a significant predictor of plant species richness **(A)**, trapped pollinator abundance **(B)**, trapped pollinator richness **(C)**, and netted pollinator richness **(D)**.

log-transformed (P = 0.001), and presence absence data (P = 0.001) (raw data visualized, **Figure 5C**).

Habitat Indicator Species

Within the trapped pollinators, out of a total of 235 species, 13 were found significantly more in the historically farmed sites, 22 species were found significantly more often in the historically grazed sites, 4 species were found significantly more often in contemporary actively managed sites, and only 1 species was found more often in contemporary unmanaged sites. Of these, the top five indicator species found significantly more frequently in historically farmed sites were *Lasioglossum disparile* (stat = 0.905, p = 0.001), *Lasioglossum tegulare* (stat = 0.820, p = 0.001), *Megachile brevis* (stat = 0.680, p = 0.002), *Lasioglossum callidum* (stat = 0.630, p = 0.006) and *Osmia Texana* (stat = 0.589, p = 0.024), while the top five species found significantly more frequently in historically grazed sites were *Lasioglossum bardum* (stat = 0.910, p = 0.001), *Lasioglossum hudsoniellum* (stat = 0.903, p = 0.001), *Lasioglossum coactum* (stat = 0.891, p = 0.001), *Diadasia rinconis* (stat = 0.856, p = 0.001), and *Pyrgus communis albenscens* (stat = 0.760, p = 0.003). The four species found significantly more in contemporary actively managed sites were *Lasioglossum callidum* (stat = 0.597, p = 0.027), *Colias eurytheme* (stat = 0.529, p = 0.014), *Lasioglossum sp. TX 20* (stat = 0.440, p = 0.046), and *Lasioglossum coreopsis* (stat = 0.408, p = 0.028), while the only significant indicator species in the contemporary unmanaged sites was *Pyrgus communis albenescens* (stat = 0.733, p = 0.003).

Within the netted pollinators, out of a total of 177 species collected, 5 species were found significantly more in historically farmed sites, 8 were found significantly more in historically grazed sites, and 3 were found more in actively managed sites than passively managed sites, which had no prevalent indicator species. The indicator species found significantly more

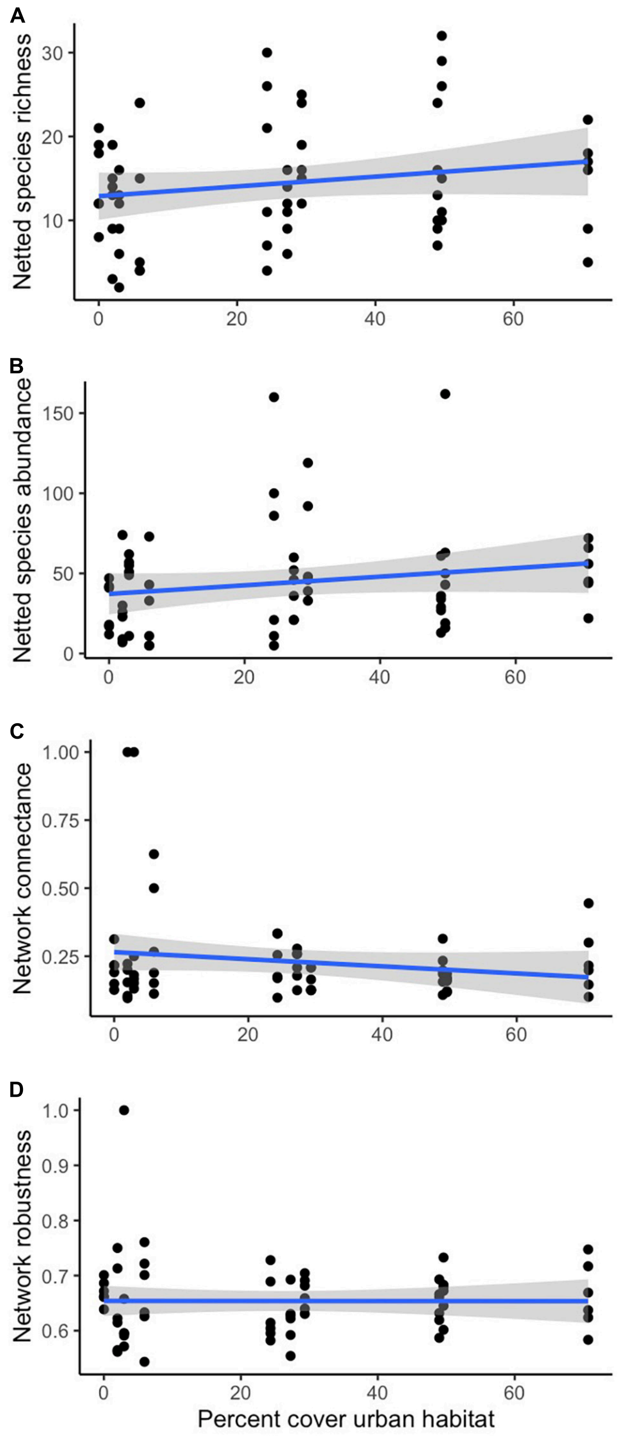

FIGURE 3 | Linear regressions for the significant relationships between percent urban cover and netted species richness **(A)**, netted species abundance **(B)**, network connectance **(C)**, and network robustness **(D)**.

frequently in historically farmed sites were *Lasioglossum disparile* (stat = 0.805, p = 0.001), *Bombus pensylvanicus* (stat = 0.718, p = 0.001), *Bombus griseocollis* (stat = 0.500, p = 0.005), *Hylephila phyleus* (stat = 0.408, p = 0.010), and *Bombus fraternus* (stat = 0.408, p = 0.038), while the top five species found significantly more frequently in historically grazed sites were *Megachile policaris* (stat = 0.735, p = 0.001), *Pyrgus communis albenscens* (stat = 0.693, p = 0.002), *Diadasia rinconis* (stat = 0.577, p = 0.004), *Nathalis iole* (stat = 0.576, p = 0.016), and *Lasioglossum coactum*. The three species found significantly more in contemporary actively managed sites were *Lasioglossum disparile* (stat = 0.613, p = 0.034), *Junonia coenia* (stat = 0.548, p = 0.006), and *Bobmus griseocollis* (stat = 0.429, p = 0.027) (**Supplementary Table 5**).

DISCUSSION

In this study, we found that pollinator richness and abundance were significantly higher in historically grazed vs farmed land and that both floral and pollinator richness decreased with phenological period. In addition, we found that while network connectance was higher in habitats with contemporary active management, robustness was lower, indicating that these mowing and burning management practices may increase species interactions but may not necessarily strengthen plant-pollinator network stability. Pollinator richness and robustness were also higher in landscapes with higher surrounding urban cover, indicating that moderately developed spaces may provide novel resources for pollinators. Finally, we found striking compositional differences in floral and pollinator communities based on both contemporary and historic land use practices.

Plant and Pollinator Richness

We found that both floral species richness and pollinator species richness was significantly greater in grassland areas that were historically grazed. Field experiments conducted within grasslands have shown that grazing, and the addition of burning to grazed plots, can increase plant species richness [Collins, 1987; Gibson, 1988; reviewed in Valkó et al. (2014)]. This is because grazing is a gradual disturbance process that removes biomass from grassland systems, allowing for colonists and seeds within the seed bank to establish. Disturbances increase environmental heterogeneity by changing soil characteristics, like nitrogen availability (Baer et al., 2016), allowing species coexistence within different patches (Roxburgh et al., 2004), resulting in patches of grassland that contain unique species composition (e.g., Collins, 1987). This benefit to the flowering plant community can have cascading effects on arthropod diversity (van Klink et al., 2015). A recent meta-analysis summarizes substantial past work that resonates with our findings, where Hymenoptera respond positively to wildfire disturbance (Carbone et al., 2019), indicating an important role of biomass removal for bee and wasp pollinators.

In our study, we also found greater pollinator richness in grasslands with greater urban land cover, perhaps driven by greater floral and nesting resource availability in these landscapes (reviewed in Sexton and Emery, 2020). Indeed, a variety of nesting and food resources are necessary for many pollinators who forage in open grasslands but nest

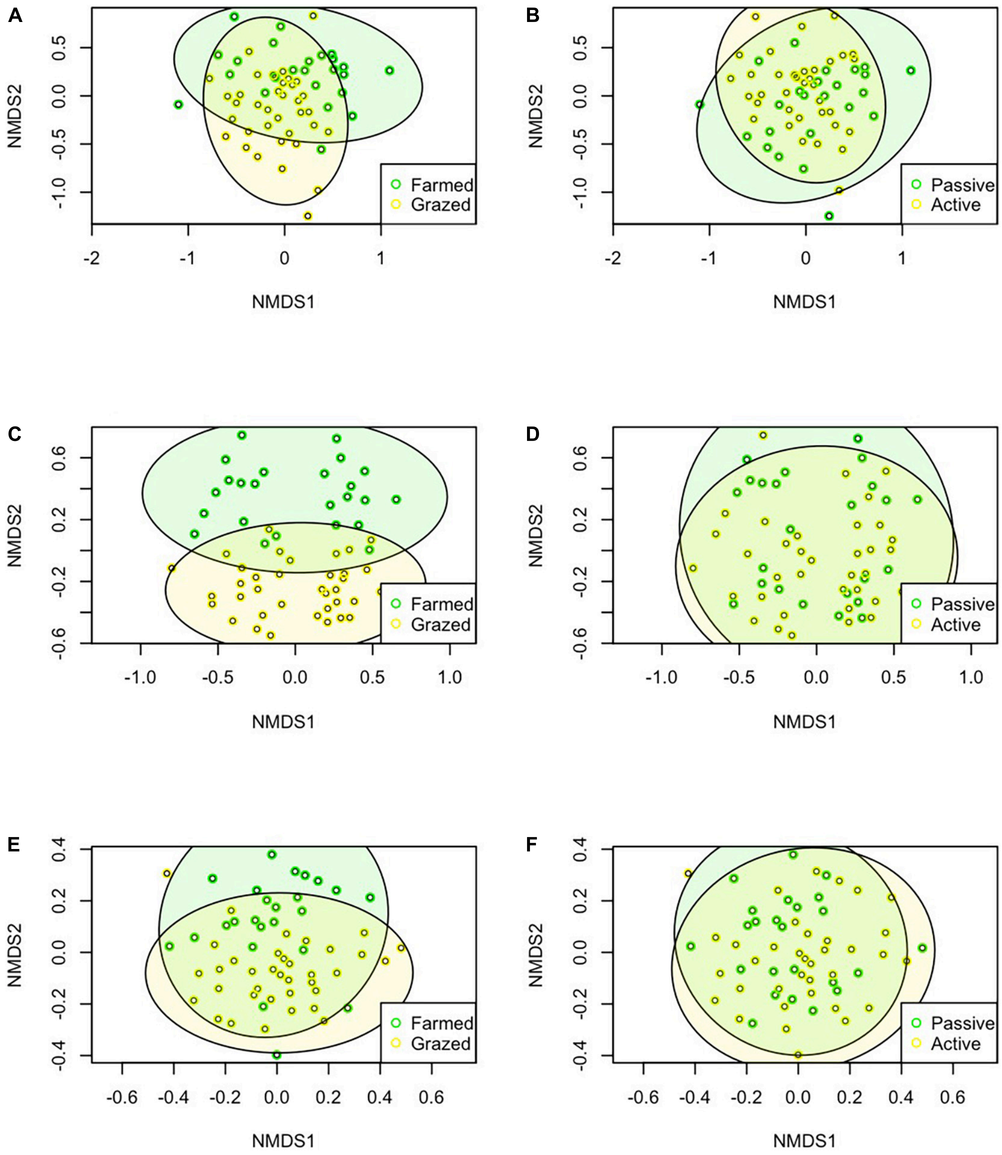

FIGURE 4 | NMDS plots showing the differences between communities for plants between the historic land-use types **(A)** and current management types **(B)**, for trapped pollinator communities between historic **(C)** and current **(D)**, and for netted pollinators for historic **(E)** and current practices **(F)**.

in wood or leaf litter (Cane and Tepedino, 2001), and past studies have documented cases where urban landscape can provide some of these critical resources (Cane et al., 2007). Even within highly urbanized areas, studies have shown that low levels of developed land can increase floral diversity and nesting habitat at a landscape-scale (Matteson et al., 2013), potentially allowing for a greater diversity of pollinators to persist in neighboring natural habitat. Lowenstein et al. (2014) found that more dense neighborhoods supported a greater diversity of flowering plants, leading to greater bee diversity in sites with greater human population density. Residential and community gardens have also been shown to be pollinator abundance hotspots due to high floral resource availability (Baldock et al., 2019). Finally, more urbanized landscapes often exhibit higher levels of habitat heterogeneity (McDonnell and Pickett, 1990) and this heterogeneity may lead to increases in diversity in many arthropods (Báldi, 2008). In our study system, urban cover was comprised primarily of low and open development (i.e., large-lot single-family homes, parks, golf courses) and this type of land use is increasing across the southern United States at rapid rate (Johnson and Beale, 1998). Outside of exurban systems, within agroecosystems, a recent meta-analysis that modeled the effects of land-use intensity on pollinator biodiversity also found that low levels of land-use intensity (e.g., minimal-use urban) are indeed beneficial for pollinator biodiversity (Millard et al., 2021), suggesting an important role of promoting habitat heterogeneity in ever-expanding low-development exurban landscapes (Cusser et al., 2018).

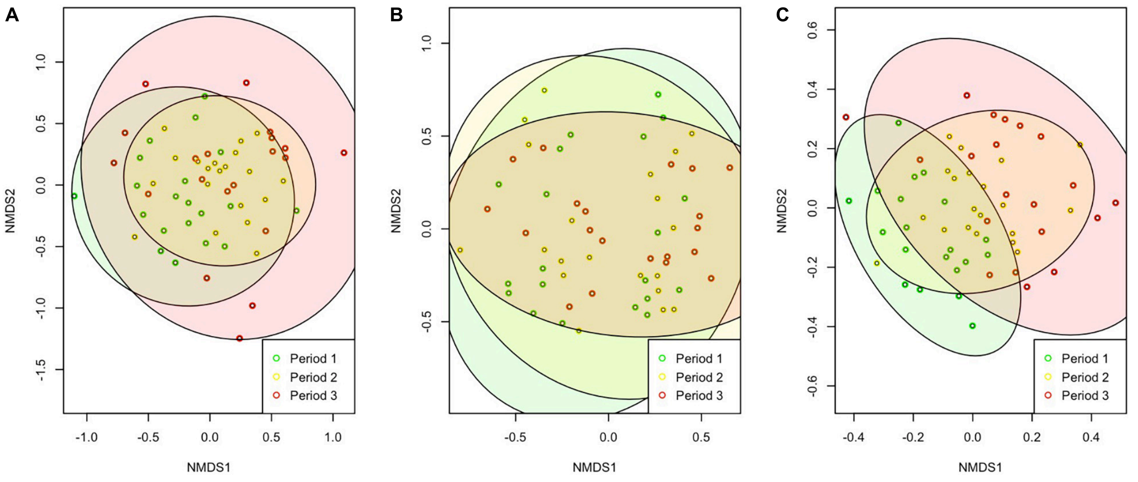

FIGURE 5 | NMDS plots showing the community dissimilarity driven by phenological periods 1, 2, and 3 for the plant **(A)**, trapped pollinators **(B)**, and netted pollinator **(C)** communities.

We also found that pollinator and floral species richness decreased significantly throughout the flowering season, from May to July, as the temperature in the Southern Plains tend to peak (Griffiths and Strauss, 1985) and the abundance of wildflowers tend to decrease. This pattern has been documented in many temperate grassland systems (Wilsey et al., 2011) as well as in our central Texas study system (Ritchie et al., 2016; Ballare et al., 2019). Such pronounced shifts in floral and pollinator richness likely influence pollinator foraging preference, as previously documented in in our study system (Ritchie et al., 2016), but also impact plant-pollinator network structure.

Network Characteristics

Indeed, we found that several network indices, including connectance, nestedness, and robustness were significantly negatively correlated with phenological period. While some studies have found similar patterns with connectance (Vázquez et al., 2009; CaraDonna and Waser, 2020) others have found the opposite relationship (Basilio et al., 2006). The studies that show similar patterns, where connectances decreases as the number of plant and pollinator species decreases, all sampled throughout the flowering season to account for variability in network structure. Specifically, CaraDonna and Waser (2020) describe interaction flexibility between species, where network structure changes from week to week, resulting in differences in connectance, nestedness, and specialization, over the summer growing season. CaraDonna and Waser (2020) also found that throughout the phenological season (where floral species richness declined), both connectance and nestedness were dependent on species richness. Interestingly, we found that nestedness (NODF) was negatively correlated with phenological period, meaning that the networks became less nested over the flowering season. This same pattern has been documented in other grassland system (Dunne et al., 2002), suggesting that networks are more robust when there are more species present. In past simulation studies, robustness has indeed been positively correlated with topological plasticity, indicating that when species are removed from the system, the network is more robust if the species are able to fill in the roles of the newly extinct species (Somaye et al., 2020).

We also found that increases in urban cover at a landscape-level led to increases in higher level robustness; meanwhile, connectance and higher-level robustness were lower and higher in passively managed regions, respectively. Our finding of positive relationships between robustness and urban cover could indicate that developed spaces can increase beneficial habitat and support disparate species, as seen in pollinator studies conducted in more urbanized landscapes (Matteson et al., 2013). While some network studies conducted in wetter regions have not documented this pattern (Moreira et al., 2015), our focus on more water-limited ecoregions, highlights the potential for urban habitat to provide floral resources not found in natural habitat within arid landscapes (Cane et al., 2006; Baldock et al., 2015; Wenzel et al., 2020). We also found that connectance was lower with contemporary passive land management and decreased with increasing regional exurban cover. Because connectance captures the proportion of realized links and can predict network stability (Poisot and Gravel, 2014), our finding indicates land management techniques such as grazing can impact stability, as seen in past studies (Vanbergen et al., 2014). Overall, our findings demonstrate that network structure between plant-pollinator interactions is primarily a function of contemporary disturbances.

Plant and Pollinator Community Composition

We found that plant and pollinator community composition were driven by both historic and contemporary land use practices, as seen in a number of past studies on plants and pollinators (Benjamin et al., 2005; Cusser et al., 2018). Indeed, focusing just on grassland systems, both contemporary (Carvell, 2002) and historic land-use (Cusser et al., 2018) also played a major role in determining species richness and community composition (Howe, 1994; Foster et al., 2003; Ellis and Coppins, 2007; du Toit et al., 2016). In other words, long-term disturbance regimes, such as wildfire burns, can invoke differences in plant species assemblages, even in the presence of contemporary burn or mowing practices (Fidelis et al., 2012), and these "land-use legacies" may be quite strong, though they are often overlooked when focusing on contemporary species patterns (reviewed in Perring et al., 2016). For example, plant communities (Mattingly and Orrock, 2013), lichen (Berglund and Jonsson, 2005; Ellis and Coppins, 2007), and even other flying insect classes, such as hoverflies and butterflies (Sang et al., 2010; Bommarco et al., 2014) have exhibited significant compositional responses to historic land-use regimes. In another study, Sang et al. (2010) found that species richness of habitat specialist butterfly and moth species was positively related to both current and historic surrounding natural habitat area within a 2 km radius (Sang et al., 2010). Our study similarly indicates that both contemporary and historic management have strong impacts on plant and pollinator community composition and species interactions.

Habitat Indicator Species

We found that more than 40 species of pollinators were significantly driving differences in community composition between historically farmed and grazed sites and 8 species significantly driving differences between contemporary actively managed and passively managed sites, likely due to differences in the landscape's ability to provide resources for bees differing in body size and nesting preferences (Ballare et al., 2019). There were more pollinator species differentially representing the historically grazed sites, 26 unique species between netted and trapped methods combined, while the historically farmed sites had 16 unique species driving differences in community composition. The indicator species for the historically grazed sites tended to be more heterogenous in nesting type and foraging specialization (combination of oligo- and polylectic species) while indicator species in the historically farmed sites tended to be more polylectic. These differences in communities could be due to lasting effects that farming and large-mammal grazing have on the respective soil and vegetation types (Foster et al., 2003; Tappeiner et al., 2020). Specifically, grazing may be more similar to the natural history of disturbances in the region (Frank et al., 1998), where specialized oligolectic pollinator species may have adapted over time. Out of the five netted indicator species that differentiated farmed from grazed sites, three of them were from the genus *Bombus.* Widely known for their large body size, sociality, and surface or underground colony nests, these bumblebee species may be doing particularly well in the historically farmed sites because of the potential to use abandoned small mammal burrows as nesting resources (Kells and Goulson, 2003). Bumblebees have been recorded to use abandoned rodent holes as the base for their nest (Harder and Real, 1987), and rodents often live near human-dominated landscapes (Purvis et al., 2020). The greater representation of this group suggests that conversion of historically farmed land back to grassland habitat may be particularly beneficial conservation strategy for this group, which is believe to be declining more than other species.

The indicator species that distinguished the actively managed contemporary habitats from the passively managed ones were diverse in body size, with 4 out of the 7 species being *Lasioglossum* and one from the genus *Bombus*. The only significant indicator species found in the passively managed habitats was the butterfly *Pyrgus communis albenescens*, which was also significantly found in the historically grazed sites.

Conclusion

Overall, we found that land use history and contemporary land management can differentially impact the community composition and species interactions of plants and pollinators in grassland ecosystems. Specifically, we show that both historic (∼80 years prior) grazing and farming practices as well as contemporary (∼10 years prior) burning and mowing have lasting impacts on the composition of plant and pollinator communities. Our results also demonstrate that landscape-level urban cover is a driver of network structure and may lead to higher levels of plant-pollinator network robustness, especially in arid grassland systems. Additionally, our results show that plant and pollinator richness decrease across phenological periods, resulting in altered network structure. Finally, we show that indicator species which characterize historically grazed sites exhibit substantial diet and nesting heterogeneity, likely driven by similarity between pastoral systems and natural disturbance regimes in the bioregion.

Our study shows that to properly evaluate an area for conservation efforts, historic land use should be considered as this can have a lasting impact on current communities. Furthermore, future research should also not neglect this factor in community ecology work. Future research directions include investigating the impacts of other disturbance regimes, such as wildfires, and the impact that a combination of disturbances in one area has on the surrounding communities.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

CC conducted analysis and interpreted the data, made figures and tables, and wrote the manuscript. SJ designed the study and reviewed and revised the manuscript at all steps. JN was responsible for all taxonomic identification and read and suggested feedback on the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.867483/full#supplementary-material

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Diversity and Relative Abundance of Insect Pollinators in Moroccan Agroecosystems

Insafe El Abdouni[1,2], Patrick Lhomme[2], Stefanie Christmann[2], Achik Dorchin[3], Ahlam Sentil[1,2], Alain Pauly[4], Laila Hamroud[1,2], Oumayma Ihsane[1,2], Sara Reverté[1], Sebastien Patiny[1], Thomas J. Wood[1], Youssef Bencharki[1,2], Pierre Rasmont[1] and Denis Michez[1]*

[1] Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium, [2] International Center for Agricultural Research in the Dry Areas, Rabat, Morocco, [3] The Steinhardt Museum of Natural History, Tel Aviv University, Tel Aviv, Israel, [4] Operational Directorate Taxonomy and Phylogeny, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

Edited by:
Rory Mc Donnell,
Oregon State University,
United States

Reviewed by:
Alistair John Campbell,
Embrapa Amazônia Oriental, Brazil
Gabor Pozsgai,
University of the Azores, Portugal

****Correspondence:***
Insafe El Abdouni
insafe.elabdouni@student.umons.ac.be

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Agroecosystems are often impoverished ecosystems, but they can host diverse communities of insects which provide ecosystem services. Specifically, crops may benefit from insect pollinators that increase their quantity and quality of yields. Basic knowledge is still needed regarding the identity, diversity, abundance, and ecology of insect pollinators in many parts of the world, especially in low and middle-income countries. In this study we investigate the potential of agroecosystems and crops in Morocco to host a high diversity of insect pollinators. We sampled insects in four eco-climatic regions encompassing a total of 22 crops for 2 years (2018–2019). After describing the general pattern of diversity and abundance of insect pollinators, we focused our comparative analyses on bees as they are known to be the most efficient and abundant group of insect pollinators. We recorded a total of 53,361 insect pollinators in all agroecosystems among which 37,091 were visiting crop flowers. Bees were by far the most abundant group visiting crops. Honeybees represented 49% of crop visitors followed by wild bees representing 33% of relative abundance. Three genera (*Lasioglossum*, *Andrena,* and *Xylocopa*) represented 53% of the total abundance of wild bees visiting crops. We identified a total of 213 species visiting crops (22% of national wild bee species richness). A comparison of the abundance, species richness, and community composition of wild bees visiting the same crops showed significant inter-regional differences for zucchini, faba bean, and eggplant. This study highlights the high diversity of pollinators in Moroccan agroecosystems and represents an important step toward exploring the Moroccan pollinator fauna. It provides basic information for future studies on pollinator conservation and pollination services.

Keywords: honeybees, wild bees, *Andrena*, *Lasioglossum*, *Xylocopa*, pollination services

INTRODUCTION

For centuries, farmers have worked to find ways to increase their yields and improve harvest quality. Several controlled factors affect crop production, such as fertilizer use (Yousaf et al., 2017), irrigation (Temesgen et al., 2018; Tura and Tolossa, 2020), and pesticide use (Zhang et al., 2015). In addition to these agricultural inputs, crops benefit from different ecosystem services that are provided "free of charge," and which have complementary or synergistic effects on crop yield such as nutrient cycling, pest regulation, and pollination (Losey and Vaughan, 2006; Garibaldi et al., 2018). A recent study comparing the effect of reduction of fertilizer input, irrigation, and pollination, found that a reduction of insect pollination has a stronger effect on crop yield than other agricultural inputs (Fijen et al., 2020). Furthermore, insect pollination enhances the yield and the quality of many crops at both the local and global scale, such as for faba bean (Aouar-sadli et al., 2008; Cunningham and Le Feuvre, 2013), strawberries (Abrol et al., 2019; MacInnis and Forrest, 2019), apple (Garratt et al., 2014a; Hünicken et al., 2021), eggplant (Jayasinghe et al., 2017), cucumber (Christmann et al., 2017, 2022), and tomato (Bashir et al., 2018; Toni et al., 2020). Moreover, many countries are becoming increasingly dependent on pollinators because production has shifted to more pollinator-dependent crops (Aizen et al., 2008; Potts et al., 2016). Pollination is therefore a key ecosystem service to conserve for crop production in and of itself, and also for agricultural development. On the other hand, many pollinator groups are experiencing widespread population declines (Powney et al., 2019; Zattara and Aizen, 2021) in different parts of the world. Dicks et al. (2021) demonstrated that land cover and land management are the major drivers of pollinator declines in most Western countries, while in Africa, pesticides, in addition to land cover was an important factor. Therefore, there is both a simultaneous increase in the demand for pollinators in agricultural regions, whilst many pollinators are concurrently declining in these same areas. The combination of these two phenomena could have a strong effect on crop production and food security (Reilly et al., 2020).

Despite the importance of pollinators, farmers often show little interest in wild pollinators and have a poor understanding about the ecosystem service they provide, especially in developing countries (Ali et al., 2020; Tarakini et al., 2020; Christmann et al., 2021). This suggests an urgent need to study pollinators and their contribution to crop production in these countries to identify measures to protect and enhance pollinator habitats. Therefore, the first step forward is to acquire basic knowledge on pollinator diversity in agricultural systems and crop-pollinator relationships (Garratt et al., 2014b).

Located in the extreme northwest of Africa, Morocco is characterized by a high diversity of bioclimatic regions. The northern regions are influenced by Mediterranean and Atlantic climates (humid, sub-humid, and semi-arid) and the southern regions have an arid to desertic climate (Mokhtari et al., 2013). Morocco constitutes an important hotspot of biodiversity and shows a high rate of endemism when compared to Mediterranean countries and in North Africa (Rankou et al., 2013, 2015). In particular, Lhomme et al. (2020) recorded a total of 961 wild bee species which ranks Morocco as the fifth most species rich country for bee richness in the Mediterranean Basin, confirming previous estimates that Morocco comprises a hotspot of wild bee diversity and pollinators in general (Patiny and Michez, 2007; Patiny et al., 2009). However, few publications exist that focus on insect pollinators and their importance in Moroccan agroecosystems (Christmann et al., 2017, 2021; Sabbahi, 2021; Sentil et al., 2021).

This study aims to test these previous assumptions with quantitative evidence based on extensive pollinator surveys, which comprised four regions in Morocco, 22 local entomophilous crops, and during two consecutive years. Our specific aims were: (1) to document the baseline abundance and diversity of the main insect pollinator groups in agroecosystems, specifically those of wild bees visiting crops in Morocco; (2) to characterize the bee assemblages visiting each crop; and (3) to compare the inter-regional variation in bee abundance, species richness and community composition associated with different crops.

MATERIALS AND METHODS

Geographical Framework, Sites, and Crop Selection

Insect surveys were conducted in four regions in Morocco as follows (**Figure 1** and **Supplementary Table 1**). (1) The Casablanca-Settat (CS) region in the north-center of Morocco, a semi-arid region dominated by cereal and legume crops. (2) The Rabat-Sale-Kenitra region (RSK) near Maamora forest (25 km from Kenitra city), characterized by sub-humid climate and small farms producing vegetables and avocado. (3) The Fes-Meknes region (FM), a mountainous zone with semi-arid climate located at the foot of the Middle Atlas in Sefrou province. This latter area is unique in Morocco for having Rosaceae orchards such as apple, plum, and cherry in addition to other crops like vegetables and cereals. (4) The Draa-Tafilalet (DT) arid region, including sites located between 4 and 20 km from Errich city and 8 and 20 km from Errfoud. Thus, sites were located between mountainous and oasis areas and characterized by apple orchard, alfalfa, some vegetables, and date palm (Ministry of Agriculture, Fisheries, Rural Development, Water, and Forests, 2020).

We considered nine main crops: faba bean, pumpkin, zucchini, melon, apple, cherry, tomato, eggplant, and okra (**Supplementary Table 1**). For each main crop in each region, we selected eight fields of 300 m^2 (30 m × 10 m) (apple and cherry orchards excepted, see below). Five of the eight fields were under the Farming with Alternative Pollinators management approach (FAP) (Christmann and Aw-Hassan, 2012; Christmann et al., 2017). Under the FAP management, the main crop was planted in the central 75% of the field area and other crops (Marketable Habitat Enhancement Plants, MHEP) were planted at the edge of the fields. The three remaining fields were monoculture of the main crop. For apple and cherry, four orchards per crop (i.e., two regular orchards and two orchards with MHEP) were selected. For each orchard, two rows with seven trees were marked. All crops were planted (or orchards were surveyed) in 2018 and replicated in 2019, except pumpkin and faba bean in some regions (**Supplementary Table 1**). Crop planting date followed local farmer's schedule in each region. Most crops were planted in different regions at the same time except for zucchini. Following the farmers' choice, this was planted in spring in DT and FM regions and in summer in RSK and CS regions.

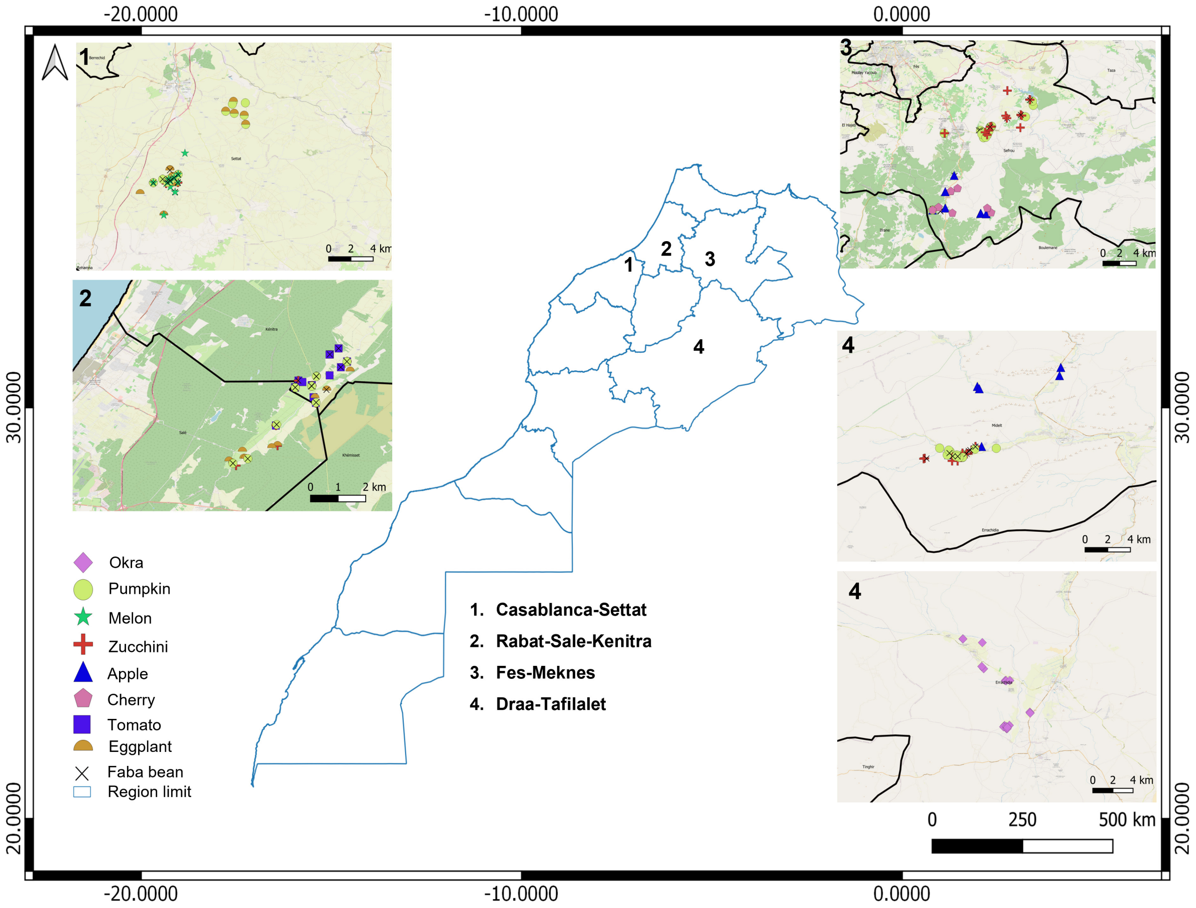

FIGURE 1 | Map of Morocco indicating locations of crop trials.

Overall, we completed 37 trials with 22 crops (including main crops and MHEP, see **Supplementary Table 1**), in a total of 263 fields.

Surveys of Pollinator Communities

For each trial, three or four insect sampling sessions were conducted depending on the flowering period of the main crop. Two types of sampling methods were used in each session: (1). Transect walks, including observations, and sweep net (about 30–35 cm in diameter) and vacuum collection on flowers (Popic et al., 2013), and (2). Trapping with colored pan traps (white, blue, and yellow) (Westphal et al., 2008).

Four transects were completed in each sampling session as follows: two inside the main crop for 10 min (5 min each), a third in the outer 25% of the field (i.e., in main crop or MHEP, for 5 min in 2018 and 10 min in 2019) and a fourth in the flowering plants (wildflowers and other crops) in the field margin outside the crop (for 5 min). The insect survey in orchards (apple and cherry) consisted of walking alongside trees of the two plots during 10 min, and visits in MHEP were assessed within a 10 min transect. All insects visiting the crops' flowers were collected or noted if they could not be collected. Pan trapping was done by using two sets of plastic bowls with three colors (blue, white, and yellow). Pan traps were filled with water and a drop of liquid soap. The three sets of pan traps were put in each field in each sampling event for 2 days (one set inside the field and the other outside the field). Floral visitors were identified visually to species level for the bee species *Apis mellifera*, *Bombus terrestris,* and *Xylocopa pubescens*, all which can be unambiguously determined in the field.

Specimens collected from transects and pan traps were prepared and labeled for the following insect groups: Hymenoptera, Aculeata (i.e., bees and wasps), Lepidoptera (i.e., butterflies), and Diptera, Syrphidae (i.e., hoverflies) (Ollerton et al., 2011). Individuals were identified in the laboratory to genus level for bees (Michez et al., 2019) then sent to expert taxonomists for identification to species level (see "Acknowledgments" section). The remaining groups were identified to the lowest taxonomic level possible in the laboratory (Borror and White, 1991).

Data Analysis

We explored the records from both FAP and control fields. We assume that MHEP did not affect the crop pollinator community in FAP fields based on previous research in the same sites. This study has demonstrated using visitation data and pollen load analysis that MHEPs affected neither the crop pollinator's abundance and richness nor the crop pollinator's pollen diet (Sentil et al., 2021, 2022).

Initially, we classified insects recorded in this study into five insect groups: wild bees, honeybees, non-bee Hymenoptera, Syrphidae, and butterflies. The mean and the standard error of the relative abundance of each insect group was calculated using (1) the data of all insects recorded in agroecosystems and (2) the data of insects recorded visiting studied crops (22 different crops). We used the relative abundance for each insect group because the sampling effort was not standardized between the different agroecosystems and crops.

For the following analyses, we focused on wild bees. Data from all fields, all sampling dates and both years were pooled to generate a species accumulation curve using the "vegan" package in R. A heatmap was constructed using the "pheatmap" package to visualize interaction between bee genera and crops using the relative abundance of each genus visiting each crop.

To compare regions, we performed the analysis for each of the main crops which were studied in more than one region (zucchini, pumpkin, faba bean, and eggplant). We used data from the two transects sampled inside the main crop and only three sampling events for each crop in each year to remain with coherent sampling effort across all crops and regions. Differences in bee species richness and abundance were analyzed using a generalized linear mixed model "glmer.nb" in the "lme4" R package (Bates et al., 2015). The data distribution used was a negative binomial and we fitted a random intercept model. We used "region" as fixed factor, "year" as crossed random factor and "sampling" as nested random factor within "field." Then we performed a pairwise comparison between crops and regions using the "glht" function in the "multcomp" R package (Hothorn et al., 2008).

Bee community composition across different regions was analyzed by Non-metric Multidimensional Scaling (NMDS) employing the function "metaMDS" in the R package "vegan." NMDS was followed by statistical analyses: PERMANOVA (Permutational Multivariate Analysis of variance) and SIMPER (Similarity Percentage Analysis) to identify those species that contributed the most to the observed differences between pollinator communities. Indicator species were also identified using Indicator Value with the "multipatt" function in the package "indicspecies" in R.

RESULTS

Insect Pollinators in Moroccan Agroecosystems

A total of 53,361 insects were collected or observed in all agroecosystems surveyed in 2018 and 2019. We recorded a total of 37,317 individuals within crop fields by direct observation and hand netting, of them 3,831 individuals vising wildflowers and additional 12,213 by using pan traps (**Supplementary Figure 1**). Honeybees and wild bees were the most abundant groups with 37 ± 2% and 45 ± 2% (mean ± SE; $N = 263$ fields), respectively. Among the other groups, non-bee Hymenoptera represented 12 ± 1%, Syrphidae with 4 ± 0.5% and Lepidoptera with 2 ± 0.5% (**Supplementary Table 2**). Among the insects visiting target crops, we counted a total of 24,553 honeybees which represented a mean of 49 ± 2%, and 8,400 wild bees that consisted of 33 ± 2% of the mean bee abundance per field (**Supplementary Table 2**).

Species accumulation curve showed that the number of bee species visiting crops did not reach an asymptote. Basing on the extrapolated values, we sampled as many as 88% of the expected wild bee species richness (**Figure 2A** and **Supplementary Table 3**).

A total of 213 species from 39 genera were identified as crop visitors (22% of the national richness) (**Figure 2**). The most abundant wild bee family visiting crops was the Halictidae ($n = 3{,}940$; 47% of the wild bees) followed by Apidae ($n = 1{,}567$; 19%), Andrenidae ($n = 1{,}530$; 18%), Colletidae ($n = 432$; 5%), and Megachilidae ($n = 368$; 4%). The most abundant genus was *Lasioglossum* ($n = 2{,}046$; 29%), followed by *Andrena* ($n = 1{,}302$; 19%), *Xylocopa* ($n = 528$; 8%), *Amegilla* ($n = 450$; 6%), *Nomioides* ($n = 436$; 6%), and *Hylaeus* ($n = 391$; 6%). Sixteen genera had an abundance less than 10 individuals and represented less than 1% of the total number of bee genera visiting crops. The most species-rich genus visiting crops was *Andrena* (53 species), followed by *Lasioglossum* (35 species), *Anthophora* (17 species), *Hylaeus* (16 species), and *Eucera* (12 species) (**Supplementary Table 4**).

Wild Bees Visiting Each Crop in Morocco

The heatmap revealed that most of the 22 crops were visited by dominant bee genera like *Lasioglossum*, *Andrena*, *Xylocopa,* and *Amegilla,* while some rare genera like *Pseudapis, Lithurgus,* and *Melecta* were visiting only one crop (**Figure 3**).

The family Fabaceae represented by faba bean, alfalfa, sweat pea, green bean, and grass pea, was visited mostly by the bee genera *Anthophora*, *Amegilla*, *Eucera*, *Megachile*, and *Xylocopa,* but also by some species of *Andrena* (such as *A. abjecta*, *A. flavipes*, and *A. fulvicornis*) and *Lasioglossum*. The Cucurbitaceae (zucchini, pumpkin, melon, armenian cucumber, and cucumber) was mostly visited by *Lasioglossum, Ceratina*, and *X. pubescens.* Crops of the family Solanaceae, including tomato and eggplant, had *X. pubescens* as the main visitor. However, we also collected bees from other genera visiting these crops like *Amegilla*, *Halictus*, and *Lasioglossum* but with a lower abundance. The Apiaceae (coriander and anise) and Brassicaceae (canola and arugula) attracted a high number of bees, these crops were visited mainly by *Andrena*, *Hylaeus*, and *Lasioglossum*. Okra (Malvaceae) was visited by a high abundance of *Amegilla*, *Ceylalictus*, *Lasioglossum,* and *X. pubescens*. In sunflower fields, the only crop which represented the Asteraceae family, we found that the most abundant species was *X. pubescens*, followed by the family Halictidae (genera *Halictus, Lasioglossum,* and *Nomioides*), but also with some bees of the genera *Amegilla* and *Bombus*. The family Lamiaceae represented by chia was frequented mostly by *Ceratina cucurbitina*, and two species of *Osmia*: *O. caerulescens* and *O. tricornis.* Finally, the wild bee species visiting apple flowers included two species of *Andrena* (*A. flavipes,* and *A. propinqua*), three species of *Lasioglossum* (*L. lucidulum, L. malachurum, L. algericolellum*)*, B. terrestris, Eucera nigrilabris,* and *Anthophora fulvitarsis*, while cherry was

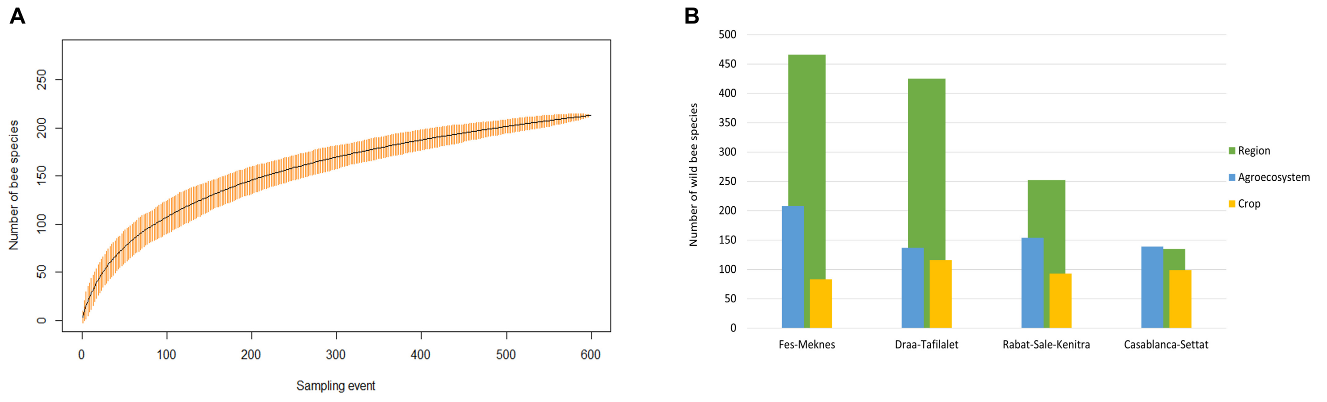

FIGURE 2 | (A) Accumulated number of bee species visiting crops in Morocco. Curve was plotted based on data grouped across all sites and all sampling events. **(B)** Number of bee species known in each of the four studied region (Lhomme et al., 2020, green bars) compared to the number of bee species collected by different methods in all agroecosystems in each regions during 2018 and 2019 (blue bars) and the number of bee species visiting crops (22 crops; yellow bars).

mostly visited by *Lasioglossum* (*L. algericolellum*) and *B. terrestris* (**Figure 3** and **Supplementary Table 6**).

Variation in Abundance, Species Richness and Community Composition of Wild Bees Visiting Crops Present in Different Regions

Accounting to how the abundance and species richness of wild bees visiting each crop differed between regions, the GLMM analysis revealed significant differences for zucchini, faba bean, and eggplant (all $p < 0.05$, **Supplementary Table 7**). Zucchini and faba bean in Draa-Tafilalet hosted the highest mean bee abundance (13.05 ± 3.2 individuals for zucchini and 10.08 ± 1.92 individuals for faba bean) and mean bee species richness (3.25 ± 0.54 for zucchini and 5.17 ± 0.65 for faba bean) followed by Fes-Meknes for zucchini (5.36 ± 1.03 individuals; 2.22 ± 0.31 species), and Casablanca-Settat for faba bean (4.49 ± 0.69 individuals, 2.20 ± 0.26 species).

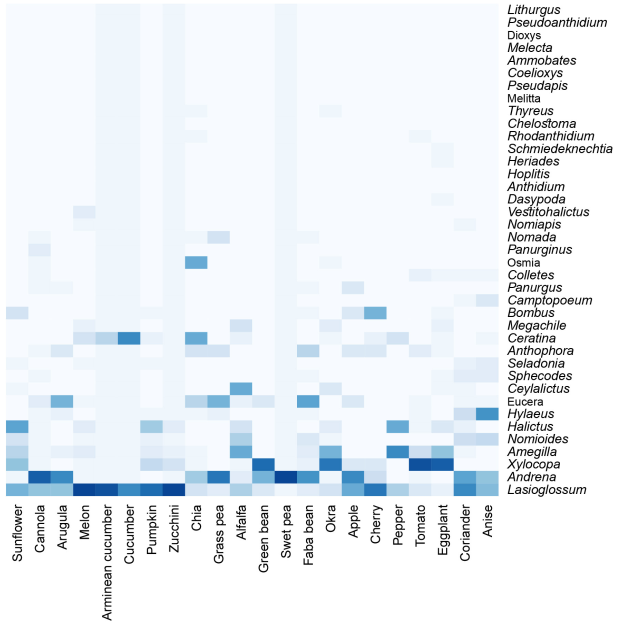

FIGURE 3 | Heatmap showing bee genera visiting each crop. Darker blue represents high abundance.

For eggplant, the mean bee abundance and species richness were found to be higher in Rabat-Sale-Kenitra (1.23 ± 0.27 individuals: 0.65 ± 0.12 species) than in Casablanca-Settat region (0.36 ± 0.11 individuals, 0.31 ± 0.09 species) (**Figure 4**). However, no statistical differences were found between regions in the abundance and species richness of wild bees visiting pumpkin and apple fields (**Supplementary Table 7**).

Wild bee composition differed between regions for all studied crops (PERMANOVA test: all $p < 0.005$). Based on pairwise analyses, we found differences in bee composition between all pairs of regions for each crop except between the regions Rabat-sale-Kenitra and Casablanca-Settat for wild bees visiting zucchini ($p = 0.15$), and between Rabat-Sale-Kenitra and Fes-Meknes for zucchini and pumpkin ($p = 0.08$ and $p = 1$, respectively) (**Figure 5**).

SIMPER analysis showed that *X. pubescens* and halictid bees, especially *Lasioglossum* species, were the major contributors to the dissimilarity between regions for zucchini and pumpkin, whereas species of *Eucera*, *Anthophora*, *Amegilla*, and *Xylocopa* contributed to the compositional differences between regions for faba bean. Comparison between the two regions where eggplant was sampled revealed that only four species had a cumulative contribution of more than 70% to the dissimilarity index (*X. pubescens* with 53%, *Anthophora* sp. with 8%, *Amegilla quadrifasciata* with 7% and *Halictus fulvipes* with 5%) (**Supplementary Table 8**).

Based on the indicator species analysis, 14 bee species visiting zucchini were strongly associated with one or several regions. Seven species were associated with Draa-Tafilalet, two with Fes-Meknes and only one species was associated with Rabat-Sale-Kenitra. Four other species were found in more than

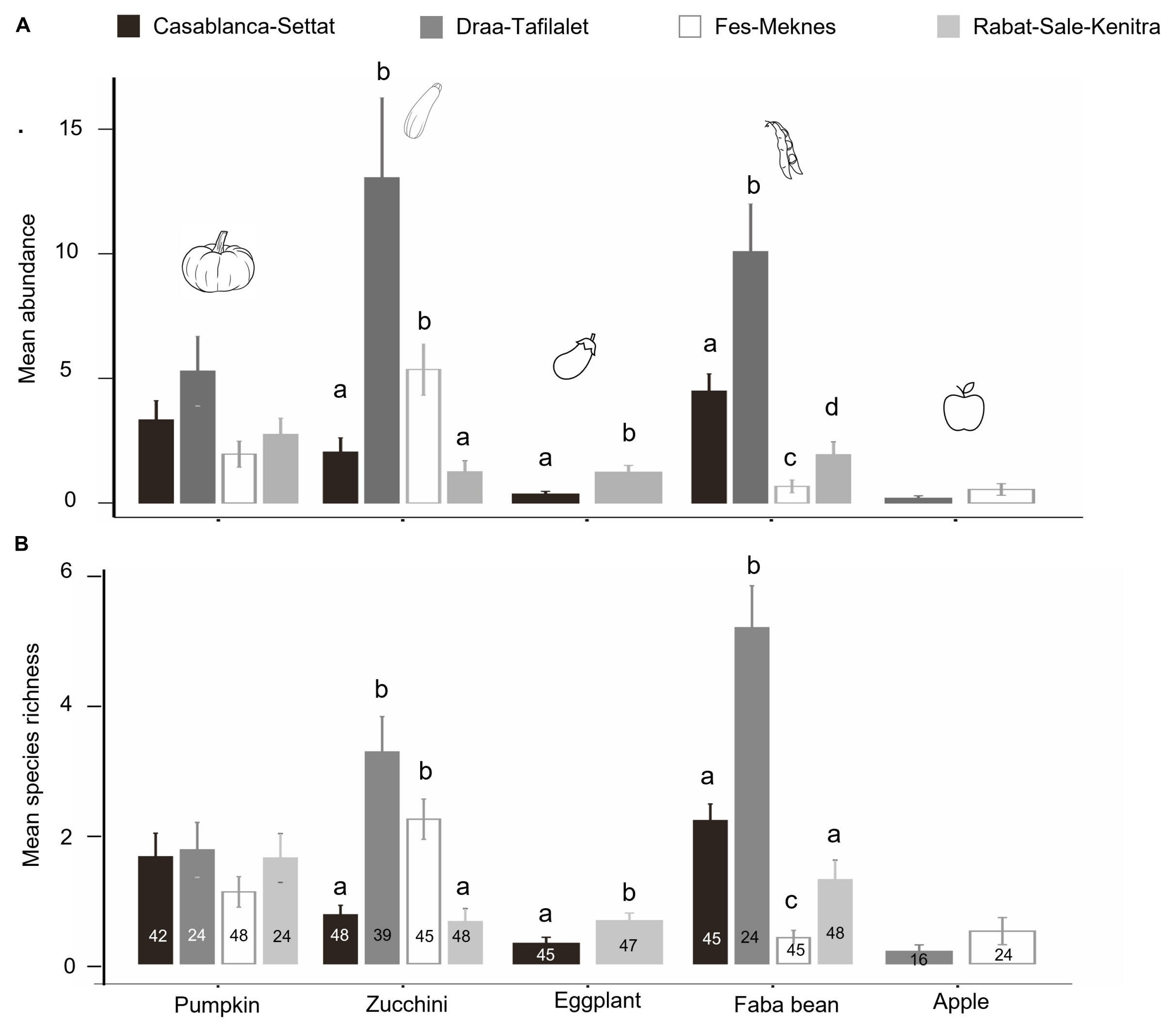

FIGURE 4 | (A) Mean number of bee individuals visiting each crop in the areas where the crop was surveyed. **(B)** Mean number of bee species visiting each crop in the areas where the crop was surveyed. Means ± SE are shown. Small letters indicate significant differences between regions for each crop present in more than one region. Numbers indicate the number of observations (combination of year/field/sampling).

one region: *Lasioglossum villosulum*, *Lasioglossum leucozonium,* and *Lasioglossum interruptum* in two regions and *Lasioglossum malachurum* in three regions (**Tables 1**, **2**). Among the bee species visiting pumpkin, only four species were significantly associated with one region (**Table 1**) whereas *Lasioglossum villosulum* and *Xylocopa pubescens* were common and widespread species across regions (**Table 2**). For faba bean, six species were associated with Draa-Tafilaltet, two species were found to be indicators of the regions Rabat-Sale-Kenitra and Casablanca-Settat (**Table 1**) and only one species was associated with both Casablanca-Settat and Rabat-Sale-Kenitra (**Table 2**).

DISCUSSION

Our study provides a first comprehensive and accurate estimation of the relative abundance and species richness of pollinators in agroecosystems in Morocco. It describes the relationship between wild bee species and crops from different plant families and across four ecoclimatic regions.

Wild Bees in Moroccan Agroecosystems

This study shows that agroecosystem in Morocco and particularly crop plants host high relative abundance of wild bee species, i.e., 45% in all agroecosystems, and 33% of all crop visitors, compared to that found in other countries. For example, in a study across five continents, wild bees accounted for 23% of crop visitors (Rader et al., 2016) and in the United States they accounted for 26% of the visitors (Reilly et al., 2020).

Furthermore, we recorded 213 wild bee species as crop visitors, i.e., 22% of national species richness. This number is high compared to the estimated global species richness of bees currently known to be crop visitors, 785 bee species, i.e., 4%

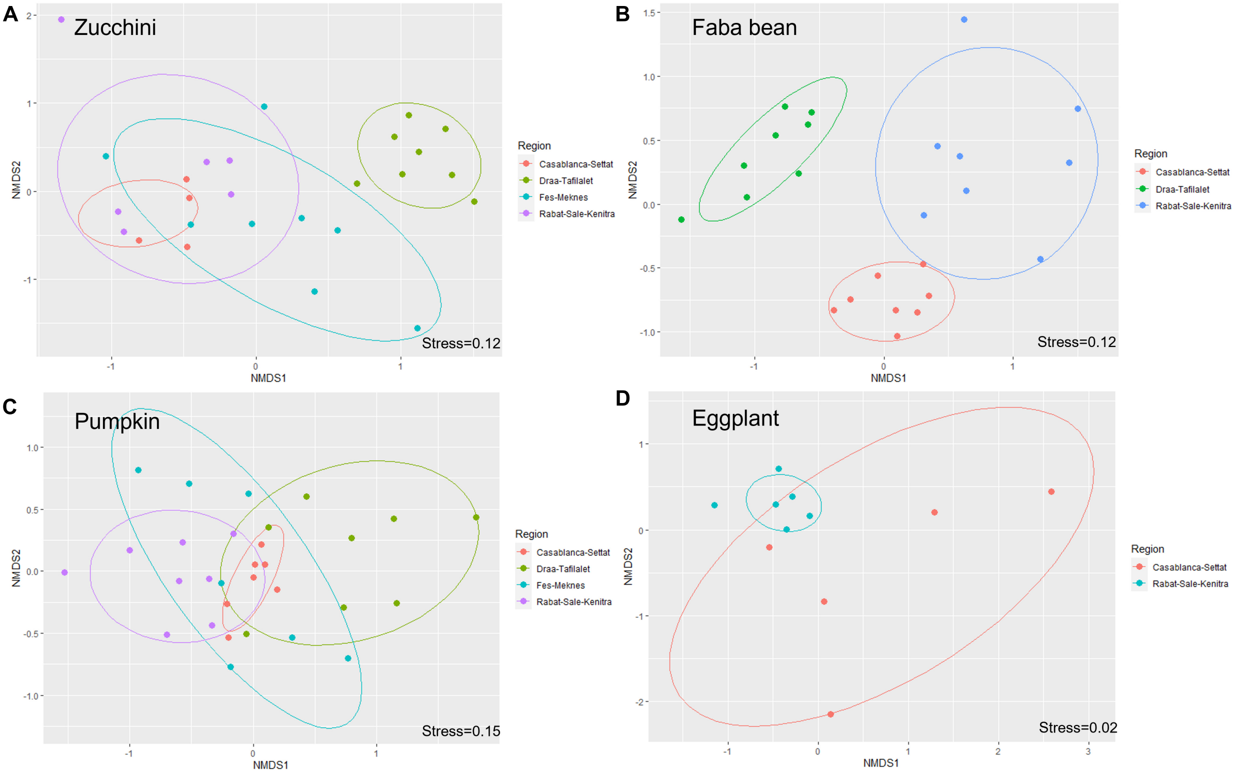

FIGURE 5 | Non-metric multidimensional scaling (NMDS) ordination showing differences in bee community composition among regions for zucchini **(A)**, faba bean **(B)**, pumpkin **(C)**, and eggplant **(D)**.

of global species richness (Kleijn et al., 2015). The high species richness of wild bees found on crops further supports Morocco's status as a hotspot of bee diversity (Michener, 1979; Lhomme et al., 2020).

Wild Bees Visiting Crops in Morocco

Our results are in accordance with many studies linking bee species and crops. We found that the most commonly represented genera in our study sites, like *Lasioglossum*, *Andrena*, and *Xylocopa*, were visiting multiple crops in agreement with the findings of Hutchinson et al. (2021).

Our results are also in line with studies showing a link between the floral traits of crops and their bee visitors (Garibaldi et al., 2015). We found that long tongued bees like *Eucera*, *Anthophora*, and *Amegilla* were more abundant at Fabaceae flowers that are characterized by flowers with a deep corolla tube (Aouar-sadli et al., 2008; Shebl and Farag, 2015; Marzinzig et al., 2018). Likewise, Solanaceae crops that typically require buzz pollination for fertilization were found to be visited mostly by *X. pubescens* and *A. quadrifaciata*, the two species which represent groups of efficient buzz pollinators (Gemmill-herren and Ochieng, 2008; Jayasinghe et al., 2017; Toni et al., 2020; Udayakumar et al., 2021).

Unlike other crops, we found a relatively small number of species and individuals visiting apple and cherry orchards. This could be explained by our sampling of these crops during cold and rainy weather conditions, when only a few species that are more tolerant to suboptimal conditions were active (including the genera *Anthophora, Bombus, Eucera*, and *Lasioglossum*). A similar pattern of floral visitation was found along a climatic gradient from Spain to the Netherlands with a growing dominance of the genera *Bombus* and *Lasioglossum* (Weekers et al., 2022). The higher abundance of *Anthophora* and *Eucera* in our study sites is typical to countries with a warmer Mediterranean climate, such as Morocco and Spain (Patiny and Michez, 2007; Weekers et al., 2022). Particularly in cherry orchards, we recorded mostly bees of the genus *Lasioglossum* while *Andrena* were less frequent, contrary to other studies in Germany and Belgium where these bees were the most abundant (Holzschuh et al., 2012; Eeraerts et al., 2020). Additionally, as woody Rosaceae are not a common element of the native Moroccan flora, the Moroccan *Andrena* community lacks many of the Eurasian species typically associated with these flowering plants, potentially explaining their low abundance in our surveys.

Regional Variability of Bee Abundance, Species Richness and Community Composition

We found that bee abundance, species richness and bee community composition were significantly different between regions for several of the studied crops.

There are several possible factors that can account for this variability between regions. Firstly, the studied regions are

TABLE 1 | Indicator bee species visiting crops significantly associated with only one region (DT, Draa-Tafilalet; FM, Fes-Mekenes; CS, Casablanca-Settat; RSK, Rabat-Sale-Kenitra).

Crop	Species	DT	FM	RSK	CS	Stat	*P-value*
Zucchini	*Xylocopa pubescens*	X				0.935	0.001
	Lasioglossum pseudoplanulum	X				0.866	0.002
	Halictus fulvipes	X				0.844	0.001
	Amegilla sp2_cf_velocissima	X				0.707	0.011
	Lasioglossum limbellum	X				0.707	0.005
	Nomioides facilis	X				0.707	0.007
	Amegilla sp.	X				0.612	0.040
	Lasioglossum algericolellum		X			0.866	0.002
	Lasioglossum mediterraneum		X			0.707	0.010
	Lasioglossum prasinum			X		0.577	0.028
Pumpkin	*Lasioglossum discum*				X	0.7	0.005
	Nomioides facilis	X				0.707	0.005
	Lasioglossum pseudoplanulum	X				0.612	0.043
	Lasioglossum callizonium			X		0.866	0.010
Faba bean	*Anthophora fulvitarsis*				X	0.984	0.001
	Eucera nigrilabris				X	0.866	0.002
	Andrena numida	X				0.887	0.002
	Andrena verticalis	X				0.854	0.003
	Andrena asperrima	X				0.791	0.005
	Andrena fulvicornis	X				0.791	0.005
	Eucera sp.	X				0.791	0.003
	Lasioglossum lucidulum	X				0.707	0.024
	Xylocopa pubescens			X		0.791	0.008
	Andrena tunetana			X		0.707	0.031
Eggplant	*Xylocopa pubescens*			X		0.966	0.005

TABLE 2 | Indicator bee species visiting crops significantly associated with more than one region (DT, Draa-Tafilalet; FM, Fes-Mekenes; CS, Casablanca-Settat; RSK, Rabat-Sale-Kenitra).

Crop	Species	DT + FM	DT + CS	CS + FM	CS + RSK	CS + FM + RSK	Stat	*P-value*
Zucchini	*Lasioglossum villosulum*	X					0.879	0.006
	Lasioglossum leucozonium	X					0.707	0.020
	Lasioglossum interruptum	X					0.661	0.040
	Lasioglossum malachurum					X	0.934	0.010
Pumpkin	*Xylocopa pubescens*		X				0.847	0.003
	Lasioglossum villosulum			X			0.818	0.018
Faba bean	*Eucera numida*				X		0.750	0.037

different in terms of the landscape composition and crop diversity that surround the surveyed fields. Studies have repeatedly shown that closer proximity of crop fields to semi-natural areas has a positive impact on wild bee diversity via augmentation of nesting resources in depauperate agricultural landscape (Ricketts et al., 2008; Kennedy et al., 2013; Chatterjee et al., 2020; Geeraert et al., 2020). In addition, higher crop diversity in agroecosystems may increase bee density (Raderschall et al., 2021). Secondly, the variability of bee diversity and community composition might be related to the climatic variability among regions in our study (sub humid, semi-arid, and arid regions). Bee species richness was found to be higher in arid and warm regions compared to wet regions (Michener, 2007; Patiny et al., 2009). This finding is in line with our result in Draa-Tafilalet (an arid region) where bee abundance and species richness in faba bean and zucchini fields were higher compared to other regions. Finally, seasonal differences throughout the year (Osorio-canadas et al., 2016). Previous studies have shown differences in bee abundance and species richness between dry and rainy seasons (Oertli et al., 2005; Samnegård et al., 2015; Balfour et al., 2018). These results may explain the differences that we found for zucchini in Draa-Tafilalet and Fes-Meknes where the crop was surveyed in early summer and was associated with a higher number of bee species as compared to Casablanca-Settat and Rabat-Sale-Kenitra, the two sites which were sampled in autumn.

Overall, 26 species were identified as indicator species of the regional crop-visiting bee community. The identified indicator species are among the most abundant species in agroecosystems

(**Supplementary Table 5**) and are widespread in Morocco (Lhomme et al., 2020). Identifying indicator species by region and by crop could form the basis for selecting conservation priority species (Bladt et al., 2008). We found for example that only *Lasioglossum* species (three species) are indicators in Rabat-Sale-Kenitra and Fes-Meknes for zucchini compared to Draa-Tafilalet, in which seven species from six genera were found: *Lasioglossum*, *Xylocopa*, *Amegilla*, *Halictus,* and *Nomioides*. This suggests that different conservation strategies may be required between regions given the identified differences in the agriculturally relevant bee faunas.

Conservation Implications and Future Recommendations

This study provides basic information about wild bees in Moroccan agroecosystems and shows the importance of these habitats in hosting a high diversity of wild bees. Identifying the bee species associated with each crop and their abundance in different regions could help to identify conservation strategies for wild bees in Moroccan agroecosystems. For example, *Lasioglossum* and *Andrena* were the most abundant and species rich bee genera recorded in this study. These ground nesting bees could be at risk from intensive agriculture because of deep tillage (Ullmann et al., 2016; Christmann, 2022), soil compaction by heavy machinery, accumulation of pesticides as well as the combined effects of these factors. Reducing machinery or adopting no tillage in farms could conserve ground nesting bees (Shuler et al., 2005). Assessment of pesticide impact and risk to solitary ground-nesting bees as demonstrated by Christmann (2022), may be particularly important for the protection of pollinators in developing countries with high pesticide use (Dicks et al., 2021). For faba bean, cherry and apple that bloom in early season, seeding adequate complementary flowering crops (Christmann and Aw-Hassan, 2012; Sentil et al., 2021), wildflowers strips (Garibaldi et al., 2011; Sutter et al., 2017; Muñoz et al., 2021) or hedgerows (Morandin and Kremen, 2013) may help to attract early flying bees like the genera *Eucera*, *Anthophora,* and *Bombus* and help to conserve these bees in the agricultural landscape.

This first comprehensive study on the bee fauna associated with crops in Morocco highlights the relatively few knowledge available and the urgent need for future research in this country including: (a) research on the value of pollination services (Blaauw and Isaacs, 2014; Christmann et al., 2017, 2021, 2022; Anougmar, 2021; Sabbahi, 2021), (b) applied research to enhance pollinator diversity, and (c) assessment of different global change drivers (i.e., climate change) and their influence on pollinator distribution and diversity (Scheper et al., 2013).

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

IE and DM developed the specific research questions of this article. IE, PL, AS, LH, OI, and YB collected field data. AD, AP, SP, TW, DM, and PR identified bee species. IE analyzed data with the help of SR. SC developed FAP, designed, and coordinated the entire project. IE wrote the manuscript. All authors contributed to revisions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.866581/full#supplementary-material

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Effect of Conservation Agriculture on Aphid Biocontrol by Generalist (Carabid Beetle) and Specialist (Parasitoids Wasp) Natural Enemy Communities in Winter Wheat

Ambre Sacco--Martret de Préville[1], Sebastian Ortiz-Martinez[1], Manuel Plantegenest[2†] and Elsa Canard[1†]*

[1] *IGEPP, INRAE, Institut Agro, Univ Rennes, Le Rheu, France,* [2] *IGEPP, INRAE, Institut Agro, Univ Rennes, Rennes, France*

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***Correspondence:**
Elsa Canard
elsa.canard@inrae.fr

†*These authors share last authorship*

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Adoption of practices that reduce the risk of pest outbreaks is one of the pillars of agroecology and is largely based on biological control. Multiple infield and landscape parameters affect biocontrol, but the effects of conservation soil management on biological control have been poorly investigated over crop season. By comparing winter wheat fields within the same landscape but with different soil management, the direct and indirect effects of soil management (conservation and conventional systems) on natural enemies' communities and their biological control on aphids was studied from the tillering stage to the harvest. In addition to aphid infestation, two families of the main natural enemies' guilds were monitored, as well as their associated services: aphid parasitoid, a specialist and flying natural enemy, with parasitism service, and carabid beetles, a generalist and ground-dwelling predator, with aphidophagy service. Soil conservation system hosted more abundant and diverse carabid beetles' assemblages, and received higher aphidophagy service in June than conventional system. However, neither parasitoid abundance, nor parasitism rates, were affected by soil management. Aphid infestation and its associated damage did not depend on soil management either. Our results suggest that ground-dwelling natural enemies are more impacted by soil management than foliage-dwelling natural enemies, which is partly reflected in aphid biocontrol. In agricultural systems with reduced soil perturbation, direct mortality on ground-dwelling communities due to tillage may be lower than in a conventional system, but habitat heterogeneity is also greater, increasing the number of ecological niches for natural enemies. Both factors are supposed to favor an early presence of natural enemies and a tendency toward a precocious aphidophagy service is indeed observed in conservation system.

Keywords: conservation agriculture, biological control, aphid, sentinel prey, parasitism, aphidophagy

INTRODUCTION

Faced with many global challenges, including increasing agricultural yields, food security, environmental protection and climate change mitigation, the European Union is aiming to promote practices combining viable agricultural production, sustainable management and territorial development with improved economic competitiveness (Kertész and Madarász, 2014). Biological control is one of the reliable levers of integrated pest management, significantly reducing yield loss caused by agricultural pests (Losey and Vaughan, 2006; Ali et al., 2018). However, by threatening biodiversity, including natural enemies' communities, agricultural intensification jeopardizes the biocontrol service they provide (Geiger et al., 2010; Winqvist et al., 2012; Carbonne et al., 2022). By promoting agricultural practices which allow subsidiary resources being available for natural enemies and improving the number of biological interactions, biological control of pests could be favored, leading to a reduction in the use of pesticides.

Several natural enemies, with specialist or generalist diet, may reduce pest abundances with complementary effects (Snyder and Ives, 2003; Thies et al., 2011; Dainese et al., 2017). Biocontrol complementarity is based on facilitation between natural enemies, but also niche partitioning, resulting from attacking different life stages of the same pest or asynchronous complementarity, by exploiting the prey at different times of the season (Snyder, 2019). However, complementarity effects of natural enemies on pests are not systematic (Letourneau et al., 2009) and the complexity of trophic networks and the risk of antagonistic interactions, such as predation, increase with natural enemy diversity and could deter biological control (Schmidt et al., 2007; Martin et al., 2013).

Aphids are a major pest of cereal fields causing yield loss through sap-sucking but also through virus transmission (Ali et al., 2018; Nancarrow et al., 2021). Aphids and their natural enemies are diverse and well-studied, and their interaction-complex has become a model-system for trophic interactions and population dynamics studies. The effects of complementarity and interactions between aphid natural enemies on aphid biological control have also been the subject of numerous studies. Indeed, facilitation between coccinellids and carabids occurs when the foraging behavior of coccinellids causes aphids to fall off plants making it easier for carabids to capture preys (Losey and Denno, 1998). Likewise, niche partitioning occurs between coccinellids and aphid parasitoids that attack aphid populations on different parts of their host plant (Straub and Snyder, 2008). However, predation events also happen between several natural enemies of aphids. For instance, Staudacher et al. (2018) showed that spiders are an important part of the diet of carabids, reflecting intraguild predation. Parasitoid DNA was also detected in the gut content of both coccinellids and carabids (Traugott et al., 2012; Ortiz-Martínez et al., 2020).

According to Gonzalez-Sanchez et al. (2015), conservation agriculture is a sustainable agriculture production system "whose farming and soil management techniques protect the soil from erosion and degradation, improve its quality and biodiversity, and contribute to the preservation of the natural resources, water and air, while optimizing yields." Conservation agriculture differs from conventional agriculture in three main ways: minimal soil perturbation (no- or reduced tillage), permanent soil cover and high diversity of cropping system with crop rotation (Bash et al., 2011; Gonzalez-Sanchez et al., 2015). Conservation systems stand out for their habitat heterogeneity as well, they provide more ecological niches and alternative preys to natural enemies' communities (Finke and Denno, 2002; Langellotto and Denno, 2004), indirectly favoring their presence and diversity and consequently the biocontrol services they provide (Dainese et al., 2019). Positive relationship between soil management reduction, diversity and abundance of natural enemy communities and biocontrol services has already been proven for weeds or some ground dwelling pests like slugs (Honek et al., 2003; Menalled et al., 2007; Bohan et al., 2011; Scaccini et al., 2020) but still has to be demonstrated on insect pests, especially aphids (but see Tamburini et al., 2016).

Damage from aphid transmission of the Yellow Dwarf Virus have also been reported to be lower on barley cultivated with crop residues and no-tillage than on barley cultivated in a conventional system. Jordan and Hutcheon (2002) suggest that this is due to (1) the inability of aphids to recognize barley plants in presence of residues and (2) a more abundant and diverse presence of natural enemies in conservation system. Indeed, direct perturbation due to tillage is reduced in conservation systems and, as a result, direct mortality of underground and ground-dwelling communities, including predators like carabids, spiders or rove beetles, is also reduced (Thorbek and Bilde, 2004; Saska et al., 2008; Soane et al., 2012).

By comparing pairs of winter wheat fields that differ in soil management but are surrounded by the same landscape structure, we aimed to assess the effects of soil management on natural enemies' communities, their biocontrol services and pest populations throughout the growing season. For the first time, both direct and indirect effects of soil management were studied diachronically, considering temporal variations in natural enemy communities, their biocontrol services and aphid infestation. From wheat tillering to harvest season, two communities of natural enemies, carabid beetles and aphid parasitoids, were monitored to illustrate the impact of soil management on both ground-dwelling and foliage-dwelling natural enemies. Carabids are ground-dwelling predators that can overwinter in fields and are supposed to be sensitive to soil management (Soane et al., 2012). Both overwintering and circulating communities of carabids were monitored to disentangle the direct and indirect effects of conservation soil management. The level of aphid infestation, the composition and abundance of natural enemies' communities and their biocontrol services were assessed. Finally, damage were estimated using the Rautapää's index (Rautapää, 1966) through the cumulative abundance of cereal aphids over time.

We hypothesized that fields cultivated under conservation soil management (1) host more abundant and diverse communities of natural enemies, especially carabid beetles, than conventional fields, and (2) benefit consequently from enhanced biological control services, especially aphidophagy, and reduced aphid infestation. At last, (3) carabid beetles' communities are expected to be more precocious in conservation fields than in conventional ones, due to overwintering carabid populations not affected by tillage, which should result in earlier predation on sentinel prey.

MATERIALS AND METHODS

Study Site

The study site was located in the western periphery of Rennes, Brittany, Western France. This site is a 50,000-ha farmland area that exhibits a "bocage" structure, with a dense hedgerow network, and is dominated by a polyculture-livestock system. Five locations were chosen in the study site, in which two paired fields in conservation and conventional soil management were selected (**Figure 1**). The distance between two locations was at least 1.9 km and at most 21.3 km. Locations were not supposed to be spatially auto-correlated, as previous studies in the same region have shown spatial independence (Puech et al., 2015; Djoudi et al., 2018). Conventional fields corresponded to crops where the soil management consisted in more than 25 cm depth tillage and inversion of the soil and conservation fields corresponded to crops where the soil management consisted in tillage of less than 10 cm depth, and non-inversed soil. Paired fields were less than 100 m apart and therefore assumed to share the same landscape environment. Reduced soil management was applied for at least 6 years in conservation fields except for one (Biodiversystem: 1 year). Fields surface varied from 0.25 ha to 2 ha. All fields were cultivated with winter wheat (*Triticum aestivum*), and previously cultivated with maize.

Arthropod Sampling

Aphids and Parasitoids Sampling

Aphid density was assessed at 6 times in each field from the start of the rise of aphid population in April to grain maturation stage in July 2019 (April 5th, April 18th, May 9th, May 28th, June 13th, July 12th). In the center of each field, seven spots were randomly selected, a total of 100 wheat tillers were sampled in 10 m transect and the sum of apterous aphid individuals was carefully collected alive by hand using small brushes. Individuals were identified to species level using taxonomic keys (Blackman and Eastop, 2000), counted and stored in laboratory under controlled conditions (20 ± 2°C, 60 ± 10% RH and LD 16:8 h photoperiod) in ventilated petri dishes up to 4 weeks or to the mummification of the parasitized ones. In the case of mummified aphids, they were isolated in individual 1.5 mL microcentrifuge tubes until the emergence of adult parasitoids, which were identified to species level (Hullé et al., 2020).

Carabid Sampling

Two carabid communities were studied: the circulating carabid community and the emerging carabid community. The circulating community is composed of carabids that are present in the field but that have not necessarily emerged in the field, and the emerging community is composed solely of carabids that have emerged in the field. Circulating carabid individuals were sampled for 1 week each month, from the start of carabid emergence, in March, to grain maturation, in July 2019 (March 21st, April 4th, May 9th, June 13th, July 11th), using pitfall traps. Those individuals compose the so-called circulating carabid community. Pitfall traps were filled to the third with a brine (100 g of salt for 1 L of water) mixed with 3–4 drops of soap (TEEPOL). Emerging carabid individuals were collected using emergence tents (60 cm^2) in parallel of circulating communities. In each tent, two traps were set up: one pitfall trap at ground level to capture ground-dwelling invertebrates and another one placed at the top of the tent (50 cm high) to capture flying invertebrates. Those individuals compose the so-called emerging carabid community. In each field, four sampling spots, 10 m apart from each other, consisting of one emergence tent and two pitfall traps (10 m apart from each other), were set up at least 25 m away from the field border, to avoid edge effect. See the SM1 for more details. Traps were active for 7 days and, trapped carabid beetles were counted and identified at lowest taxonomical level using taxonomical keys (Roger et al., 2013).

Service and Damage Assessment

Aphid Damage

Damage was estimated using the Rautapää's index (Rautapää, 1966) through the cumulative abundance of cereal aphids over time, all species combined.

Parasitism Service

Parasitism potential was measured by the ratio of parasitized aphids among all the aphids that were collected during wheat threshing and kept in isolation in the laboratory.

Aphidophagy Service

Predation potential on aphids was assessed using sentinel preys, once a month, from March to July 2019. Eight 2-weeks old broad bean (*Vicia faba*) plants infested with 10 apterous pea aphids (*Acyrthosiphon pisum*) were exposed in each field for 3 days. After 3 days, aphids remaining on the sentinel plants were counted and predation rate assessed. The aphids exposed in the fields were in their fourth nymphal stage to avoid reproduction and a skewed predation rate during their period in the field.

Statistical Analysis

All statistical analyses were carried out with the software R (R Core Team, 2019), version 3.6.1.

Impact of Soil Management on the Dynamics of Arthropod Communities

The sum of larvae and adult carabids were considered for abundance data, but only adults were considered for biodiversity index due to inability to identify larval state. Effects of soil management and the sampling session on the abundance of all arthropod communities of interest were assessed using generalized linear models fitted with the "nb.glmer()" function from "lme4" package (Bates et al., 2019), considering a negative binomial distribution and a log link function. Field factor, nested in location factor, was included as a random factor. If models did not converge, we followed the recommendations of the package's authors (Bates et al., 2019), i.e., 3 corrections were applied one after another until models converged: first, scaling

FIGURE 1 | Paired fields location in the vicinity of Rennes, Brittany, Western France.

variables, second, increasing models' iterations number, last changing optimization function from "nlminbwrap" to "bobyga." Detection of overdispersion was tested using Pearson residual χ^2 statistic. Significance of the explanatory variables was checked using the "Anova()" function from "car" package (Fox et al., 2019). Pairwise comparisons between levels of each factor were carried out using "emmeans()" and "pairs()" functions from "emmeans" package (Lenth et al., 2020).

Sampling completeness was estimated by calculating Chao1 index with the "specpool()" function from "vegan" package (Oksanen et al., 2019). The effect of soil management and of sampling session on Species richness and Shannon index of carabid communities was assessed using a generalized linear model fitted with the "glmer()" function from "lme4" package, considering a Poisson distribution and a log link function for Species richness, and with the "lmer()" function and a Gaussian distribution for Shannon index (Bates et al., 2019). Field factor, nested in Location factor, was also included as a random factor. Model convergence, overdispersion checking, significance of variable and pairwise comparisons were treated in the same way as for abundance models.

Permutational Multivariate Analysis of Variance (PERMANOVA) applied on Cao distance matrix was used to test the significance of differences in carabid assemblages between emerging communities captured in pitfall traps and circulating communities, between conservation and conventional systems and between sampling sessions, using the "adonis2()" function from "vegan" package (Oksanen et al., 2019). In a second time, PERMANOVA was applied separately on Bray-Curtis distance matrices of circulating and emerging communities to test the significance of differences in carabid assemblages between conservation and conventional systems and between sampling sessions, using the same function. Association between farming practices, or sampling sessions, and species patterns was tested with a multi-level pattern analysis, using the "multipatt()" function from "indicspecies" package (De Cáceres et al., 2010).

Due to the low number of species observed, differences in species richness, Shannon values and assemblage composition were not considered for parasitoids and aphids.

Impact of Soil Management on the Dynamics of Ecosystem Services and Damage

The effect of soil management and sampling session on aphid predation and parasitism rates were assessed using a generalized linear model fitted with the "glmer()" function from "lme4" package (Bates et al., 2019), considering a binomial distribution and a logit link function. The effect of soil management and sampling session on damage (cumulated number of aphids) were assessed using a generalized linear model fitted with the "nb.glmer()" function from "lme4" package (Bates et al., 2019), considering a negative binomial distribution and a log link function. Field factor, nested in Location factor, was also included as a random factor. Model convergence, overdispersion checking, significance of variable and pairwise comparisons were treated in the same way as for abundance model.

RESULTS

Variations in Arthropod Communities of Agricultural Interest Over Time and According to Soil Management

Carabid Communities

A total of 6,334 carabid individuals belonging to 71 species were captured in pitfall traps and emergence tents. Observed specific richness of circulating and emerging carabid communities were 66% and 67% of Chao1 estimated richness respectfully (SM2). Considering the highly J-shaped structure of carabid communities in agroecosystems, those rates suggest good sampling completeness, so statistical analysis are presented on observed specific richness.

Differences Between Assemblages of Pitfall-Trapped Emerging and Circulating Carabids

The two carabid communities were trapped with different sampling methods, so the observed differences might be due to differences in carabids assemblages or differences in trapping efficiency and should be interpreted with caution. Permutation test showed that carabid assemblages significantly differed between emerging and circulating communities (R^2 = 0.077, P = 0.001), but also between types of soil management (R^2 = 0.007, P = 0.003). The difference in their interaction was also significant (emerging versus circulating, R^2 = 0.005, P = 0.007), which is hardly attributable to the trapping method alone. Sampling session, and its interaction with soil management, had a significant influence on carabid assemblages (R^2 = 0.126, P = 0.001, respectively, R^2 = 0.017, P = 0.001). Some carabid species were significantly associated with specific soil management and nature of the community (**Figure 2**): *Ophonus subquadratus* was significantly associated with emerging communities in conservation fields (P = 0.035). *Poecilus cupreus* was significantly associated with circulating communities regardless of soil management (P = 0.005). Finally, *Anchomenus dorsalis*, *Amara sp* and *Harpalus affinis* were significantly associated with circulating communities regardless of soil management as well as with the emerging communities in conservation fields (respectively, P = 0.005, P = 0.005 and P = 0.045).

Circulating Carabid Communities

A total of 4,855 carabid individuals belonging to 61 species were captured in pitfall traps. Soil management, sampling session and their interaction all had a significant effect on the number of captured carabids per trap. Only soil management and sampling session had a significant effect on species richness per trap, and only sampling session and its interaction with soil management had a significant effect on Shannon index (**Table 1** and **Figure 3**).

Overall, carabid abundance was greater in fields under conservation soil management than in fields under conventional soil management (P = 0.004). Differences were significant on March 21st (P = 0.020) and on June 13th (P = 0.030). Carabid abundance also increased over time (P < 2.2e-16) and significant differences were observed during Spring (March 21st < April 4th < May 9th/June 13th/July 11th, P < 0.0001 for all comparisons with March 21st and P = 0.012/0.006/0.001). Species richness was greater in fields under conservation soil management than in fields under conventional soil management (P = 0.017) and also increased from Spring to Summer (March 21st < April 4th < June 13th, July 11th, respectively, P < 0.0001, P = 0.0008, P = 0.0002). Finally, Shannon index also increased significantly over time from Spring to Summer (March 21st < April 4th < June 13th, July 11th, respectively, P = 0.0001 for all comparisons with March 21st, P = 0.0001, P < 0.0001).

Permutation test showed that carabid assemblages significantly differed between soil management (R^2 = 0.006, P = 0.011), sampling session (R^2 = 0.133, P = 0.001) and

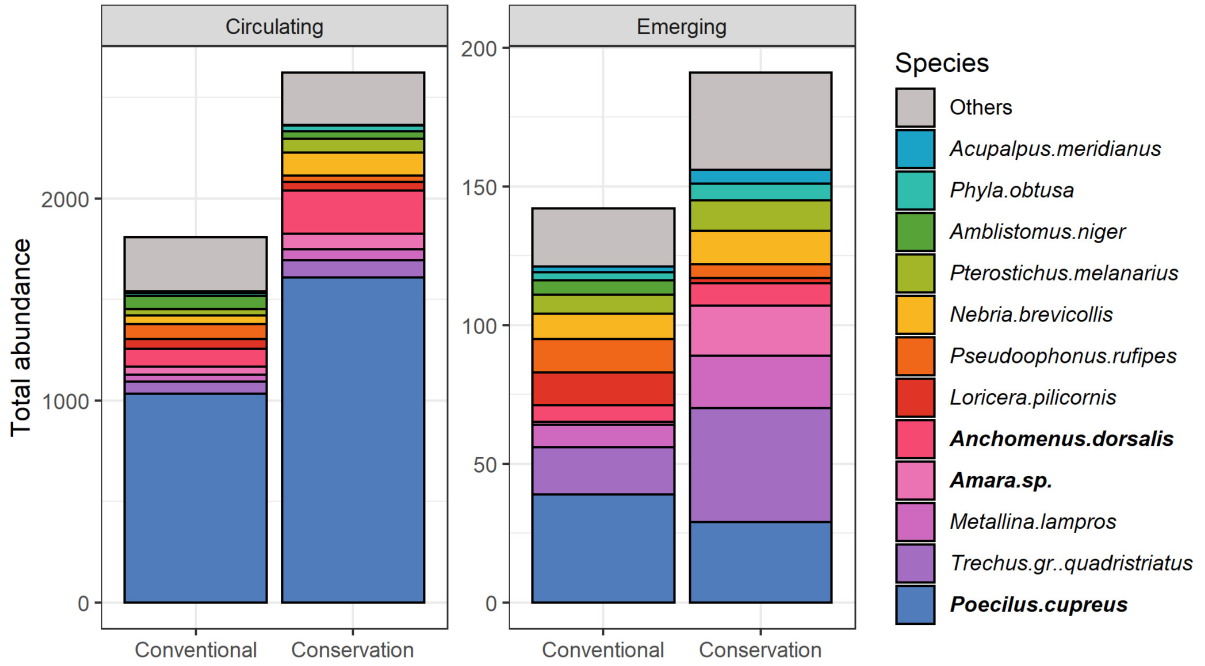

FIGURE 2 | Variations of cumulative abundance of carabid species representing at least 75% of the total abundance depending on soil management and type of community. Indicator species are bolded. Take note that the scales are different between circulating and emerging carabid communities.

their interaction ($R^2 = 0.017$, $P = 0.009$). Two species were significantly associated with each type of soil management: *Nebria brevicollis* ($P = 0.005$) and *Notiophilus biguttatus* ($P = 0.025$) for conservation soil management, and *Demetrias atricapillus* ($P = 0.005$) and *Drypta dentata* ($P = 0.025$) for conventional soil management (**Figure 4**). Differences between fields in conservation and conventional soil managements were significant on June 13th only ($R^2 = 0.030$, $P = 0.029$), but no species was specifically associated to a type of soil management. On May 9th and July 11th, differences in community composition between soil management are close to significance ($P = 0.072$ and $P = 0.06$) with *D. atricapillus* significantly associated with conventional soil management on May 9th ($P = 0.005$), and *N. brevicollis* with conservation soil management both on May 9th and July 11th ($P = 0.005$ and $P = 0.02$). Several carabid species were also significantly associated with a sampling session (SM3).

Emerging Carabid Communities

A total of 1,479 carabid individuals belonging to 43 species were captured in emergence tents. Sampling session and its interaction with soil management had significant effects on the number of

TABLE 1 | Deviance table for the effect of soil management, sampling session and their interaction on arthropod communities, their ecosystem services and damage.

Dependant variable	Fixed effect	Chisq	Df	P (> Chisq)
Circulating carabid communities				
Abundance per trap	Intercept	8.195	1	0.004 **
	Soil Management	5.418	1	0.020 *
	Session	96.188	4	< 2.2e-16 ***
	Soil Management:Session	11.560	4	0.021 *
Species Richness per trap	Intercept	0.378	1	0.539
	Soil Management	5.656	1	0.017 *
	Session	70.332	4	1.931e-14 ***
	Soil Management:Session	9.456	4	0.051
Shannon index per trap	Intercept	1.964	1	0.161
	Soil Management	2.957	1	0.086
	Session	97.132	4	< 2.2e-16 ***
	Soil Management:Session	17.336	4	0.002 **
Emergent carabid communities				
Abundance per trap	Intercept	0.0001	1	0.863
	Soil Management	0.0001	1	0.870
	Session	261.453	4	< 2.2e-16 ***
	Soil Management:Session	10.510	4	0.023 *
Species Richness per trap	Intercept	6.235	1	0.013 *
	Soil Management	3.094	1	0.079
	Session	56.208	4	1.813e-11 ***
	Soil Management:Session	3.229	4	0.520
Shannon index per trap	Intercept	18.667	1	1.557e-05 ***
	Soil Management	2.138	1	0.144
	Session	26.223	4	2.854e-05 ***
	Soil Management:Session	2.402	4	0.662
Parasitoid communities				
Abundance per field	Intercept	15.933	1	6.563e-05 ***
	Soil management	0.016	1	0.901
	Session	35.624	5	1.129e-06 ***
	Soil management:Session	2.506	5	0.776
Aphid communities				
Abundance per field	Intercept	97.792	1	< 2.2e-16 ***
	Soil management	0.945	1	0.331
	Session	66.417	5	5.694e-13 ***
	Soil management:Session	5.114	5	0.402
Biocontrol services and damage				
Aphidophagy	Intercept	14.558	1	1.359e-04 ***
	Soil management	0.080	1	0.778
	Session	77.683	4	5.390e-16 ***
	Soil management:Session	9.779	4	0.044 *
Parasitism	Intercept	0.302	1	0.583
	Soil management	2.137	1	0.144
	Session	30.640	5	1.103e-05 ***
	Soil management:Session	4.956	5	0.421
Damage (Cumulated abundance of aphids)	Intercept	172.253	1	< 2e-16 ***
	Soil management	1.588	1	0.208
	Session	327.671	5	< 2e-16 ***
	Soil management:Session	6.209	5	0.286

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

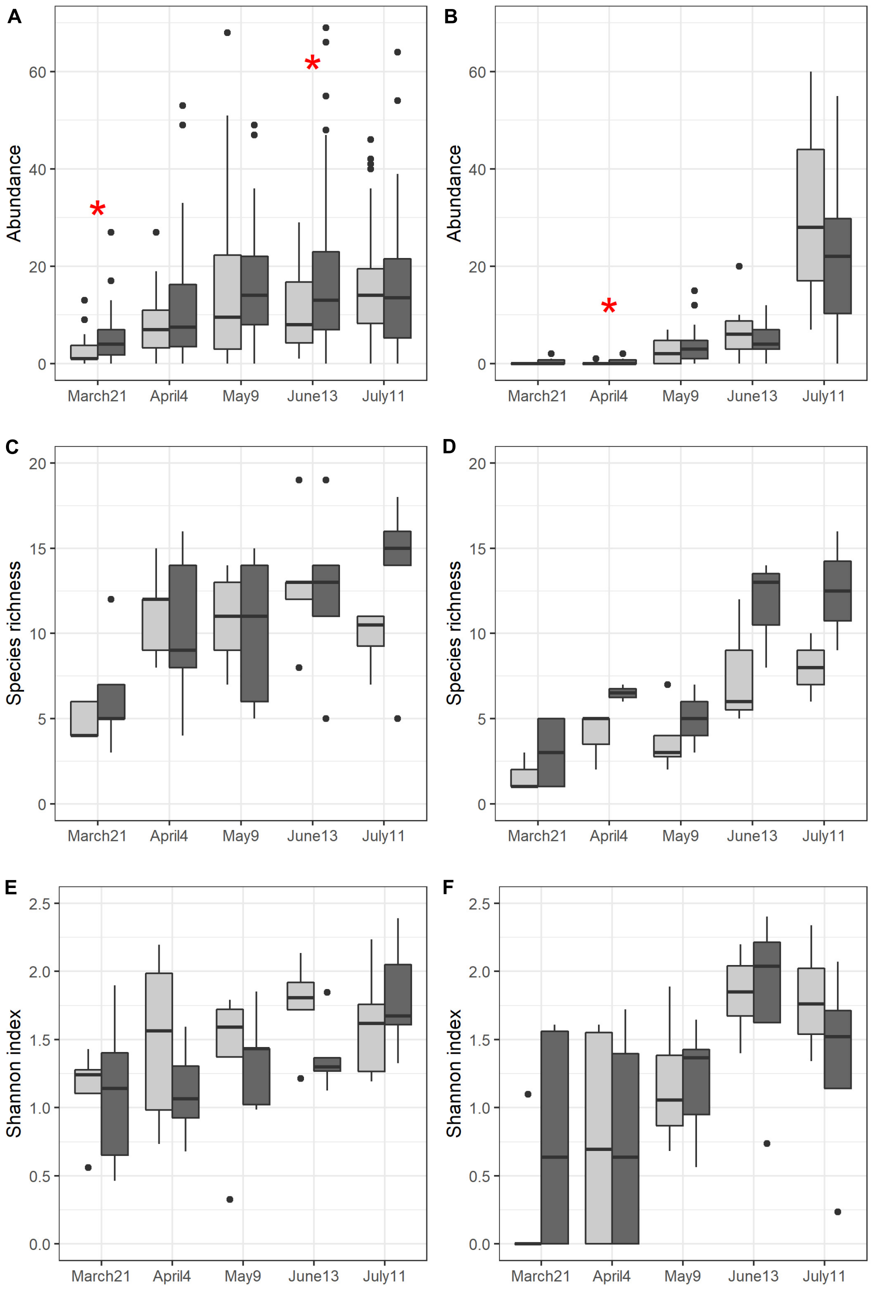

FIGURE 3 | Variations of Abundance per trap **(A,B)**, species richness per field **(C,D)** and Shannon index per field **(E,F)** for circulating **(A,C,E)** and emerging **(B,D,F)** carabid communities over time and according to soil management (light gray: conventional, dark gray: conservation). Red asterisk reflects significant difference between soil managements at a specific sampling session. To improve the readability of the figure, 2 outliers were ignored in panel **(A)** (1 in May in conservation and 1 in June in conservation), 1 outlier was ignored in panel **(B)** (in July in conservation) and 1 outlier was ignored in panel **(C)** (in July in conventional).

carabids captured per tent, but only sampling session significantly influenced species richness per tent and Shannon index (**Table 1** and **Figure 2**).

Emerging carabid abundance significantly increased over time (April 4th < May 9th < June 13th < July 11th, respectively, $P < 0.0001$, $P = 0.0005$, $P < 0.0001$). In Spring (March 21st, April 4th, May 9th), the number of captured emerging carabids was greater in fields under conservation soil management than in fields under conventional soil management (significant difference on April 4th only, $P = 0.044$). In Summer (June 13th, July 11th) the trend was reversed. Species richness and Shannon index increased over time with significant differences between early Spring and Summer (Species richness: March 21st < May 9th < June 13th, July 11th, respectively, $P = 0.002$, $P = 0.0001$ and $P < 0.0001$; Shannon index: March 21st, April 4th, May 9th < June 13th, July 11th, respectively, March 21st: $P < 0.0001$

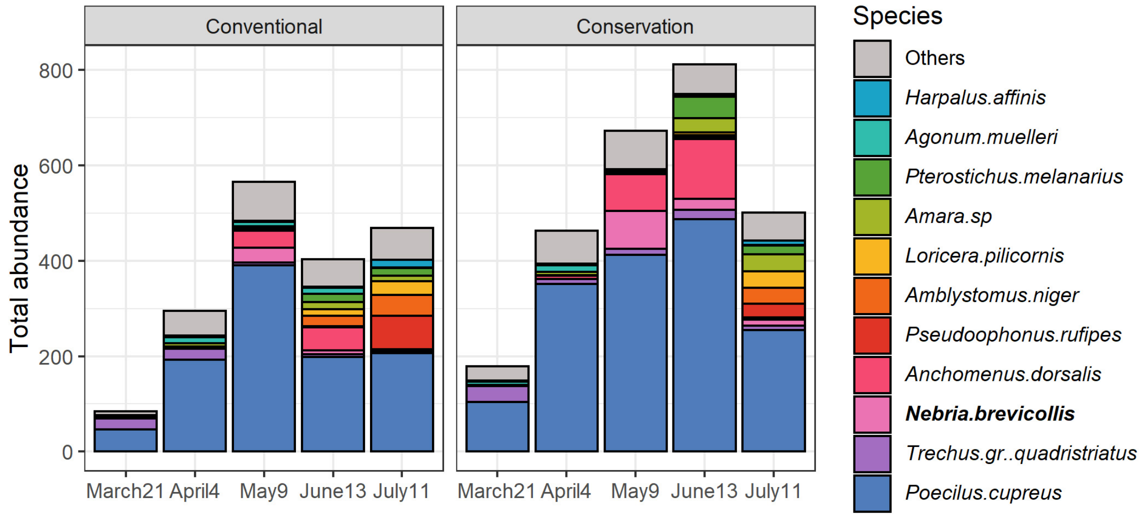

FIGURE 4 | Variations over time and soil management of cumulative abundance of carabid species in the circulating community representing at least 80% of the total abundance. Indicator species are bolded.

and $P < 0.0001$, April 4th: $P = 0.001$ and $P = 0.002$, May 9th: $P = 0.014$ and $P = 0.031$).

Permutation test showed that emerging carabid assemblages significantly changed depending on soil management ($R^2 = 0.020$, $P = 0.005$) and sampling session ($R^2 = 0.107$, $P = 0.001$) and but not with their interaction (**Figure 5**). One species was associated with conservation soil management: *Amara sp.* ($P = 0.040$). When analyses were repeated for each sampling session individually, the difference between fields in conservation and conventional soil managements was significant only on May 9th ($R^2 = 0.069$, $P = 0.041$), and this month, *Metallina lampros* was associated with conservation soil management ($P = 0.035$). Some carabid species were also significantly associated to a sampling session (SM4).

Parasitoid Communities

A total of 403 parasitoids emerged from collected aphids. Sampling session had a significant effect on parasitoid abundance, but not soil management (**Table 1** and **Figure 6A**). During the first three sampling sessions (April 5th, April 18th, May 9th), 6 species of parasitoids were identified (**Figure 6B**). Parasitoids were significantly more abundant at peak infestation (June 13th) compared to previous sampling sessions regardless of the type of soil management ($P < 0.001$ for all comparisons with previous sessions). Regardless of the sampling session, no difference between soil managements was significant.

Aphid Communities

A total of 1,147 aphids were collected, comprising 610 *Sitobion avenae*, 464 *Metopolophium dirhodum* and 73 *Rhopalosiphum padi* (**Figure 7**). The colony structure of aphid populations resulted in a strong aggregation of data. Hence, despite the use of binomial negative distribution a significant remaining overdispersion ($P = 0.003$) after model fitting was observed. Therefore, the estimated probabilities should be interpreted with caution.

Only sampling session had a significant effect on aphid abundance (**Table 1**). Aphid abundance peaked on May 28th and June 13th and was significantly higher at these times than during all previous and subsequent sampling sessions ($P < 0.001$ for all comparisons with previous and subsequent Sessions).

Variations in Aphid Biological Control and Damages Over Time and According to Soil Management

Sampling session and its interaction with soil management had significant effects on aphidophagy, but only sampling session had a significant effect on parasitism service (**Table 1**). Aphidophagy significantly increased over time (March 21st, April 4th < May 9th, June 13th < July 11th, $P < 0.0001$ for all comparisons, **Figure 8A**), and on June 13th, aphidophagy service was greater in fields under conservation soil management than in fields under conventional soil management ($P = 0.018$). Reversely, parasitism service decreased over time (**Figure 8B**) with significant differences between April 5th and May 28th ($P = 0.006$), and between May 9th and June 13th ($P = 0.0001$).

Only sampling session had a significant effect on aphid damage (**Table 1**). Damage caused by aphids tended to be lower in fields under conservation soil management in Spring than in conventional fields but the trend was reversed from the end of May onward, however, the differences between soil management were not significant (SM5).

DISCUSSION

In the present study, the influence of soil management practices on natural enemy communities was assessed. Simultaneously, the ecosystem service of biological control of aphids and their population dynamics were evaluated. In general, compared to conventional soil management, conservation soil practices had a

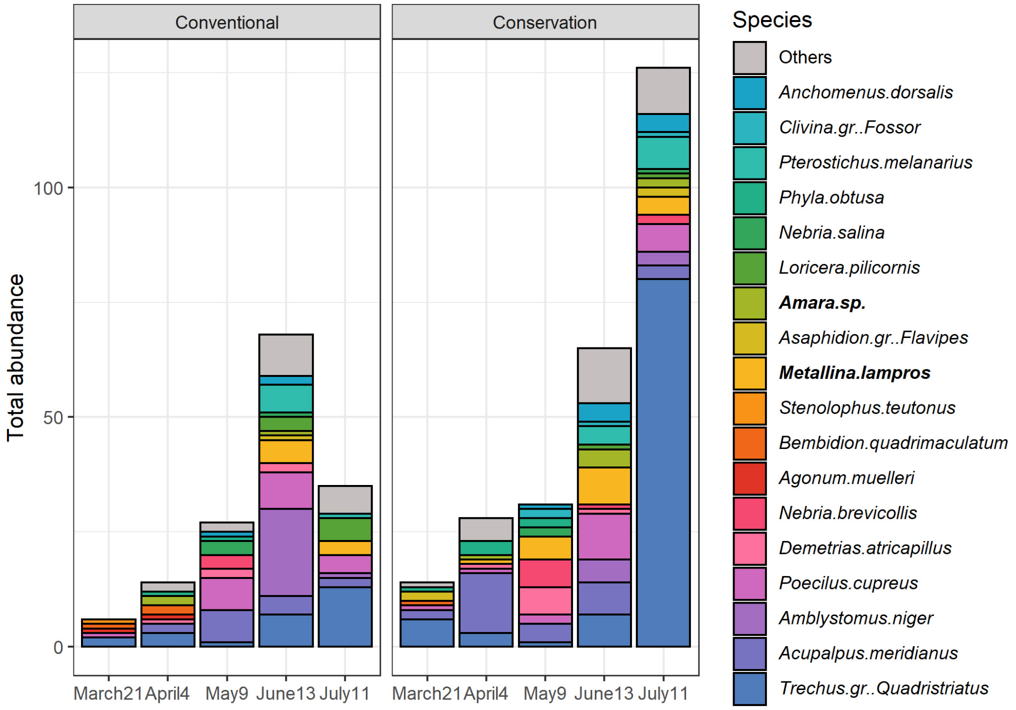

FIGURE 5 | Variations over time and soil management of cumulative abundance of carabid species in the emerging community representing at least 80% of the total abundance. Indicator species are bolded.

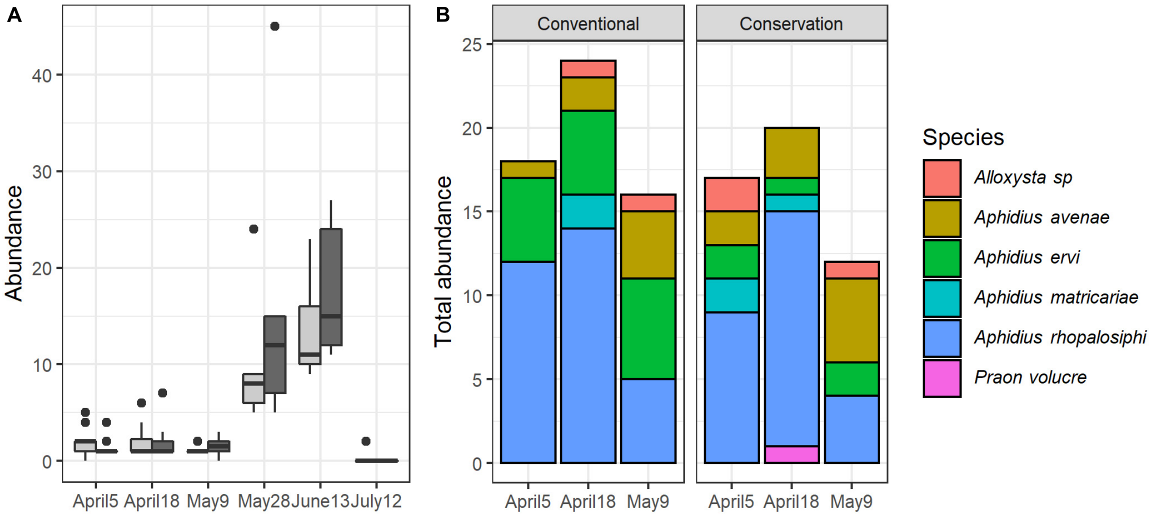

FIGURE 6 | Variations of parasitoid abundance per field over time and according to soil management **(A)** and of the cumulative abundance of parasitoid species for the first three sampling sessions **(B)**.

positive effect on the abundance and diversity of carabids (ground dwelling natural enemies). Conversely, parasitoids (aerial natural enemies) were not influenced by soil management practices. Some variations over time in carabid community's structure were observed and the effect on natural enemy communities was partially reflected in biocontrol services with higher aphidophagy services at peak aphid infestation. No differences were observed for parasitism rates and aphid abundances remained similar between the two types of soil management.

Influence of Soil Management on Natural Enemy Abundance and Diversity

Soil management is an important factor in determining soil biotope properties and its biocoenosis (Kladivko, 2001).

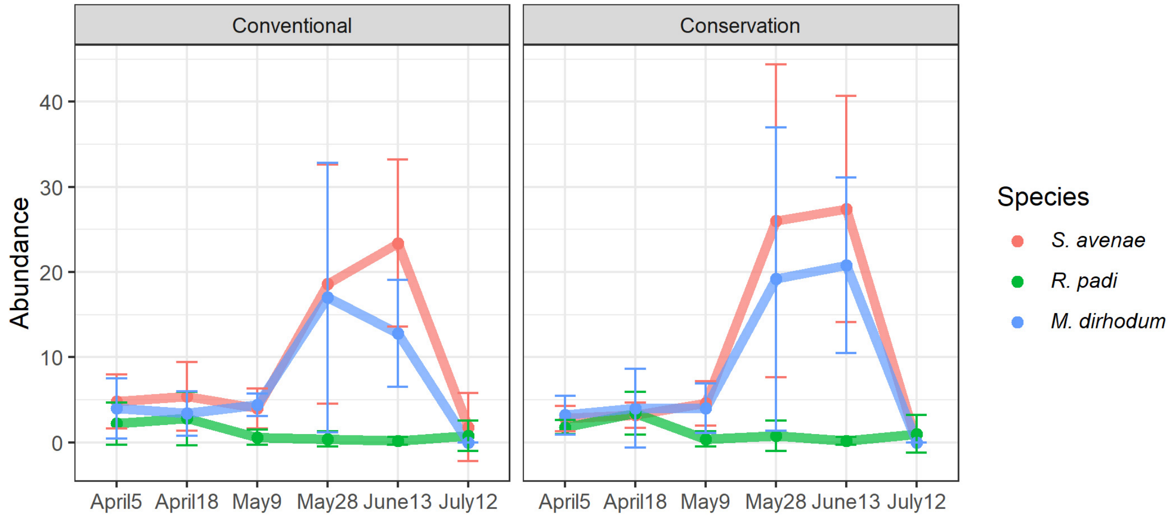

FIGURE 7 | Temporal variations of the abundance per field of the three detected cereal aphids depending on soil management.

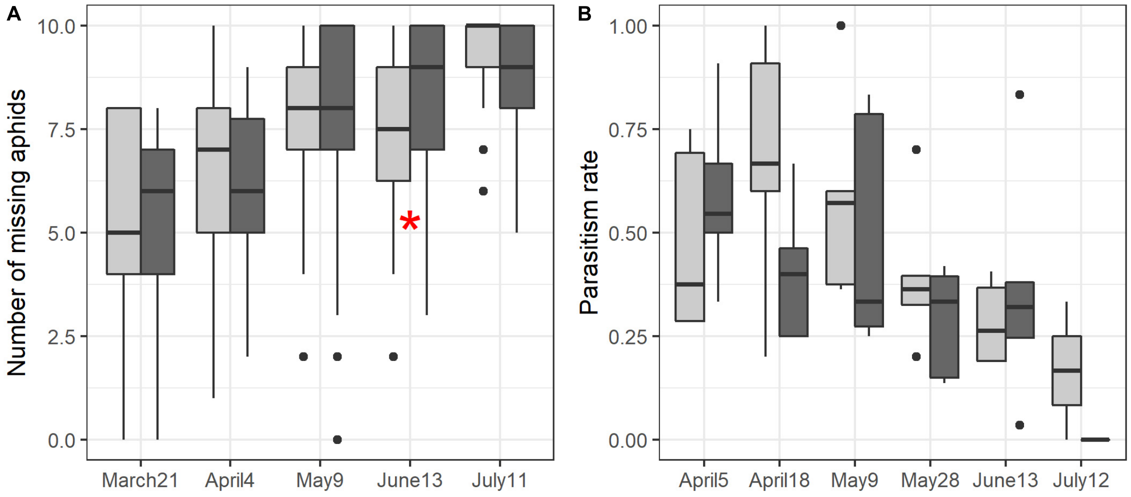

FIGURE 8 | Variations of aphidophagy service **(A)** and parasitism service **(B)** over time and according to soil management (light gray: conventional, dark gray: conservation). Red asterisk reflects significant difference between soil managements at a specific sampling session.

Ground dwelling arthropods, such as carabids, are especially sensitive to soil management. In accordance, conservation fields with reduced perturbations harbored more diverse and abundant carabid communities than conventional fields in this study. Regarding diversity indices similar findings have been consistently observed in previous studies (Baguette and Hance, 1997; Hatten et al., 2007; Menalled et al., 2007; Shearin et al., 2007) but abundance responses to conservation soil management varied. Most previous studies also showed a positive impact of conservation practices with higher abundances of carabids in conservation systems than in conventional systems (Kromp, 1999; Holland and Reynolds, 2003; Kosewska et al., 2014). However, in some cases higher abundances were found in conventional systems (Baguette and Hance, 1997; Hatten et al., 2007; Menalled et al., 2007), but this was usually due to a single dominant species that was either indifferent to disturbance or resilient to it thanks to good colonization capabilities (Baguette and Hance, 1997; Hatten et al., 2007). Allema et al. (2019) also hypothesized that in disturbed open habitat, such as conventional fields, carabids exhibit a more intense foraging activity than in complex habitat, resulting in increased trapping efficacy rather than increased abundance.

In general, conservation soil management is expected to have direct beneficial impacts on communities that overwinter in fields (Soane et al., 2012). Direct mechanical disturbances, such as deep tillage applied in conventional fields, result in high mortality of field-overwintering carabid individuals. In fact, emerging communities of carabids have been recorded to be more abundant in conservation fields or in less disturbed habitats than in conventional fields (Holland and Reynolds, 2003;

Thorbek and Bilde, 2004; Mesmin et al., 2020). Similarly, the number of individuals that emerged in conservation fields in our study was 1.3 more abundant than in conventional fields (SM6). Carabid species overwintering as larvae are suspected to be more sensitive to tillage than overwintering adults (Purvis and Fadl, 1996; Mesmin et al., 2020) with the exception of those that overwinter below plough depth (Barney and Pass, 1986). In our case, almost all emerged carabid species overwintered as adults, but *Trechus gr. quadristriatus*, the most abundant species in the emerging community overwintered as both larvae and adults and was 2.45 more abundant in conservation fields than in conventional ones (SM6), which may indeed suggest a greater sensitivity of the larval stage to tillage. Emergence dynamics also depended on soil management: emerging communities were more abundant in conservation fields than in conventional ones in Spring (see **Figure 3**), but the trend was reversed in Summer. This temporal change in carabids response to tillage can be explained by two non-exclusive hypotheses: first, the soil is more loosened in conventional fields than in conservation ones due to tillage which facilitates carabid burial and Summer-emerging species overwinter below plough depth escaping direct mortality. For instance, *Pseudoophonus rufipes*, that starts emerging and being active in July in our study, have previously been found buried at 45 cm and below (Briggs, 1965). Second, carabid emergence in conservation fields is earlier because of changes in soil properties. Indeed, heat diffuses faster in conservation soils than in conventional soils (Potter et al., 1985), leading to faster development of carabid eggs and larvae (Tenailleau et al., 2011) which could explain earlier emergence.

One of the main benefits of conventional deep tillage is weed suppression, which leads to habitat homogenization, in contrast to the more weed-colonized conservation fields (which was actually observed in our fields pairs), and to indirect negative impacts on natural enemies. MacArthur (1972) hypothesized that the number of available ecological niches increased with habitat complexity, which is expected to have a positive effect on the co-existence of species (Tews et al., 2004; Stein et al., 2014). A high habitat heterogeneity supports a wide range of alternative preys (Langellotto and Denno, 2004; Finke and Snyder, 2008) and brings stability in natural enemies food webs (Staudacher et al., 2018) and biocontrol services (Langellotto and Denno, 2004; Birkhofer et al., 2008; Finke and Snyder, 2008) by lessening interspecific competition (Finke and Denno, 2002; Staudacher et al., 2018). In consequences, high habitat heterogeneity is expected to be beneficial for generalist predators like carabid beetles, by providing a wider range of prey and refuge, which is in accordance with the higher abundance and species richness observed in circulating communities of conservation systems in our study. Parasitoids are also expected to benefit from habitat heterogeneity. Conservation fields by providing a greater abundance and diversity of weeds (Soane et al., 2012) might provide more nutritional resources, overwintering sites and alternative preys (Rusch et al., 2010; Araj et al., 2011). The number of parasitoids was slightly higher in conservation fields than in conventional ones at peak aphid infestation, in late May and early June in our study. On the contrary, more parasitoids were present at the beginning of the season in conventional fields than in conservation ones, possibly due to a bottom-up control of parasitoid populations by aphid resources since aphids were more abundant in April in those fields. In any case, no significant differences in parasitoid abundance were found according to the type of soil management, suggesting that if an effect exists, it is of low magnitude.

Influence of Soil Management on Biocontrol Services and Aphid Infestation

Aphid predation by carabid beetles increased over time and was similar between soil managements, except in June when this service was greater in conservation fields than in conventional fields. The mechanisms governing temporal variations in aphids place in the carabid diet have not been studied but they have been for seed predation. Given the generalist diet of carabid beetles, the same mechanisms could be at play for aphid predation. According to Saska et al. (2008), temporal variations in seed predation result from three mechanisms. First, there is a turnover in carabid communities; even if carabids are generalist predators, there is a wide gradient of diet among species and a different food demand is expected depending on community composition. Second, food demand is expected to change with phenological stage, even within the same species. Third, changes in environment temperature lead to changes in activity and metabolic rates of carabids and in consequences, in predation rates. Aphidophagy service was linked to carabid abundance in a previous study that used aphids as sentinel prey (Boetzl et al., 2020) and, regardless of soil management we can assume that carabid community turnover, changes in phenological stage and in environment temperature, all contributed to changes in aphidophagy rates, with an increase over time in our study. In June, aphid predation rate was higher in conservation fields than in conventional ones while no significant difference in carabid abundance was observed at this time. However, in June, a significant difference in carabid community composition was noted between fields managed with conservation practices and fields managed with conventional practices, suggesting that species composition rather than total abundance determines aphid predation rate.

In our study, parasitism rates did not differ according to soil management. Contrarily, Tamburini et al. (2016) found higher parasitism rates in fields under conservation soil management than in fields under conventional management. However, in this study, parasitism rates also depended on the proportion of semi-natural habitat in the landscape, but only for conventional fields, suggesting that habitat heterogeneity within the field could compensate for poor landscape quality. Brittany's agricultural landscape exhibits a "bocage" structure with semi-natural field-borders that may provide refuge and resources to arthropod communities. This type of structure could compensate for the poor habitat heterogeneity in conventional fields and mitigate the positive response of parasitoids to conservation practices. Parasitism rates decreased over time in our study. Until May, aphid infestation remained low resulting in a shortage of resources for the already present parasitoids and parasitism rates reaching over 50% (see **Figure 8**). At peak aphid infestation, parasitism rates decreased, probably due to a rapid increase in resources, and varied between 25% and 35% regardless of soil management, an order of magnitude similar to a previous study performed in Brittany (Derocles et al., 2014). Although differences were not significant, peak parasitism rate in conservation fields was earlier than in conventional fields, which makes us wonder about the potential benefits of conservation practices on early service of biocontrol.

Aphidophagy and parasitism have reversed temporal dynamics suggesting a temporal complementarity of carabid and parasitoid communities in the provision of aphid control service. An effective complementarity between flying and ground dwelling guilds of natural enemies had already been observed through lower aphid infestation when both guilds were present (Schmidt et al., 2004; Tamburini et al., 2016), despite the risk of negative interactions between natural enemies, such as consumption of mummified aphids by predators (Traugott et al., 2012; Ortiz-Martínez et al., 2020). These opposed dynamics between natural enemies might be due to the removal by predators of aphid resources which the parasitoids can no longer access. Similarly to the delay between complex and simple landscape context, conservation soil management, by favoring a precocious presence of predators compared to conventional soil management, might explain the delay in parasitism response in conventional system (Ortiz-Martínez and Lavandero, 2018). Regardless of the interaction between natural enemies, aphid population dynamics or their damage do not change with soil management. Indeed, despite higher carabid abundances and aphidophagy, especially in June, conservation fields displayed similar aphid infestation dynamics as conventional fields.

Services were measured with various methods, inducing some biases: parasitism rate was assessed directly on local aphid populations, while aphidophagy was measured using introduced sentinel prey, pea aphids. As a result, the measures of aphid control by parasitoids and by predators are not strictly identical, making it difficult to compare the two. Furthermore, sentinel prey have been criticized for their immobility and suspected of not truly reflecting natural trophic interactions (Birkhofer et al., 2017) but they have been shown to be an efficient proxy for assessing predation in an ecosystem (Boetzl et al., 2020) and immobility bias was removed in our study by using live aphids as sentinel prey.

Natural Enemy Assemblage and Implications for Biocontrol Services

Parasitoid community did not seem to be impacted by soil management: both assemblages were composed of Aphidiinae parasitoids. In contrast to parasitoid assemblages and in accordance with previous studies, soil management influenced carabid assemblages (Baguette and Hance, 1997; Holland and Reynolds, 2003; Thorbek and Bilde, 2004; Kosewska et al., 2014). One species was dominant in circulating communities regardless of soil management (*Poecilus cupreus)*, suggesting that this species mainly overwinters outside the fields and recolonizes them afterward. Some others were specific to the circulating communities and to the emerging community in conservation soil management (*Anchomenus dorsalis, Amara sp, Harpalus affinis*), suggesting that these species overwinter both inside and outside the fields and are directly negatively impacted by autumn tillage. Similar results have already been observed for Amara and Harpalus genera (Tonhasca, 1993; Baguette and Hance, 1997; Thorbek and Bilde, 2004; Pretorius et al., 2018) and for *A. dorsalis* (Thorbek and Bilde, 2004 but Baguette and Hance, 1997) and corroborate the existence of direct mortality caused by tillage. It is worth noting that those three taxa were especially active in June, when aphidophagy was significantly higher in the conservation fields than in the conventional ones, and are recognized biocontrol agents. Indeed, *A. dorsalis* is a well-known aphid consumer (Chiverton, 1987, p. 0; Sunderland and Vickerman, 1980; Staudacher et al., 2018) and could explain the difference in aphidophagy between systems. *Amara sp* and *H. affinis* have also been observed consuming aphids but at lower predation rates (Sunderland and Vickerman, 1980; Staudacher et al., 2018). However, Amara and Harpalus genera are known for their predation service on weed seeds (Honek et al., 2007; Saska et al., 2008), highlighting that other services not assessed in our study could be affected by conservation practices.

When considering only circulating carabid communities, some species were also characteristic of the soil management mode. *Demetrias atricapillus* and *Drypta dentata* were more abundant in conventional fields, while *Nebria brevicollis* and *Notiophilus biguttatus* were more abundant in conservation fields. Spring breeders that overwinter as adults, such as *D. atricapillus* and *D. dentata*, should be resistant to soil disturbance. In contrast, species that breed in autumn and overwinter in their larval form are more sensitive to perturbation (Purvis et al., 2001; Ribera et al., 2001 but Kosewska et al., 2014), which is consistent with the observed higher abundance of *N. brevicollis* and *N. biguttatus* in conservation fields as they breed in both Autumn and Spring. In addition, the ability to fly of *D. atricapillus* and *D. dentata*, which had functional wings, gives them good dispersal capabilities, which is advantageous in conventional fields to escape disturbance and recolonize (Ribera et al., 2001). *D. atricapillus* has been shown to be a good aphid-consumer (Sunderland and Vickerman, 1980) but the diet of *N. brevicollis* includes a wide variety of pests in addition to aphids: lepidopterans, crane flies or even slugs (Sunderland and Vickerman, 1980; Seric Jelaska et al., 2014; Reich et al., 2020), confirming the presence of multiservice-providing species in conservation fields.

CONCLUSION

This study assessed the impact of conservation soil management on some selected natural enemy characteristics of a single pest and their corresponding complementary services. Our results contributed to a better understanding of the sensitivity to soil management of ground-dwelling natural enemies like carabids and suggested a correlation between both abundance and assemblage of carabids and aphid predation. In contrast, foliage-dwelling natural enemies like parasitoid wasps had a moderate response to soil management, and so had their parasitism service. Despite an overall positive effect of conservation soil management compared to conventional soil management, aphid infestation remained similar between farming systems, suggesting soil management is not the only factor affecting aphid biological control.

Considering that one of the natural enemy communities was composed of generalist predators, variations of this community due to conservation soil management could also have an impact on the biocontrol of other pests. Other ecosystem services could be impacted altogether by conservation practices, such as decomposition of organic matter or pollination, and a multiservice approach would allow for the assessment of synergies and trade-offs between services.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

EC and MP conceptualized and designed the study. ASMP, EC, and SO-M acquired fieldwork data. ASMP analyzed the data and wrote the first version of the manuscript. All authors contributed to 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/fevo.2022.893787/full#supplementary-material

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REVIEW
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Vineyard Management and Its Impacts on Soil Biodiversity, Functions, and Ecosystem Services

Brice Giffard[1]*, Silvia Winter[2], Silvia Guidoni[3], Annegret Nicolai[4,5], Maurizio Castaldini[6], Daniel Cluzeau[7], Patrice Coll[8], Jérôme Cortet[9], Edith Le Cadre[10], Giada d'Errico[11,12], Astrid Forneck[13], Elena Gagnarli[14], Michaela Griesser[13], Muriel Guernion[7], Alessandra Lagomarsino[6], Silvia Landi[14], Yves Le Bissonnais[15], Elena Mania[3], Stefano Mocali[6], Cristina Preda[16], Simone Priori[17], Annette Reineke[18], Adrien Rusch[19], Hans-Josef Schroers[20], Sauro Simoni[14], Magdalena Steiner[21], Elena Temneanu[16,22], Sven Bacher[21], Edoardo A. C. Costantini[23], Johann Zaller[24] and Ilona Leyer[25]

[1] Bordeaux Sciences Agro, INRAE, SAVE, ISVV, Bordeaux, France, [2] Institute of Plant Protection and Institute of Integrative Nature Conservation Research, University of Natural Resources and Life Sciences Vienna (BOKU), Vienna, Austria, [3] Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco, Italy, [4] UMR 6553 EcoBio, Station Biologique de Paimpont, Université Rennes 1, Paimpont, France, [5] Living Lab CLEF, Plélan-le-Grand, France, [6] Research Center for Agriculture and Environment – Council for Agricultural Research and Economics (CREA- AA), Florence, Italy, [7] UMR CNRS ECOBIO, OSUR, Station Biologique, Université Rennes 1, Paimpont, France, [8] Viti-Oeno Conseil, Perpignan, France, [9] UMR CEFE 5175, University of Montpellier, EPHE, University Paul-Valéry Montpellier, Montpellier, France, [10] Institut Agro, INRAE, SAS, Rennes, France, [11] Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy, [12] CNR – Istituto per la Protezione Sostenibile delle Piante (IPSP), Portici, Italy, [13] Department of Crop Sciences, Institute of Viticulture and Pomology, University of Natural Resources and Life Sciences, Vienna, Austria, [14] Research Centre for Plant Protection and Certification-Council for Agricultural Research and Economics (CREA–DC), Palermo, Italy, [15] LISAH, Univ Montpellier, INRA, IRD, Montpellier SupAgro, Montpellier, France, [16] Faculty of Natural and Agricultural Sciences, Universitatii Alley, Ovidius University of Constanta, Constanta, Romania, [17] Dipartimento di Scienze Agrarie e Forestali (DAFNE), Università degli Studi della Tuscia, Viterbo, Italy, [18] Department of Crop Protection, Geisenheim University, Geisenheim, Germany, [19] INRAE, UMR 1065 Santé et Agroécologie du Vignoble, ISVV, Université de Bordeaux, Bordeaux Sciences Agro, Bordeaux, France, [20] Agricultural Institute of Slovenia, Ljubljana, Slovenia, [21] Ecology and Evolution Unit, Department of Biology, University of Fribourg, Fribourg, Switzerland, [22] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania, [23] CNR-IBE – Department of Biology, Agriculture and Food Sciences, Sesto Fiorentino, Italy, [24] Institute of Zoology, University of Natural Resources and Life Sciences Vienna (BOKU), Vienna, Austria, [25] Department of Applied Ecology, Geisenheim University, Geisenheim, Germany

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***Correspondence:**
*Brice Giffard
brice.giffard@gmail.com*

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Healthy soils form the basis of sustainable viticulture, where soil characteristics have a direct impact on wine quantity and quality. Soil not only provides water and nutrients to vines, but is also a living medium containing micro- and macroorganisms that perform many ecological functions and provide ecosystem services. These organisms are involved in many processes, from decomposing organic matter to providing minerals to vine roots. They also control diseases, pests, and weeds, in addition to improving the soil structure in terms of its capacity to retain water and nutrients. Related to decomposition processes, the carbon content of vineyard soils influences fertility, erosion and biogeochemical cycles, with significant implications for the global climate. However, common agricultural practices represent strong threats to biodiversity and associated ecosystem services provided by vineyard soils. As consumers increasingly consider environmental aspects in their purchase decisions, winegrowers have to adapt their vineyard management strategies, raising the demand for sustainable pest- and weed-control methods. This article presents a comprehensive review of the impacts of vineyard practices on the soil ecosystem, biodiversity, and biodiversity-based ecosystem services, and provides future prospects for sustainable viticulture.

Keywords: microarthropods, earthworms, gastropods, nematodes, plants, predatory arthropods, microflora, pest control

INTRODUCTION: THE IMPORTANCE OF SOIL IN VITICULTURE

Vineyards are planted all over the world, mostly between 30 and 50 degrees latitude, in both the Northern and Southern hemispheres. In 2020, the total area under vines was about 7.3 million hectares (wine grapes, table grapes or dried grapes) and total wine production was 260 million hectoliters. The global market (total exports of all countries) reached 105.8 million hectoliters and 29.6 billion Euro in terms of value (Organisation Internationale de la Vigne et du Vin [Oiv]., 2021). Despite this significant economy, there is a growing awareness and concern among winegrowers and consumers that some agricultural practices, and in particular the use of pesticides and intensive tillage, can have detrimental impacts on biodiversity (Paiola et al., 2020), soil quality and soil associated biodiversity and on sustainable wine production (Viers et al., 2013). Hence, winegrowers must consider and promote soil quality in their vineyards, defined as "the capacity of a soil to function within ecosystem boundaries, sustain biological productivity, maintain environmental quality and promote plant and animal health" (Doran and Zeiss, 2000; Riches et al., 2013).

However, knowledge about the threats to the soil in vineyards and their consequences on wine quality is scarce. Thus, efforts are required to better understand and protect soil to maintain respective ecosystem services. Interactions between soil biological communities as well as chemical and physical properties of the soil environment are fundamental to many soil processes, functions and services, such as carbon storage and cycling, nutrient cycling, soil structure formation, and pest regulation (Pulleman et al., 2012). Soils also play a key role in climate regulation and, thus, in mitigation and adaptation to climate-change, particularly in regulating greenhouse gases (Pulleman et al., 2012). This contrasts with the considerable body of knowledge on the relationships between soil characteristics and wine quality, driven by the prestige and profitability of high-quality wine production. Future research must focus on a better understanding of threats associated with viticultural management practices, as well as options for protecting soil biodiversity, functions and services while maintaining high-quality wine production and the aesthetic value of vineyard landscapes. The current work summarizes existing knowledge concerning the effects of vineyard practices on soil biodiversity, and how related ecosystem functions and services may enhance vine growth and yield. Subsequently, it offers some prospects for mitigating threats to the soil ecosystem and improving conditions for biodiversity, drawn from current research projects on these topics.

RELATIONSHIP BETWEEN TERROIR CHARACTERISTICS, SOIL MANAGEMENT, AND WINE PRODUCTION

The economic importance of wine production within a particular territory is one of the most effective motivations for protecting wine typicity (Costantini et al., 2012; Vaudour et al., 2015). Consumer perception that wine quality is closely linked to its geographical provenance creates the basis for preserving rural environments, not only in terms of productivity, but also in terms of landscape aesthetics and lifestyle. Consequently, winegrowers in geographically defined production areas place particular importance on preserving the quality of the land, thus adding further value to the wine. Wine marketing often uses the beauty of the vineyard landscape as an effective medium for promoting sales and increasing the market value of the product, developed through oenotourism (Tempesta et al., 2010). The use of the expressions "terroir," "typical," "identity," and "sense of place," mostly used in the wine sector, strictly connect the value of geographic origin and the landscape to the intrinsic value of a wine (van Leeuwen et al., 2004). In contrast, viticulture is often practiced as an extensive monoculture within a region, reducing the cover and quality of natural or semi-natural habitats. This has a negative impact on landscape aesthetics and associated biodiversity (Costantini and Barbetti, 2008). Management of the entire agroecosystem should be integrated, including ecological and cultural practices, to improve sustainability and habitat protection, as well as biodiversity and the associated ecosystem services (Viers et al., 2013; Chrysargyris et al., 2018). Most of these conservation actions concern the soil, which is not only one of the most threatened habitats in vineyards (Costantini et al., 2015, 2018), but also provides fundamental services to wine production.

Terroir may be defined as the interaction of all ecosystem characteristics in a given place that affect the crop phenotype, including vines (grape varieties and rootstocks), climate, and soil (van Leeuwen and Seguin, 2006). Grapevines are planted in a huge variety of different soils throughout the world, but soil is one of the most important factors in wine quality (van Leeuwen and Seguin, 2006). The wine economy of an area, including the focus on marketing high- or low-priced wine, is associated with soil characteristics, as well as environmental factors such as climate, geomorphology, and landscape quality (Costantini et al., 2016). However, only rarely the impact of soil management on biodiversity is taken into account.

Based on the most recent classification proposed by the Common International Classification of Ecosystem Services (CICES - La Notte et al., 2017), many soil processes contribute to functions associated with Ecosystem Services (ESs). For example, grape production and the decomposition of organic matter are related to provisioning and regulating ESs. According to Lal (2001), any soil degradation impairs soil quality or deteriorates the functions that contribute to ESs provision. It is, therefore, essential to consider the effects of soil management practices in vineyards on ecosystem functions (Herrick, 2000), particularly in the context of climate change (Chrysargyris et al., 2018).

In the Mediterranean or comparable water-limited climates, rainfall is generally concentrated in the winter season and does not usually exceed 400–500 mm per year. In this context, where water is a very limited resource, traditional management is based on intensive weed control to avoid competition with grapevines for water and nutrients. Herbicide use increased considerably with the global intensification of pesticide use in the 1950–1960s. However, nowadays, tillage and herbicide use are the two most-widespread systems under water-limited conditions (e.g., Biarnès et al., 2004). These practices are associated with a decline in soil quality, e.g., soil erosion, soil compaction, and loss of organic matter (Steenwerth and Belina, 2008; Salome et al., 2014, 2016; Biddoccu et al., 2016). Herbicide use also negatively influences biodiversity through the reduction of resources (Kazakou et al., 2016; Hall et al., 2020) or direct effects on the metabolism of organisms and groundwater quality (Louchart et al., 2001).

In temperate regions, especially under maritime influence, the climate is characterized by higher rainfalls, more evenly spread throughout the year, and soils are more fertile with higher nutrient availability (Peregrina et al., 2012). Hence, tillage or herbicides to reduce competition between vines and weeds is unnecessary and may even be undesirable, due to the risk of intensified erosion and nutrient leakage from bare soil in heavy rainfall (Biddoccu et al., 2016). In addition, it may be difficult to drive machinery for other pesticide applications. Permanent plant cover between rows has been widely adopted, at least during winter and spring, since vineyards are more often prone to precipitation-related soil erosion than weed competition. A further possibility is to adopt a permanent grass cover in alternate inter-rows, while the intervening inter-rows are tilled one or more times a year, depending on precipitation conditions and any potential negative competitive effects of weed species on grape yields or quality (Peregrina et al., 2012).

Another intermediate management technique, called "green manure," combines tillage or herbicide and permanent cover, with sown cover crops in winter or spring. These cover crops help to improve nutrient supply to the vines (especially nitrogen fixation by legumes species), store carbon, and mitigate soil erosion during winter. This practice includes the cultivation of sown cover crops destroyed by tillage before it starts competing with the vines for nutrients and water. Nevertheless, the soil type and, in particular, its lime content and texture, strongly influence the effectiveness of cover crops in providing these benefits (Ruiz-Colmenero et al., 2011; Salome et al., 2016). Consequently, strategies combining various management practices are more effective for maintaining and improving soil quality (Ruiz-Colmenero et al., 2011). Therefore, flexible, innovative practices are required to face the key issues of vineyard complexity and counteract the negative effects of climate change on soil fertility (Salome et al., 2014, 2016).

Wine-growing areas are more exposed to several environmental risks, as other agricultural landscapes: soil erosion, depletion of biodiversity, as well as water and air pollution due to the use of pesticides (Viers et al., 2013). In addition, vineyard topography (especially steep slopes), the abundance and distribution of precipitation (modified by climate change), and anthropogenic factors increase soil exposure to various threats, particularly erosion and landslides (Arnaez et al., 2007; Martínez-Casasnovas and Ramos, 2009; Biddoccu et al., 2016; Chrysargyris et al., 2018). As highlighted by the analysis of long-term soil-loss measurements under natural rainfall (Cerdan et al., 2010), vineyards exhibited the highest soil losses in Europe, compared to other crops and land uses. Vineyard planting also strongly impacts soil and involves slope reshaping, deep plowing, which mixes topsoil and subsoil, as well as stone crushing and removal. These operations entail a high risk of decreasing soil functionality and biodiversity, as well as degrading the beauty of the landscape and long term sustainability of the vineyard (Le Bissonnais et al., 2002; Costantini and Barbetti, 2008; Martínez-Casasnovas and Ramos, 2009; Costantini et al., 2015). During high-intensity rainfall events, the accelerated erosion damages fields, reduces organic carbon stocks and soil fertility, exposes roots, and causes rill erosion, as well as downstream damage, with flooding, mudslides, and pollution (Le Bissonnais et al., 2002; Arnaez et al., 2007). As this accelerated erosion modifies the carbon dynamics, it may also have a broader environmental impact, such as reducing carbon sequestration and biodiversity (Lal, 2001). Furthermore, climate change may lead to the extension of viticulture at higher altitudes and latitudes, potentially impacting upland ecosystems and eliminating natural or semi-natural vegetation (Hannah et al., 2013).

SOIL BIODIVERSITY IN VINEYARDS: TROPHIC INTERACTIONS AND ECOLOGICAL SERVICES

Soils are living environments and include a large variety of microhabitats, characterized by different physicochemical properties (Coleman et al., 2004). A countless diversity of organisms lives in these habitats, generally divided into 4 categories according to their body size (Lavelle and Spain, 2001): microorganisms (Fungi, Bacteria, Protists, and Archaea), microfauna (e.g., Collembola, Acari, Amoeba, Nematoda, Annelida Enchytraeids) and macrofauna (e.g., earthworms, gastropods and larger arthropods).

All these organisms have a textural (changes in the distribution of mineral and organic particles) or structural (formation of aggregates and soil horizons) influence on soil functions. Soil texture and structure influence above-ground vegetation and animal communities through changes in fertility, related to biogeochemical cycles and plant nutrition (Coleman et al., 2004; Blouin et al., 2013). Soils provide nutrients and water to support the growth of both crop and spontaneous plant species: the latter are often considered weeds (**Figure 1**). The provisioning service of soil fertility associated with the regulation service of nutrient cycling is directly or indirectly associated with below-ground organisms, from macro- or mesofauna, the primary decomposers of organic matter, to microorganisms (**Figure 1**). Agricultural practices, such as tillage, pesticide and heavy machinery use, interfere with vegetation diversity and cover and are of primary importance for the soil trophic networks that drive the diversity and abundance of all taxa.

Most of the studies exploring the relationship between soil biodiversity and its functions in agricultural landscapes have focused on arable crops, but less on vineyards. The following paragraphs examine aboveground biodiversity that is strongly linked to soil conditions, firstly represented by vascular plant communities in vineyards and the services they provide. Organisms will be then considered in order of size, from the larger taxa of animals, macrofauna and mesofauna living in soil or on the ground surface, to microorganisms. We describe the state of the art concerning these taxa, as well as the services provided in vineyards, particularly nutrient cycling and pest regulation, as well as the influence of agricultural practices and soil parameters on the diversity and abundance of these taxa.

Very little research exists on larger soil organisms such as small mammals (moles and small rodents), and focuses mostly on their negative effects on vine yield, and their biological control by predatory birds such as owls in Napa valley using next boxes in vineyards (e.g., Wendt and Johnson, 2017).

Vascular Plants

Herbicides and tillage are still the most common techniques for soil management in viticulture as they are supposed to eliminate the competition between weeds and vines, thus improving grape yields and quality in water-limited conditions (Peregrina et al., 2012). However, no significant competitive effects of inter-row

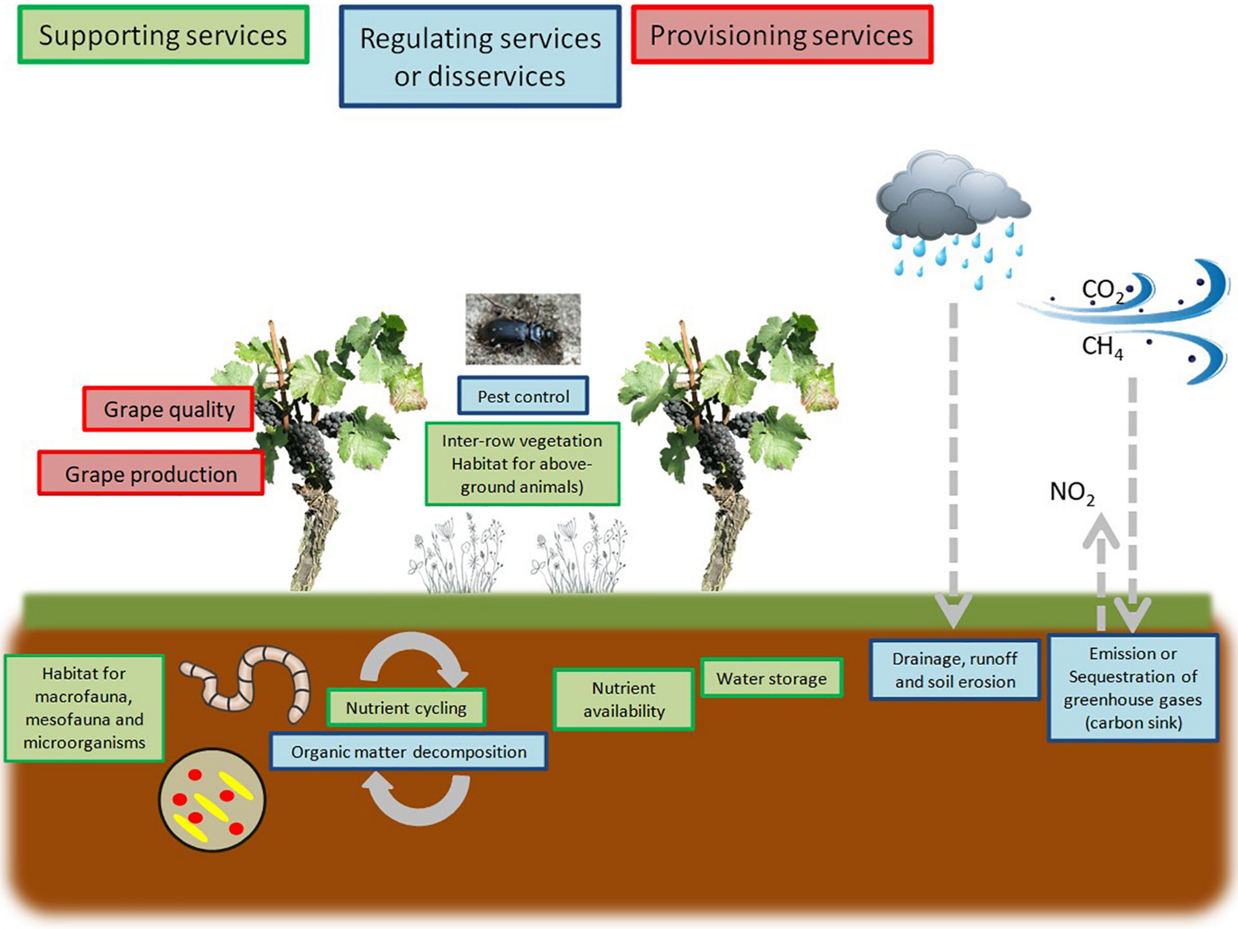

FIGURE 1 | Ecosystem services provided by vineyard soils. Biodiversity is strongly linked to habitats availability in this agroecosystem: soil and plant cover above-ground. In turn, biodiversity strongly drives several regulating services (blue boxes), related to supporting services (green boxes), and influences some provisioning services (red boxes).

vegetation cover have been observed in oceanic climates or irrigated vineyards, especially where soil water-holding capacity was high (Wheeler et al., 2005; Monteiro and Lopes, 2007; Baumgartner et al., 2008; Steenwerth et al., 2013; Irvin et al., 2016). Nowadays, many winegrowers are aware of the benefits of plant cover for the vines and the vineyard agroecosystem. The vineyard should be considered as a consociation, formed by the vines and the underlying grasses, both complementing the functions and ecosystem services of the vineyard. Associated plant diversity in vineyards may contribute to multiple ESs, such as mitigating soil erosion and, preserving soil fertility, biocontrol of vine pests by promoting colonization of vine rows by their natural predators (see in the following paragraphs and Nicholls et al., 2000), aesthetic value of viticultural landscape (Hervé et al., 2020), and conservation of endemic species (Gillespie and Wratten, 2012). Studying the benefits and ecosystem services supported by spontaneous flora in vineyard agroecosystems is a quite novel topic (Garcia et al., 2018), but findings suggest that vegetation cover should be used wherever soil and climate conditions are favorable.

Vineyards are mostly associated with a moderate level of plant diversity; nevertheless, like other permanent, heterogeneous systems with a multi-strata design such as orchards (Simon et al., 2010), they have a high potential for preserving agroecosystem biodiversity and providing habitats for xerothermic species. In recent years several studies have evaluated plant diversity in vineyard ecosystems. Examining varying numbers of sampling plots and environmental conditions, 32 plant species were found in one vineyard in Napa Valley (Baumgartner et al., 2008), 86 species in 25 vineyards (averaging 22.2 ± 2.9 per site) in northern Switzerland (Bruggisser et al., 2010), 177 species in 33 vineyards (ranging from 24 to 46 average number of plant species per vineyard) in the Piemonte region of north-western Italy (Mania et al., 2015), 211 species in 18 vineyards (averaging 32.8 in organic and 25.1 in conventional vineyards) in the Veneto region in north-eastern Italy (Nascimbene et al., 2012), over than 200 species in 120 vineyards of Dão winegrowing region of Central Portugal (Monteiro et al., 2012), and 259 species in 48 vineyards in southern Switzerland (Trivellone et al., 2014).

Soil conditions and cultural practices selected several physiological, morphological, and dispersal traits among plant communities (Kazakou et al., 2016), e.g., the proportion of perennial species increased with less-intense management (Gago et al., 2007; Kazakou et al., 2016). Plant diversity and community composition depend on inter-row plant management, i.e., mowing or mulching frequency, fertilization and tillage intensity, chemical weed control, and sowing of a mixed cover crop or fostering spontaneous vegetation (Gago et al., 2007; Bruggisser et al., 2010; Nascimbene et al., 2013; Trivellone et al., 2014), as well as the management and characteristics of adjacent areas (Thomson and Hoffmann, 2009; Simon et al., 2010; Mania et al., 2015). Weed-control techniques influence species richness and composition of plant communities. For example, shredding inter-row vegetation had a more negative effect on plant diversity than mowing but this effect was lower than local abiotic conditions, such as vineyard altitude and slope that drive the community composition (Bruggisser et al., 2010). In a landscape with small vineyard plots, the diversity of microhabitats also enhances the conservation of rare species. For example, 9% of sampled species in Swiss vineyards were regarded as near threatened or vulnerable, according to the national red list (Bruggisser et al., 2010). Extensive management practices in vineyards, like organic farming, have been found to increase plant diversity in Italy (e.g., Nascimbene et al., 2012), probably due to the ban on herbicide use. The negative effect of herbicides was also confirmed by Sanguankeo and León (2011) in California. However, Bruggisser et al. (2010) and Kehinde and Samways (2014) did not find any significant effect of organic farming on plant diversity in a set of South African and Swiss vineyards, where no herbicides were used, even in the conventional plots. Inter-row vegetation management also influences dominant plant traits, such as basal buds or underground regeneration in case of frequent mowing (Gago et al., 2007) or a higher specific leaf area in tilled vineyards (Kazakou et al., 2016). Several studies identified a significant reduction in vascular plant diversity in vineyards with intensive soil tillage (Sanguankeo and León, 2011; Bagella et al., 2014; Kazakou et al., 2016). This technique benefits certain species that regenerate from plant fragments, like *Spergula arvensis* (segetal species), and annual plant species with short development cycle (Gago et al., 2007). Changing from mulching to mowing led to an increase in the proportion of plant species with basal rosettes (Bruggisser et al., 2010). Traditional species-rich communities, featuring bulbous geophytes, have become very rare due to changes in soil management in Central European vineyards (Bruggisser et al., 2010). Mowing frequency also modifies dominant plant traits and particularly enhances the cover of rosulate and reptant species (Nascimbene et al., 2012). Nevertheless, since the seed germination of ruderal species benefits from bare soil created by tillage, a low frequency of soil disturbance seems to benefit plant diversity (Gago et al., 2007), in accordance with the intermediate disturbance hypothesis (Grime, 2006).

First tested in annual crops, there is a growing interest in cover crops or sown vegetation in vineyards, at least in inter-rows (Garcia et al., 2018). As already mentioned, one common technique is to establish permanent vegetation in alternate inter-rows, while the intervening inter-rows are tilled several times a year, depending on precipitation or the sowing of cover crops. Annual cover crops may be divided into two main classes: winter cover crops, sown in autumn, adapted to winter conditions and removed by tillage in the following spring or dry season, and summer cover crops, sown in spring. Cereals and legumes are the two main categories of plants used as cover crops. Spontaneous as well as sown species may provide several services to winegrowers: their management and the choice of species are very important for simultaneously maintaining production and regulating services in the vineyard.

Although using resident natural vegetation or sowing cover crops is viewed partly critically due to potential competition effects on vines, this management is beneficial from an environmental point of view (Marques et al., 2010) and it is supported by agro-environmental schemes. This becomes especially obvious if a permanent vegetation cover is permanently maintained over the entire surface throughout the year to reduce soil erosion, one of the major ecosystem disservices in many sloping vineyards (Gyssels et al., 2005; Blavet et al., 2009; Marques et al., 2010). Plant species adapted to local conditions may also provide numerous other ESs for the agroecosystem, including buffering soil nitrogen availability (Martínez-Casasnovas and Ramos, 2009; Messiga et al., 2015; Pérez-Álvarez et al., 2015), increasing soil organic matter content (Steenwerth and Belina, 2008; Blavet et al., 2009; Mazzoncini et al., 2011; Salome et al., 2016), regulating water use (Monteiro and Lopes, 2007), soil temperature and vineyard microclimate (Fourie and Freitag, 2010), as well as in fertile soils, controlling vine vigor and enhancing grape quality (Wheeler et al., 2005; Monteiro and Lopes, 2007; Giese et al., 2014). Furthermore, fostering spontaneous vegetation or use of cover crops not only increases plant diversity (Hall et al., 2020) but also inhibits troublesome weeds (Monteiro et al., 2012; Kazakou et al., 2016; Steenwerth et al., 2016). For example, the use of cover crops reduced the cover and frequency of spontaneous, ruderal plant species, like *Digitaria sanguinalis* and *Chenopodium album* (Gago et al., 2007; Smith et al., 2015). Spontaneous vegetation enhances the biodiversity of the whole ecosystem at different trophic levels, as it provides the main energy source for decomposers and heterotrophic organisms (Danne et al., 2010; Kehinde and Samways, 2014; James et al., 2015; Buchholz et al., 2017; Geldenhuys et al., 2021).

Macrofauna: Earthworms

Among all the taxa, earthworms are a well-studied group of macroinvertebrates, representing the largest component in the animal biomass in soils (0.1–12 g dry weight per square meter) and are very important organisms for maintaining soil fertility (Jeffery et al., 2010). They are commonly known as "ecosystem engineers," thanks to their burrowing, mixing and casting activities (Jones et al., 1994; Blouin et al., 2013). Earthworms can be divided into three ecological categories, based on their distribution within the soil: epigeics, anecics and endogeics (Bouché, 1977 in Blouin et al., 2013). Epigeic species, also known as litter or surface-dwelling species, live on the soil surface, in leaf litter and humus layers, and sometimes in the first few soil centimeters. Anecic species, also known as topsoil species or soil-dwelling species, live in permanent, vertical burrows, connected to the soil surface, which are important for soil drainage. Endogeic species, also known as subsoil or soil-dwelling species, live mainly within the soil and are important for maintaining its granular structure. All these groups and species are known to strongly influence plant growth by creating and connecting pores (burrows, aestivation or hibernation nests), that modify the physical matrix for roots, aggregating or disaggregating particles, and moving them within the soil profile (Wurst et al., 2018). These activities are conditioned by soil organic matter content and quality, soil compaction, species interactions, and seasons (Pérès et al., 1998; Capowiez et al., 2009; Blouin et al., 2013).

Ecological functions provided by earthworms are mainly pedogenesis, soil structure development, water regulation, nutrient cycling, primary production, climate regulation, and pollution remediation (Blouin et al., 2013). Soil management practices in vineyards have direct and indirect impacts on the ecology and physiology of earthworms (Schreck et al., 2012). While the impacts of tillage on earthworms have been studied intensively in arable systems, little is known about vineyards. However, the consequences of soil tillage are quite similar, as it severely reduces earthworm abundance, biomass, and variety (Paoletti et al., 1998). Conversely, Buchholz et al. (2017) showed a higher species diversity in infrequently tilled plots, which could be related to a higher plant diversity and soil fertility in these plots. Earthworm biomass was negatively, but only slightly, impacted by reduced tillage methods, such as grubbing, rotary hoeing and harrowing, particularly in spring under dry soil conditions. Furthermore, when soil is tilled only every second inter-row, rapid re-colonization may occur between disturbed rows and rows with permanent herbaceous cover (Faber et al., 2017).

Earthworms are useful bioindicators, as they respond quickly to environmental inputs, such as chemicals, and can therefore be used to monitor the effects of farming practices and soil contamination (Paoletti et al., 1998; Pérès et al., 2011). Earthworms are impacted by copper, used as fungicide, which decreases earthworm survival, growth, and behavior (measured as a decreasing burrowing rate, Eijsackers et al., 2005; Pelosi et al., 2014; Karimi et al., 2021). Herbicides may also affect the activity and reproduction of earthworms, especially anecic species (Gaupp-Berghausen et al., 2015). A laboratory experiment showed that fungicide and insecticide spraying was neurotoxic for endogeic earthworms (Schreck et al., 2008). In contrast, organic fertilization increased the abundance, biomass, and diversity of the earthworm community (Pérès et al., 1998).

Macrofauna: Gastropods

Unlike earthworms, gastropods are part of a less studied group of macroinvertebrates, including isopods and myriapods, comprising mainly litter- and topsoil-dwelling species, as well as a few endogeic ones. ESs provided by gastropods are mainly linked to the decomposition of organic matter and soil structure improvement (Wolters and Ekschmitt, 1997). By feeding on organic matter and promoting microflora through burrowing organic material, as well as producing enzymes and feces, gastropods contribute 1% to the total decomposition activity of soil biota (Schaefer, 1990).

In natural systems, gastropod richness and diversity decreased with lower soil pH values, due to lower calcium availability (Nekola, 2010). Soil moisture, texture, and vegetation composition are also strong drivers of their abundance and diversity (Dvořáková and Horsák, 2012; Hettenbergerová et al., 2013). In vineyards, as in agricultural fields in general, most gastropods are xerothermophilic (dry- and warm-adapted, mostly open-land species), but communities tend to be less species-rich than in grasslands. Cultivation involves several types of disturbance: (i) mechanical disturbance that increases exposure to predators and destroys microhabitats used for reproduction and shelter, (ii) simplification of vegetation that leads to loss of shelter, moisture, and food, (iii) use of mineral fertilizers that acidify the soil, such as nitrates, and (iv) biocides that reduce plant cover and/or are toxic to gastropods (Wolters and Ekschmitt, 1997).

The decrease in abundance and richness within gastropod communities may foster pest organisms: the simplification of plant cover favors pest species with corresponding feeding preferences and the loss of predators and competitors (Wolters and Ekschmitt, 1997). Most of the introduced gastropod species are considered vineyard pests, such as (i) the *Cepaea nemoralis* snails in Ontario, Canada, which use vines as a daytime roost and are picked with the grapes, thereby fouling the wine (Martinson, 1999), (ii) *Meghimatium pictum* slugs in Brazil, that feed on fruit pulp (Baronio et al., 2014), (iii) *Bradybaena similaris* snails in Taiwan, that destroy leaves and buds (Chang, 2002), and (iv) *Cornu aspersum* and *Theba pisana* snails in South Africa, which feed on developing foliage and leaving a mucus trail on grapes, deteriorating the aesthetics of table grapes, or contaminating dried-fruit products with their shells in Australia (Sanderson and Sirgel, 2002). The last two species may reach densities of up to 400 individuals per vine and cause economic losses of up to 25% (Sanderson and Sirgel, 2002).

Soil management practices that minimize the disturbance of gastropod communities, such as low tillage and the use of organic fertilizers, help to maintain and restore the gastropod community and reduce or eliminate pest problems (Wolters and Ekschmitt, 1997). The maintenance of a complex landscape in wine-growing regions may also favor the persistence of gastropod communities able to recolonize vineyards if soil management intensity decreases. For example, in Austria, two rare gastropod species, *Zebrina detrita* and *Caucasotachea vindobonensis*, had refugia in managed grassy stripes bordering vineyards (Duda, 2016). However, changing from intensive soil management to an extensive system may also attract pest species (e.g., in Australia - Sanderson and Sirgel, 2002). Vegetation or mulch cover provides shade and moisture advantageous for gastropod development and thus increasing pest slug densities (Baronio et al., 2014).

Macrofauna: Predatory Arthropods

Among the arthropod communities that provide ESs in agroecosystems, generalist predators may act as biological control agents of crop pests (Symondson et al., 2002). Several groups of generalist predators are found in vineyards. Spiders (Araneae), rove beetles (Staphylinidae), ground beetles (Carabidae) and ants (Formicidae) are among the most abundant taxa living on the ground or near the soil surface in vineyards (Thomson and Hoffmann, 2009). Soil dwelling spiders are excellent hunters and mostly belong to the Lycosidae and Gnaphosidae families. They prey on various vineyard pests, e.g., caterpillars (grapevine moths) or Hemipteran species living on vine leaves. Ground beetles such as carabids or rove beetles may also be abundant in vine plots. They are well-studied arthropod predators in agroecosystems and consume various insect pests and weed seeds (Holland, 2002). Moreover, they are well-adapted to open habitats and intensive agricultural landscapes, dominated by perennial cropping systems, such as vineyards (Rusch et al., 2016a). Ants may also exert top-down control of arthropods in natural and agricultural systems (e.g., Chong et al., 2010), but mealybug-tending ants could also hamper parasitoid biocontrol of certain pests such as vine mealybug (Cocco et al., 2021).

Several factors related to soil, vegetation diversity and management are known to affect generalist predators and regulation associated services (Rusch et al., 2010; Winter et al., 2018; Pfingstmann et al., 2019).

Firstly, habitats with higher plant diversity or a more complex structure feature more abundant predators, smaller herbivore populations, and less plant damage, compared with plant monocultures (Langellotto and Denno, 2004; Letourneau et al., 2011; Rusch et al., 2017). Vegetation diversity around vineyards and in-field grass cover is thus expected to favor the diversity of arthropod communities with a high level of resources (Nicholls et al., 2001; Hogg and Daane, 2010), and sustains biological pest control and seed predation (Rusch et al., 2017; Winter et al., 2018). Perennial agroecosystems provide important resources and functions for all these generalist natural enemies, such as overwintering sites, alternative hosts, and food sources. Rusch et al. (2017) demonstrated that inter-row plant cover is a strong driver of grape moth density in vineyards: it was significantly lower in vineyards with full compared to partial grass cover. Higher richness and activity-density of rove beetles, carabids, and hoverflies were found when natural vegetation was preserved, at least between the rows (Sanguankeo and León, 2011; Pétremand et al., 2017). However, several studies detected little or no effect of vegetation cover on carabid communities: the presence of surrounding vegetation may influence trapping success (Hanna et al., 2003; Sanguankeo and León, 2011). Community changes may also occur, with higher abundances of macropterous species with higher dispersal capacities than dimorphic or brachypterous species, which may be associated with an increase in tillage (Rusch et al., 2016b). The relationship between arthropod diversity and ES delivery is, however, variable, suggesting that rare species may contribute little to the agroecosystem functions. Thus dominant species are probably the main drivers of ES delivery such as pest control (Winfree et al., 2015; Winter et al., 2018).

Secondly, disturbance on the field scale, especially soil tillage, is known to be a key driver of the abundance and diversity of predatory arthropods (Holland, 2004). Several characteristics of soil tillage may affect aboveground arthropods, including intensity, frequency, the type of tool used, and the timing of the operation (Rusch et al., 2010; Pfingstmann et al., 2019). Larval stages of several predatory Coleopteran families are also strongly dependent on above-ground resources and disturbance intensity (Holland, 2002). Tillage may affect arthropods through indirect effects on habitat quality, by modifying their biochemical and biophysical characteristics or changing prey distribution and resource availability such as microarthropod prey (Pfingstmann et al., 2019). In addition, soil tillage may have direct lethal effects through mechanical damage, burying organisms or forcing them to migrate (Holland, 2004; Rusch et al., 2010).

Thirdly, it is reported that organic farming enhances the abundance and diversity of several taxa, from plants to birds, as compared to conventional arable farming (Tuck et al., 2014). However, somewhat contrasting results have been reported in viticulture: some studies reported a neutral effect

(Bruggisser et al., 2010; Uzman et al., 2020) and others a positive effect of organic farming on certain predatory arthropods (Caprio et al., 2015; Ostandie et al., 2021). These may be due to the taxa considered, as well as differences in terms of farming practices labeled "organic" or "conventional", which are highly variable (Ostandie et al., 2021). Brachypterous carabids, ambush spiders, ground-hunter spiders and other hunters preferred organic vineyards, whereas macropterous carabids, specialist spiders (mostly ant-eating spiders), and sheet web weavers selected conventional vineyards (Caprio et al., 2015). Landscape context is also known to be an important predictor of arthropod assemblages and the level of biological control in agroecosystems (Bianchi et al., 2006; Chaplin-Kramer et al., 2011; Rusch et al., 2016b). For example, it is well documented that semi-natural habitats are key habitats for many arthropods, as they foster individual fluxes, provide overwintering sites, refuges from disturbance, and alternative food and hosts (Thomson and Hoffmann, 2009; Hogg and Daane, 2010; Paiola et al., 2020).

Mesofauna: Collembolans and Mites

Soil mesofauna mediates soil functioning through a wide range of engineering processes such as distribution of organic matter, bioturbation, comminution, incorporation of litter into soil, determining structural porosity, and the formation of soil aggregates through burrowing, casting, and nesting activities, as well as feeding on microbial communities (Lavelle et al., 2006; Brussaard et al., 2007). These functions contribute to improving water infiltration, as well as mitigating soil erosion and nutrient provision. Collembola and some taxa in the mite (*Acari*) subclass, such as Oribatida are the most extensively studied microarthropods involved in detritivore food webs. The suborder Oribatida (order Sarcoptiformes) among the mites comprises over 10,000 species worldwide and is the most important group in providing decomposition ES in forests and grasslands (Culliney, 2013). They are also very abundant in vineyards (Gagnarli et al., 2015). They are involved in decomposition as direct consumers of organic matter, as well as indirectly, *via* a catalytic effect, by consuming saprophytic fungi and bacteria. This has a major impact on the composition of microorganism communities and makes a significant contribution to the fragmentation of plant residuals (Brennan et al., 2006). Generally, these organisms are quite sensitive to the quantity of resources in their habitat, i.e., the organic matter content of upper soil layers (Gagnarli et al., 2015).

Mesofauna abundance and biomass in soil differ among land use and habitat types. Few studies have identified microarthropods in soil samples at the species level and used standardized data on individuals per surface. However, a recent study in France compiling over 750 samples collected under different land-use conditions, including forests, grasslands, arable lands, vineyards, urban vegetable gardens, and urban soils, concluded that vineyard soils were by far the most negatively impacted by human activities (Joimel et al., 2017). Even if total microarthropod densities were higher in vineyards than in other agroecosystems, these soils presented the lowest Collembola species richness and evenness, the lowest Collembola ecomorphological index, and the highest Acari/Collembola ratio (Joimel et al., 2017). However, the samples were collected from Mediterranean vineyards (Languedoc and Banyuls appellations), where the dry climate may lead to generally low mesofauna density and diversity (Joimel et al., 2017). Renaud et al. (2004) found very wide variations in Collembola abundance, from a few hundred to over 12,000 individuals per square meter, depending on the soil treatment and season, with considerably smaller populations where herbicides were used. In undisturbed ecosystems, Oribatida densities easily reach several thousand individuals per m^2 comprising between 20 and 50 different species. In agricultural fields, their density is about ten times lower, mainly because of the cultivation practices that have negative effects on these mites (Behan-Pelletier, 1999). In vineyards, maintaining herbaceous vegetation in inter-rows fosters Oribatida adult densities (Nannelli and Simoni, 2002). Other vineyards in Europe need to be sampled to extend our knowledge of these groups, particularly their diversity and abundance. The abundance of some species seem to be enhanced by organic management as observed by Seniczak et al. (2018). Furthermore, vineyard management includes many different levels of impact (i.e., fertilization, mowing frequency) that affect this mesofauna (Fiera et al., 2020). Surprisingly, springtail communities were more diverse in tilled inter-rows, and springtails were more abundant in the inter-rows with herbicide use in vineyard rows of Romanian vineyards (Fiera et al., 2020), which is probably due to disrupted competitor/predator relationships. In Bordeaux vineyards, Ostandie et al. (2021) also found that tillage combined with organic farming and the reduction of insecticide use fostered springtail abundances, however, not their diversity. Due to their sensitivity to changes in the environmental conditions, mesofauna is increasingly used as a quality bioindicator of quality in agroecosystems. The Soil Biological Quality index QBS-ar assumes that soils of higher quality will shelter more abundant, varied mesofauna (Parisi et al., 2005). The advantage of this method is that specimens are not classified on a species level, but grouped into specific taxa (Classes, Superorders and Orders). Furthermore biological forms are determined according to their morphological adaptation to soil environments and scored to calculate the QBS-ar index (Parisi et al., 2005). The few studies conducted in vineyards found QBS-ar values ranging from 137 to 230, indicating potential higher quality than in arable soils (e.g., Rüdisser et al., 2015). Values were lower in Portugal vineyards and higher in ground cover treatments (Gonçalves et al., 2020). For example, some biological forms symptomatic of undisturbed soil, such as Diplura, Protura, Pauropoda, and Chilopoda, were found in many vineyards. Organic management even recently adopted (3-year period after conversion) fostered microarthropod community diversity as observed by Ghiglieno et al. (2020) in Lombardy vineyards. Several other parameters influence microarthropod communities such as vegetation cover, soil characteristics, degree of compaction, and organic matter content related to weed and soil management. In agricultural fields, the long-term exposure to synthetic or organic pesticides and their accumulation in soils also contributes to the reduction of the abundance and the diversity of mesofauna, particularly euedaphic forms (Karimi et al., 2020, 2021; Ostandie et al., 2021). A comprehensive risk assessment of the degree of soil contamination (by heavy metals and pesticides; excess of nitrates and phosphates) and declining soil biodiversity is usually indirectly assessed.

Mesofauna: Nematodes

Nematodes are probably the most abundant multicellular animals on earth, occupying a broad range of trophic levels. Several taxa of nematodes occupy important trophic positions in the soil detritus food web; many graze on bacteria and fungi, thus regulating decomposition and nitrogen mineralization. Other free-living or plant-parasitic nematodes are useful bioindicators of soil health. Nematode assemblages act as disturbance indicators for assessing the effects of pollution on soil and studying food web dynamics (Ferris and Tuomisto, 2015). The following tools are used to measure the ecological status of soil communities: the Maturity Index (MI), an ecological measure of environmental disturbance based on nematode species composition (Bongers, 1990), and the Ferris indices, based on trophic levels and food web systems (Ferris et al., 2001). ESs provided by soil nematodes include nutrient cycling (supporting service) and controlling pest species (regulating service). Predatory and omnivorous nematodes are involved in these services through a process of predation. Recently, Ferris and Tuomisto (2015) developed a new index named "diversity-weighted abundance" index, to evaluate the efficiency of these ESs.

Overall, agricultural intensification affects nematode fauna causing important multitrophic effects (Yeates et al., 1999; Govaerts et al., 2007). Nematodes are not necessarily adversely affected by cultivation practices and are, for example, less sensitive to tillage than larger soil animals (Wardle, 1995). As reported by Coll et al. (2012), compaction reduces nematode abundance and disturbs omnivores and predators in vineyards.

Few studies have investigated the whole nematode community associated with vineyards. Overall, free-living nematodes: bacterial feeders, omnivores, and predators, are more dominant in shallow soil (0–10 cm), whereas plant-parasitic nematodes are mainly located deeply and in the immediate vicinity of the root surface (Malossini et al., 2011; Rahman et al., 2014). Most studies have focused on plant-parasitic nematodes that cause serious damage in vineyards. Most severe losses are caused by *Xiphinema* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp. and *Criconemoides* spp. For example, Grapevine fanleaf virus (GFLV), transmitted by *Xiphinema index*, is responsible for a gradual degeneration of grapevines that occurs in most vineyards worldwide, causing yield losses of up to 80% (Andret-Link et al., 2004). *Xiphinema index*is the sole natural vector of GFLV and the transmission process is characterized by a specific association between *X. index* and GFLV. Their actual management is preventive: infested grapevines including their roots should be removed at an early stage and destroyed to avoid contamination of replants (Villate et al., 2008; d'Errico et al., 2014). Generally, under uncultivated soil and set-aside management, the nematode colonizer species, mainly bacterivores, increased in abundance and richness while plant-parasitic nematodes and predators remained constant (Landi et al., 2018). Soil resting strategies following vine uprooting is therefore applied currently especially in infested vineyards to reduce abundances of plant-parasitic nematodes. Traditionally, the most effective control of plant-parasitic nematodes has been based on the use of chemical fumigants and nematicides, today banned for toxicological and environmental reasons (d'Errico et al., 2014).

Several studies have reported that increased soil organic matter content has a direct effect on nematode community, reducing plant-parasitic and increasing saprophytic species. Amendments, such as poultry-litter biochar, green manures, and compost, decreased the abundance of plant-parasitic nematodes (Rahman et al., 2014). In a long term study, Coll et al. (2012) estimated that organic practices promoted fungal-feeding nematodes, which are key species in organic matter turnover and nutrient cycling in soils. Cover crops or vegetation strips with plants attracting parasitic nematodes is also a considered solution, although not well-explored (Villate et al., 2008).

Microorganisms and Microfauna

Soil is described as being the most diverse environment but the least known habitat, in terms of microbial diversity and its role in ecosystem functioning (Nielsen et al., 2015; Orgiazzi et al., 2016). Recent advances in genomics have made sampling and monitoring more standardized and affordable[1]. The major challenge is still to assign species to ecosystem functions. Nevertheless, it is well known that soil microorganisms are key drivers of most of the main soil processes (Nannipieri et al., 2003; Wagg et al., 2014) and play diverse and often crucial roles in soil-related ESs. Microbial communities drive soil nutrient dynamics and any changes in their activities and functions may compromise soil biogeochemical cycles and nutrient availability to plants. Soils harbor an enormous microbial diversity, mainly represented by bacteria and fungi that represent the largest proportion of the total microbial soil biomass. Thus, maintaining a taxonomically and functionally diverse microbial community is a key factor in supporting sustainable agricultural management (García-Orenes et al., 2016).

Microbes have been collected throughout the soil profile, although they are more abundant in upper soil layers and the rhizosphere (Bundt et al., 2001; Fierer et al., 2007). Rhizodeposition affects both the structural and functional diversity of microbial communities (Mendes et al., 2011; Dias et al., 2013; Philippot et al., 2013). Notably, soil bacteria can live in plant tissues as endophytes and modulate plant growth and development, with direct implications on plant physiological status and its response to biotic and abiotic stress (Bulgarelli et al., 2013) or on the beneficial properties of medicinal plants (Chiellini et al., 2014). Plant-soil-microbe interactions are complex, with plant species (even specific genotypes or varieties), soil type, and agricultural practices exerting the most influence on the composition of the microbial communities (Wu et al., 2008; Dias et al., 2013; Burns et al., 2015; Vega-Avila et al., 2015). Repeated tillage is known for its long-lasting impacts on soil microbial communities (Buckley and Schmidt, 2001; López-Piñeiro et al., 2013) and Pinguel et al. (2018) observed a strong composition difference in both bacterial and fungal communities between tilled rows and those with permanent

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[1] http://www.earthmicrobiome.org/

grass cover. Vega-Avila et al. (2015) also observed an increase in bacterial diversity in the rhizosphere of organically managed vineyards. Conversely, reduced tillage, nutrient application, and the reduction of pesticide inputs exert a positive effect on soil biology, resulting in a significantly higher level of biological activity (Mäder et al., 2002; Girvan and Bullimore, 2003; Morlat and Jacquet, 2003; López-Piñeiro et al., 2013; Martins et al., 2013). Moreover, long-term application of organic fertilizers compared to mineral fertilization induced a further increase (Treseder, 2008; Geisseler and Scow, 2014) or a slight decrease in microbial biomass with an increase in bacterial diversity (Francioli et al., 2016). Bokulich et al. (2014) proposed the term "microbial terroir" to emphasize the importance of regionally-specific microbial communities in fermentation processes that contribute to the specific characteristics of wine (see also Gilbert et al., 2014; Burns et al., 2015; Vaudour et al., 2015; Franco et al., 2021). Microbes colonizing roots may migrate through the plant to colonize aerial tissues (Bulgarelli et al., 2013), influencing plant traits and grape quality. Further studies are needed to elucidate the ecological role of specific soil- and plant-associated microbial taxa identified in vineyards. For example, soil protists are receiving increasing attention for their important and hitherto underestimated roles in soil food-web dynamics and nutrient cycling processes (Geisen et al., 2016). However, relatively few studies have investigated soil protists and none of them was conducted in vineyards.

Fungi and Mycorrhiza

Fungi drive ecological dynamics in soils as decomposers of organic biomass and nutrient recyclers. Macromolecules, including lignin and cellulose, are mostly depolymerized by fungi using peroxidases and laccases and are, therefore, essential for humification processes (Zavarzina et al., 2011). The filamentous nature of fungi and their production of polysaccharides contribute to aggregate formation and soil stability, especially in degraded soils (Rashid et al., 2016), and mineralization of nitrogen from organic biomass and rhizosphere depositions, thus recycling nitrogen for plant uptake. As a part of the food web, fungal hyphae provide a direct food source for numerous mesofaunal components, such as Collembola and mites (Jonas et al., 2007).

Numerous soil fungi are known for their opportunistic lifestyle, including strong saprotrophic compatibility and plant-related traits as endophytes or root associates. Certain *Trichoderma* species or strains activate plant defenses and induce root resistance to necrotic plant pathogenic fungi or restrict their proliferation through mycoparasitism. However, waterlogged situations or soil compaction in vineyards may trigger Blackfoot disease in grapevines, caused by other opportunistic species of the Nectriaceae with equally wide ecological amplitudes, including strong saprotrophic compatibility and plant-pathogenic abilities (Halleen et al., 2004). Permanent grass cover increased organic matter content in vineyard soils and, consequently, increased fungal counts (Whitelaw-Weckert et al., 2007). Furthermore, not only synthetic fungicides in conventional vineyards but also copper-based fungicides used in all vineyards including organic are used to control vine foliar pathogens and these can impact fungal soil communities and associated ESs (Probst et al., 2008; Karimi et al., 2020, 2021).

Among the most important soil fungi affecting the vineyard ecosystem are symbiotic arbuscular mycorrhizal fungi (AMF) (Trouvelot et al., 2015). AMF increase grapevine growth and nutrition by providing better access to soil nutrients and activating the regulation of plant transport proteins, particularly phosphate solubilization through acid phosphatases and the uptake of carbohydrates and lipids produced by plants and their dissemination into the soil (Trouvelot et al., 2015; Rich et al., 2017). The nutrient providing service of AMF is not the only one of primary interest in production systems where external inputs of fertilizers are limited (Gosling et al., 2006). It has also been demonstrated that AMF increase tolerance to abiotic stresses, such as water stress, soil salinity, iron chlorosis, and heavy metal toxicity, as well as protecting grapevines from root diseases (Trouvelot et al., 2015). Herbaceous plants used as cover crops may favor AMF in vineyards (Radic et al., 2012) whereas herbicides have been shown to decrease root mycorrhization (Zaller et al., 2018) but also soil AMF spore biomass, vesicles, and propagules in a model system (Zaller et al., 2014). AMF also induce resistance to root nematodes in grapevines (Li et al., 2006), as well as above-ground pathogens (Jung et al., 2012), as they alter biochemical pathways in above-ground grapevine tissue (Krishna et al., 2005). Surprisingly, AMF also alter the content and composition of secondary metabolites in grapevines, including phenols (Krishna et al., 2005).

Ecological Functions Associated With Soil Enzymes

Soil enzymes catalyze biochemical reactions and rate-limiting steps in organic matter decomposition and nutrient cycling in soil, thus controlling whether organic substances are decomposed or stored (Fansler et al., 2005) and influencing plant nutrient availability (Allison and Vitousek, 2005). Microorganisms mainly produce them, but plant debris, root exudates, and soil fauna also contribute to a lesser extent. Enzyme action may be intracellular or extracellular (both in the presence or absence of their originating cells), as well as free or immobilized (Gianfreda and Rao, 2004). Complex macromolecules (e.g., cellulose, lignin, pectin and hemicellulose) are not directly incorporated into cells, and need to be degraded by extracellular enzymes to yield small enough substrates (ca. 600 Da) for absorption into cell (Arnosti, 2011).

Extracellular enzymes, immobilized through association with clay minerals, humic acids, and particulate organic matter, retain significant levels of activity for prolonged periods (Burns, 1978; Dick, 1984; Fansler et al., 2005). The proportion of extracellular enzymes in soil largely varies depending on the type of enzyme and soil (reviewed by Burns et al., 2013). Anyhow, they represent a significant reservoir of potential activity and may even function as the first catalytic response to changes in substrate availability in soils, and they are also generating signal molecules for the microbial community (Caldwell, 2005; Fansler et al., 2005; Burns et al., 2013). Soil-bound enzymes may also be a source of substrate turnover during periods when microbial biomass is low or shut down due to stressed conditions (Stursova and Sinsabaugh, 2008). Their concentration in soils as well and microbial diversity and abundance is highly variable, as proposed by the use of "microbial hotspots and moments" by Kuzyakov and Blagodatskaya (2015). The concentration of substrates induces soil microbial synthesis of extracellular enzymes and one approach to assessing the physiological capabilities of microbial communities is to assay substrate degradation. In this context, measuring the activity of several soil enzymes may be useful for understanding organic matter turnover and inorganic nutrient availability (Dick, 1984).

Soil enzyme activities are a proximal driver of soil functioning, contributing to biogeochemical cycling, organic matter transformation, and nutrient availability. They are also widely recognized as indicators of soil health and candidate "sensors" of changes in soil management or fertility (Badiane et al., 2001; Vepsäläinen et al., 2001). In particular, they have been increasingly used to investigate changes in functions due to anthropogenic impacts (Dick, 1984; Vepsäläinen et al., 2001; Naseby and Lynch, 2002; Lagomarsino et al., 2008). The effectiveness of alternative vineyard strategies on higher enzyme hydrolytic activities has been demonstrated in several experiments applying green manure (Okur et al., 2009), no-tillage (Lagomarsino et al., 2008), grass-covering (Schreck et al., 2012), compost made from the organic fraction of municipal solid waste (Calleja-Cervantes et al., 2015), and biochar-compost mix (Mackie et al., 2015). The most extensively studied group of enzymes with ecological importance in soil are hydrolases, which are involved in the main biogeochemical cycling of elements and release of carbon, nitrogen, phosphorus and sulfur compounds (Caldwell, 2005). Other classes of enzymes exhibit oxidative activities such as laccases described by Theuerl and Buscot (2010); they can enhance the degradation of the most recalcitrant components of organic matter and are closely linked to soil carbon sequestration (Caldwell, 2005). Phenol oxidases and peroxidases, in particular, are key in cellulose and lignin degradation and further condensation of phenols, peptides and carbohydrates leading the formation of secondary humic compounds (Sinsabaugh, 2010). A final group of soil enzymes, poorly assessed, despite their importance for nutrient release, are lyases, which play a fundamental role in the detoxification of phenolics and reactive metals, antimicrobial defense, and the degradation of lignin, polysaccharides, and humic acids (Theuerl and Buscot, 2010).

AGRICULTURAL PRACTICES IN VINEYARDS AND THREATS TO BIODIVERSITY AND SERVICES

The soil ecosystem is one of the most complex habitats, due to the huge diversity of species, and the huge number of individuals present on a very small surface (Jeffery et al., 2010). A majority of studies mentions the following agricultural practices as threats to soil biodiversity: plowing or tillage, mineral fertilization, pesticide use, irrigation and drainage systems, and weed management (Brussaard et al., 2007; Holland et al., 2013; Tsiafouli et al., 2014; Paungfoo-Lonhienne et al., 2015). Perennial crops—particularly those planted as monocultures in large areas—are frequently associated with an increase in the use of machinery and pesticides, and with a decrease in vegetation cover. All these practices result in soil compaction, and a decrease in soil organic matter, that often is associated with structure disruption and erosion (Lal, 2001; Power, 2010; Tsiafouli et al., 2014).

In agroecosystems, intensive agricultural practices may cause soil degradation or deteriorate soil quality, leading to a decrease in the magnitude of one or more ESs (Lavelle et al., 2006). Agricultural practices increase the level of disturbance (e.g., tillage, mowing) and pollution (fertilizer and pesticide inputs), thereby not only changing physical soil properties but also affecting its biological communities and reducing the complexity of soil food webs and associated ESs (Bommarco et al., 2013; Tsiafouli et al., 2014). These reductions in biodiversity further modify soil functioning and, thus, the services it provides, as well as its resilience and ability to face the consequences of climate change, such as extreme weather events (Wall et al., 2015).

Soil erosion may represent losses of up to several millimeters of soil each year (Quiquerez et al., 2008; Rodrigo Comino et al., 2016). These values are much higher than those observed in natural systems and severely reduce the habitat suitability for a large number of taxa. A 4-year, field-scale experiment comparing four soil management systems revealed that permanent, inter-row grass cover reduced runoff and soil loss, compared to herbicide application resulting in bare soil, whereas results for superficial tillage and temporary grass cover with delayed herbicide treatment were intermediate (Le Bissonnais and Andrieux, 2007).

Chemical fertilizer and pesticide application are responsible for a global decrease in soil biodiversity, functions and services (Wall et al., 2015). The direct effects of mineral fertilizers on soil organisms have generally been described as limited, but their application may enhance soil biological activity through an increase in system productivity, crop residue return, and soil organic matter (Bünemann et al., 2006; Paungfoo-Lonhienne et al., 2015). However, nitrogen fertilization may lead to soil acidification, with considerable negative effects on soil organisms. In addition, a high input of mineral fertilizers negatively impacts plant diversity and community composition (Nascimbene et al., 2013; Paungfoo-Lonhienne et al., 2015). Organic amendments such as manure and compost provide a direct source of carbon for soil organisms and an indirect carbon source *via* increased plant growth and plant residue returns (Bünemann et al., 2006). For example, in Turkish vineyards, the application of green manure resulted in greater enzyme activity, and microbial biomass and activity (Okur et al., 2009). Compost applications or mulching were shown to have positive effects on soil structure comparable to permanent grass cover (Nachtergaele et al., 1998; Pinamonti, 1998). However, the costs involved in transport and the need for frequent applications may be high. The vineyard management techniques that are most efficient at preventing soil loss are also most favorable for preserving soil organic matter content and associated soil properties, such as aggregate stability (Le Bissonnais and Andrieux, 2007)and biodiversity (Duarte et al., 2014).

Water management in vineyards mainly consists of irrigation, but drainage may also be necessary in vineyards located in floodplains (Abbona et al., 2007). Water management aims to provide grapevines with a more amenable environment and prevent water stress. However, particularly in medium-dry environments, the implementation of water-saving irrigation techniques (e.g., partial rootzone drying) may lead to changes or decreases in the diversity and abundance of soil organisms that prefer a higher moisture content such as earthworms and generalist predators, spiders and ground beetles (Thomson, 2006; Holland et al., 2013).

Pest management techniques aim to reduce the competition between crops and weeds or reduce plant pest and pathogen densities. However, plant diversity and vegetation cover are important drivers of belowground processes: different plant species in crop rotation were shown to influence faunal, microbial, and soil organic matter dynamics *via* the diversity of root architecture, the quantity and quality of rhizodeposits, and residue biochemistry (Ingels et al., 2005). McDaniel et al. (2014) reported that introducing diversity with inter-row cover crops significantly increased microbial biomass. In addition, vegetation in vineyards also provides food and structure for many arthropod taxa (Altieri et al., 2005), and indirectly reduce pest abundance and support natural enemies (Lewis et al., 1997; Danne et al., 2010). Comparing the effects of pre- and post-emergence herbicide application, surface soil tillage, and no vegetation removal treatments, Collembola were most abundant in plots with vegetation cover, slightly less in post-emergence herbicide application and tillage plots, and least abundant in pre-emergence herbicide plots (Sturm et al., 2002; Renaud et al., 2004). However, herbicide application may increase mesofauna feeding activity in the short term, by providing dead plant material as food for detritivores (Reinecke et al., 2002). Due to the severe disturbance it causes, tillage may be more detrimental for mesofauna than herbicide application (Reinecke et al., 2002). Herbicide applications in vineyards can also affect soil biota with knock-on effects on other parts of the grapevine system, detectable even several months after its application (Mandl et al., 2018; Zaller et al., 2018).

Insecticide application generally reduces the abundance and, in the long term, the diversity not only of target pests but also of non-target species. For example, leafhopper abundance and diversity decreased strongly with insecticide use in Swiss vineyards (Trivellone et al., 2012). However, in a 2-year study comparing 5 vineyards in Arkansas (United States), carabids were more diverse and abundant in vineyards sprayed with insecticides than in abandoned, non-sprayed vineyards, while Hymenopteran parasitoids suffered from insecticide treatments (Williamson and Johnson, 2005). The authors hypothesized that, in the short term, insecticide spraying resulted in more food available for carabids on the vineyard floor.

Little research has been done into the effects of fungicides on biodiversity in vineyards. While some studies showed an effect of sulfur spraying on predacious mites, other studies did not find any negative effects (Walton et al., 2012). Fungicide application alters the grape surface microbial communities, and fungicides used in organic farming (sulfur, copper) have stronger effects than synthetic fungicides (Milanović et al., 2013). In particular, copper fungicides are considered to be among the most toxic, persistent fungicides (Bünemann et al., 2006) and impact several biodiversity groups (Korthals et al., 1996; Karimi et al., 2020, 2021; Ostandie et al., 2021). Copper residues are reported to be responsible for significant reductions in microbial biomass due to stressed microbes in orchards (Merrington et al., 2002) and cause long-term reductions of earthworm populations (Bünemann et al., 2006). Copper has also a major influence on nematodes and this effect was generally enhanced with decreasing soil pH. High copper contaminations significantly reduced the number of bacterial-feeding nematodes, whereas the number of hyphal-feeding nematodes increased (Korthals et al., 1996). Omnivorous and predacious nematodes showed the most sensitive response, until extinction. Plant-feeding nematodes also showed large variations in their abundances that could be related to the effects of copper and pH on primary production (Korthals et al., 1996). As copper accumulates in surface soils, these negative effects are likely to persist for many years.

One of the principles of organic farming states that it protects and benefits the common environment, including biodiversity. However, organic farming regulations do not oblige farmers to use inter-row vegetation cover and scientific evidence for the benefits of organic farming on biodiversity in vineyards is conflicting. In a large-scale study, including vineyards throughout Italy, predatory mite biodiversity was higher in organic and untreated vineyards than conventional ones (Peverieri et al., 2009). A slight increase of spider diversity has been observed in organic vineyards in Germany but no significant effect was reported on spider abundance (Kolb et al., 2020). However, other studies showed less biodiversity in organic vineyards: soil bacteria were less diverse on organic farms than conventional ones (Bonanomi et al., 2016). In a Swiss study, Bruggisser et al. (2010) also failed to detect an increase in plant and spider diversity in organic vineyards, while grasshopper diversity was even significantly lower in organic compared to conventional vineyards. Most of the benefits of organic farming for plants seem to be attributable to the absence of herbicide use (Sanguankeo and León, 2011; Nascimbene et al., 2012; Kehinde and Samways, 2014).

SUGGESTIONS FOR MANAGING SOIL BIODIVERSITY TO ENSURE SUSTAINABLE VITICULTURE

Trade-Offs Between Grape Production and Biodiversity

Viticulture has a long tradition and management practices have changed over the centuries. The economic importance of wine production, especially of high-quality wines, has also increased and winegrowers tended to focus on consistent production and consider that ecological aspects were of minor importance.

A range of farming practices that increase species diversity and associated functions and services have gradually been adopted by farmers in arable crops: conservation tillage, organic fertilization, natural vegetation cover or cover crops. These techniques can maintain higher levels of biodiversity by providing continuous plant cover and improving organic matter content (Bommarco et al., 2013). In addition, practices such as agroforestry, manure and residue inputs, and soil resting (a long period before replanting of vines) are gaining importance and may help to prevent soil degradation, reduce pesticide inputs and nutrient losses to adjacent habitats. They also indirectly promote the maintenance and conservation of biodiversity and multiple ESs such as pest control on a larger scale (Lewis et al., 1997; Bommarco et al., 2013). Such techniques, trade-offs between services they imply as well as the level of provided services are not well assessed and known for vineyards, nonetheless fundamental if we are to develop sustainable grapevine cropping systems.

Diversifying the management around viney ards or landscape diversification outside the actual production area influences biodiversity in vineyards. They can consist of buffer strips or riparian hedges. For example, remnants of natural habitats within vineyards promoted spider diversity in South African vineyards (Kehinde and Samways, 2014). However, islands or patches of beneficial plant species do not necessarily have to be in the vineyards (Altieri et al., 2005). Semi-natural areas in the surroundings of vineyards have a positive impact on habitat diversity but need to be connected by habitat corridors (Altieri et al., 2005). Alternating vegetation cover in inter-rows, when technically feasible, can maintain a high-biodiversity habitat within a single vineyard. This idea should be combined with current research on pesticide use adapted to local conditions, at the row or even vine-stock level (Llorens et al., 2010).

However, certain management practices require trade-offs between biodiversity and ESs (Bommarco et al., 2013). For example, the use of manure and agricultural compost increases organic matter content, but should be avoided close to water sources such as rivers and lakes, due to possible increased nitrate seepage, resulting in deteriorated water quality. At the same time, farmers may perceive the loss of certain ESs as negligible, while the reduction in yield or crop surface results in a decrease in provisioning ES (Verbruggen et al., 2012), directly linked to tangible economic losses. Such trade-offs, and especially, their consequences should be at the center of future research programs exploring links between biodiversity, ESs, and wine production.

Recently, the ecological aspects of wine production have received more attention, with higher numbers of organic and biodynamic winegrowers and greater awareness among consumers and politicians of the concept of sustainability and the negative effects of high-input viticulture. Sustaining ecosystem functions and services, as well as testing sustainable farming practices, are key issues in recent agroecological and biological conservation research and policy (Batáry et al., 2011). Winegrowers need to find a balance between ecological and economic aspects that may be symbiotic although contradictory in some parts of the production process.

However, environmental protection is often considered to impede the prosperity of enterprises, especially in the agricultural sector. Winegrowers tend to think of environmental-friendly actions as counterproductive for the quality of their wine and their earnings (Gemmrich and Arnold, 2007). Sustainability offers a solution for this conflict by integrating profitability with environmental and social issues. It offers a wholesome approach toward winegrowing and wine making (Gemmrich and Arnold, 2007). One of the major advances achieved by farmers in recent years is the perception that only a small percentage of the invertebrates present in their fields have a negative impact on crops (Lavelle et al., 2006).

There is also a gap between scientific literature and winegrowers' definitions of the ES concept and its application to viticulture. Both farmers and scientists recognize biodiversity and genetic diversity conservation as important (Brussaard et al., 2010). Viers et al. (2013) linked the sensitivity of wine-makers to ESs with the importance that they seem to attach to the terroir concept and their understanding of soil characteristics. The challenge for winegrowers is to identify the aspects of biodiversity that are desirable to maintain and/or enhance in their vineyards, as they provide specific ecological services, and then determine the best practices for encouraging this biodiversity and the associated ESs (Altieri, 1995; Gliessman, 1998; Winkler et al., 2017).

Lack of Knowledge and Research Gaps in Vineyards

Some aspects of the interaction between biodiversity conservation, management practices, ecosystem functions, and grape quality/yield are obvious and clearly understood, while many others are uncertain or even unknown (see previous sections). For example, insect functional biodiversity is assessed on plot and field scales with a focus on natural enemies to tackle biological control of vine pests (e.g., Danne et al., 2010; Caprio et al., 2015), while others investigate functional micro-biodiversity in the soil, involved in plant health management (Whitelaw-Weckert et al., 2007; Trouvelot et al., 2015). Future research should focus on the impact of viticultural landscape diversification and natural vegetation or cover crop mixtures in vineyard inter-rows on overall biodiversity and associated ESs. The interrelations and trade-offs between the responses of different taxa on field and landscape scales and the consequences for provisioning ES are still largely unexplored. In addition, only a few studies have investigated multiple trophic levels and ESs in vineyards. Furthermore, studies implementing a standardized research protocol in different climate zones are important to assess possible trade-offs and synergies between ESs in vineyards. In addition, in order to promote biodiversity conservation measures, further research is required on the relationships between certain plant species and host-disease transmitting vectors, which may impede the positive effects on biodiversity (Costa et al., 2004).

Relevant topics include the ecological role of specific soil- and plant-associated microbial taxa in vineyards. Plant growth-promoting rhizobacteria increase growth, but may also induce system resistance, thereby enhancing plant defenses (Beneduzi et al., 2012). Some of these mechanisms have been described in model plants, but current knowledge of these aspects in vineyards is limited (Beneduzi et al., 2012). Recently, certain microorganisms have been promoted as soil quality enhancers and responsible for particular organoleptic qualities (Zarraonaindia et al., 2015). These aspects need to be investigated under different soil and climate conditions keeping an eye also on nutrient cycles and nutrient availability. This topic also nicely illustrates the complexity of research tasks and the different disciplines which need to get involved.

Future Management Options and Research Directions

Currently vineyard management range from no disturbance at all (permanent plant cover) to high disturbance (regular soil tillage or herbicide application). Permanent cover and cover crops provide numerous benefits in terms of ESs, which are beneficial for the vineyard environment and increase grape quality (Biddoccu et al., 2016; Chrysargyris et al., 2018; Garcia et al., 2018). Cover crops have been found to increase soluble solids, anthocyanins and other phenolic components of grapes (Guerra and Steenwerth, 2012), increase microbial biomass of soil (Ingels et al., 2005) while controlling grapevine canopy growth (Tesic et al., 2007). However, they may compete for nutrient supply and nitrogen as observed in several European vineyards by Griesser et al. (2022).

Cover crops could be also implemented for in-row weed control, even if knowledge about in-row cover crops is scarce (Garcia et al., 2018). Use of in-row vegetation cover would avoid herbicide applications or mechanical weed control (Garcia et al., 2018), thereby indirectly and positively affecting inter-rows through the reduction of soil compaction and the number of tractor passages. Winegrowers are concerned about cover crops that may compete with vines for nutrients and water, but specific mixtures may avoid these negative effects and could provide higher biodiversity within vineyards (Garcia et al., 2018; Griesser et al., 2022). Using native plants and selecting particular species and traits within the natural pool has been also proposed as a potential solution for managing the soil under the vine rows (Tompkins, 2010). Furthermore, the application of mulch under vines during winter has been demonstrated to disturb the cycle of the gray mould fungus, *Botrytis cinerea*, and reduce its pressure by up to 70% (Jacometti et al., 2007). Studies using different plant mixtures and evaluating their impact on vine physiology, as well as grape quality, are pending, but are likely to produce important findings for adapting in-row management toward increased sustainability, less disturbance, and, possibly, promoting biodiversity.

The combined need for consistent yields of high-quality grapes, a reduction in external inputs, and climatic challenges with prolonged periods of drought, heavy rainfall, and prolonged growing seasons, makes it difficult for winegrowers to make informed decisions (Chrysargyris et al., 2018). It is necessary to develop risk management tools and adapt them to local conditions, including aspects of soil preservation, plant physiology, and pesticide management. Existing models have proved their effectiveness for pest management, but do not provide a thorough evaluation of the effects of pesticide applications on soil organisms.

Furthermore, awareness of sustainable crop production has increased among consumers, as well as producers. Winegrowers need reliable, basic knowledge of the effects of their production systems on biodiversity and other ecosystem functions, and implementing new measures need to be knowledge based. This knowledge has to be provided by scientists in terms of cultivar selection (cultivars resistant to biotic and abiotic stresses), trellising systems, canopy and cover-crop management, soil cultivation, winemaking processes, storage, and the use of renewable energy. Due to the increasing influence of international wine organizations, threats linked to climate change and new market opportunities, the wine sector has become more aware of the concept of sustainability (Hannah et al., 2013; Viers et al., 2013; Litskas et al., 2020). The global environmental footprint of wine production could be quantified and other impacts of practices on the use of resources or human health have to be measured and compared to biodiversity and environmental impacts (Litskas et al., 2020). Growing numbers of sustainable winemaking projects on local, regional, and national scales identified a number of ESs provided by vineyard soils that led to the development of locally-adapted methods and educational programs (Viers et al., 2013; Santiago-Brown et al., 2014).

AUTHOR CONTRIBUTIONS

BG, SW, SB, EAC, JZ, and IL conceived of the presented idea, based on European projects ReSolVe, PromESSinG and VineDivers. All authors contributed to the article and approved the submitted version.

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EDITED BY
James Moran,
Atlantic Technological University,
Ireland

REVIEWED BY
Paulo A. V. Borges,
University of the Azores, Portugal
Astrid Sturm,
Brandenburg University of Technology
Cottbus-Senftenberg,
Germany

*CORRESPONDENCE
Philippe Jeanneret
philippe.jeanneret@agroscope.admin.ch

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Spiders indicate delivery of an agri-environment scheme at multiple diversity levels

Philippe Jeanneret[1]*, Stefano Pozzi[2] and Carlos Martinez Nuñez[1]

[1]Agricultural Landscape and Biodiversity, Agroscope, Zurich, Switzerland, [2]Chemin de Pré-Gentil 64, Satigny, Switzerland

Agri-environment schemes (AES) are expected to counteract the negative impacts of intensive agriculture on biodiversity. These schemes were specifically designed to target farmland biodiversity and included, for instance, ecological focus areas (EFAs). In Switzerland, in order to qualify for direct payments, farmers must manage 7% or more of their land as biodiversity promotion areas (BPAs). BPAs encompass extensively managed and low intensity hay meadows, fallows (wildflower strips), traditional orchards with high-stem trees and hedgerows. Evaluation of AES delivery for biodiversity is of crucial importance but must be performed across several years and considering the various components of species diversity to avoid incomplete or wrong conclusions. From a complex study design comprising 478 fields in three regions and sampling over 7years with four sampling times, spider assemblages of BPA habitats were compared to corresponding conventionally managed fields. A battery of investigations was performed including alpha- and beta-diversity analysis, multivariate dispersion, indicator species and species specificity to understand what BPAs deliver for spiders in the habitat scale and farming landscape. Results showed that alpha-diversity (average number of species) was usually higher in BPA habitats than in conventionally managed fields but the species composition (beta-diversity) had more power to perceive AES impact. Furthermore, the various environmental conditions of BPAs in the farming landscape led to highly diverse spider assemblages (multivariate dispersion) emphasizing that not only the agricultural management plays a role in determining species diversity but the environmental heterogeneity. Indicator (and rare) species were mostly found in woody BPAs (hedges and high-stem tree orchards) revealing the high importance of these BPA habitats for spider conservation. At regional scale, BPA hedges contributed most to the regional diversity of spiders in grassland and mixed regions while BPA meadows and wildflower strip BPAs were first delivering in the region of arable crops. Recommendations highlight the role of the woody habitats and of the environmental heterogeneity in the farming landscape as well as of regional planning to make AES effective.

KEYWORDS

agri-environment scheme, semi-natural habitats, Araneae, alpha diversity, beta diversity, multivariate dispersion, indicator species

Introduction

Agriculture occupies about 34% of the total land area of the member countries of OECD and more than 50% for about half of the member countries, most of them European (OECD, 2008). These figures explain why Europe's biodiversity is inextricably linked to agriculture and agricultural practices. Yet, agricultural intensification is among the main drivers of biodiversity loss in the last decades (Diaz et al., 2019). The main impacts resulting from modern agriculture are related to habitat loss and fragmentation as well as environmental degradation, which reduces the amount of habitat available to sustain wild species in farming landscapes (Dudley and Alexander, 2017). To counteract these negative impacts of agriculture, agri-environment schemes (AES) offered by the OECD member countries were designed to specifically target farmland biodiversity (OECD, 2011). AES are considered the most important policy instruments to protect biodiversity in agricultural landscapes and are associated with considerable spending (Herzog et al., 2005; Batáry et al., 2015). However, as they seem to have had inconsistent environmental success (Batáry et al., 2010; Mccracken et al., 2015; Biffi et al., 2021) there is a need for evaluation and improvement (Peer et al., 2019; Candel et al., 2021; Tyllianakis and Martin-Ortega, 2021).

In Switzerland, an AES scheme comprising biodiversity promotion areas (BPA; Supplementary Table 1) was introduced in year 1993 and perpetuated in a cross-compliance mechanism since 1999. In order to qualify for direct payments, farmers must manage 7% or more of their land as biodiversity promotion areas including extensively managed and low intensity hay meadows, traditional orchards with high-stem trees, fallows (sown with seed mixtures of 20 to 40 herbaceous plant species), and hedgerows. These are the most important and frequent BPAs implemented in Switzerland, and their management is strictly regulated (late cut of meadows, restrictions in fertilization, pesticide use, etc.) in order to achieve environmental goals.

The role of AES in preserving and promoting biodiversity has been subject to debate in Europe, since their success seems to be landscape and context-dependent (Kleijn et al., 2006; Batáry et al., 2010; Whittingham, 2011; Martínez-Núñez et al., 2020). In Switzerland, diverse evaluation projects have been conducted to assess the effectiveness of the BPA scheme for several taxa (vascular plants, bryophytes, birds, hares, pollinators, bugs, butterflies, carabid beetles, spiders and grasshoppers) and results have been published (Jeanneret et al., 2003a; Herzog et al., 2005; Knop et al., 2006; Birrer et al., 2007; Roth et al., 2008; Aviron et al., 2009; Albrecht et al., 2010; Riedel et al., 2019; Ravetto Enri et al., 2020; Bisang et al., 2021). Almost all studies showed that AES can be effective in protecting and promoting biodiversity. Still, the effect also depends on the group of organisms investigated and on the landscape context.

Most evaluations of the effectiveness of AES have traditionally consisted of assessing *in situ* biodiversity in fields under scheme compared to conventionally managed control fields. However, unproductive and less productive perennial woody elements such as orchards with high-stem trees and hedgerows have received little attention although they may play a substantial role in preserving biodiversity (Garratt et al., 2017). Also, most of our knowledge about the effectiveness of AES to date comes from single year studies, which does not consider temporal variation and the possible ecological time lag in community responses (time necessary for species to respond to conservation measures; Watts et al., 2020). Therefore, studies addressing the effectiveness of AES and the persistence of their benefits through years are key to improve our understanding on this matter. Finally, intensified agricultural landscapes can affect diversity at different levels beyond local species loss (i.e., decreased alpha diversity) such as, for instance, a reduced beta-diversity leading to biotic homogenization across sites (Gossner et al., 2016; Larsen et al., 2018). Although an increasing number of studies recently examined the effects of AES on complex components of diversity such as beta-diversity (Warzecha et al., 2021), functional diversity (Gallé et al., 2020; Feng et al., 2021; García-Navas et al., 2022) or interaction networks (Martínez-Núñez et al., 2019) they are still scarce and rarely use a multilevel approach. In this study we aim to fill some of these gaps of knowledge by following the conceptual framework developed by Legendre et al. (2005) and further discussed by authors (e.g., Legendre et al., 2008; Tuomisto and Ruokolainen, 2008) for analyzing data with respect to diversity components. In this regard, we recognized three levels of possible impact of BPAs implementation and management in the farmland landscape across 7 years. By deciding to manage a field as BPA instead of a production field at the same location, farmers will change the abiotic conditions of fields, thereby affecting the local communities in the farm. Consequently, the average number of species in BPAs and production fields (alpha-diversity) may differ (first level). Similarly, the species assemblages (beta-diversity) may vary between both types of habitats (second level). In addition, or in combination to the management effect, farmers will increase environmental heterogeneity by placing BPAs and production fields at various locations in the farmland landscape which may also influence the number of species and the species assemblages at the landscape level (Kovács-Hostyánszki et al., 2021). This is caused by the environmental control of the species distribution (*sensu* Whittaker, 1956) determining then the species assemblages in fields. Not only the difference in alpha- and beta-diversity between BPAs and production fields may be influenced by the environmental heterogeneity but also the variation in beta-diversity among groups of fields (so called "variation in variation in community composition data," Legendre et al. (2008).

Beyond investigating diversity patterns of species assemblages, the identification of characteristic or indicator species is key to assist efficient conservation and management. This is particularly important due to the increasing focus on result-based agri-environmental payment frameworks (Chaplin et al., 2021). This approach arises as a more cost-effective alternative, but there is no consensus among farmers and it might be logistically difficult to implement (Zabel and Roe, 2009; Niskanen et al., 2021). The use of indicator species could solve some of these problems (Ruas et al., 2021), but few studies have tried to exploit this tool in the context of AES until now (but see Wittig et al., 2006; Martínez-Núñez et al., 2020).

Here, we use a unique and large data set collected in 478 fields over 7 years (four sampling times) in three regions of Switzerland, to study the effect of BPAs on spider assemblages. Focusing on spiders is particularly interesting, since they are a highly diverse and sensitive group that can play a key role in ecosystems as both predators and prey. Surprisingly, studies focusing on spider assemblages in the context of AES are underrepresented. Our approach aimed, first, at integrating traditional semi-natural habitats like orchards and hedges as part of the AES of the farming landscape in the evaluation, besides extensively managed fields in Switzerland. Second, we examined how AES performs over time in the mid-term with data collected bi-annually from 1997 to 2003. Third, we extended the usual analysis of alpha-diversity (i.e., the number of species) to further components of diversity (i.e., beta-diversity and the variability of alpha-diversity) because AES are expected to act in diverse ways on biodiversity. Fourth, we searched for indicator species that inform on the habitat conditions in BPA so that recommendations for the management can be derived. Fifth, we investigated the relative contribution of BPA to the diversity of spider assemblages at regional scale, so that recommendations for landscape planning can be made. Lastly, this study represents a good opportunity to set a baseline to compare the effect of future successive agricultural policy reforms, and to analyze long term trends of spider communities in multiple habitat types.

Based on ecological theory, we hypothesize that: i) alpha-diversity (species replacement) of spider assemblages will be higher in BPA than in production fields because these habitats provide more resources, more niches, and less perturbation, favoring the establishment and coexistence of more species (Benton et al., 2003; Chesson, 2000); ii) beta-diversity (community composition) and the variability of beta-diversity (multivariate dispersion) among spider assemblages of BPA and production fields will differ, because these different habitats will provide complementary niches for different species (Schoener, 1974; Chesson, 2000); and iii) we will be able to detect bioindicator species for each BPA and the contribution of each habitat to regional diversity: we expect BPA to contribute more specialized (or habitat-dependent) species because conventionally managed agricultural habitats will mainly support generalist frequent species (Robinson and Strauss, 2020).

Materials and methods

Study regions

We carried out investigations in three regions representative of the different farming types (arable, mixed arable–grassland, and grassland) in central Switzerland.

The study areas were each 8–10 km^2. They were located in the Swiss lowlands, in (1) region one, mixed arable–grassland (7.2 km^2, 6°49′ 30″ N/46°46′30″ E, 650 m above sea level [asl]; annual precipitation 900 mm; average annual temperature 8.4°C), (2) region two, grassland dominated (8.8 km^2, 8°7′ N/47°6′ E, 750 m asl; annual precipitation 1,400 mm; average annual temperature 6.8°C), and (3) region three, arable land dominated (8.1 km^2, 8°32′ N, 47°35 30″ E; 450 m asl; annual precipitation 900 mm; average annual temperature 8.5°C).The types of BPA and their share (percentage) of the farmland in the three regions were representative of the three larger biogeographic regions in which they were located.

Study design

The diversity of spiders was investigated bi-annually between 1997 and 2003 in both BPA and conventionally managed fields (total number of fields = 478). As not all BPA and fields were sampled every year and in every region due to study constrains, i.e., wildflower strips BPA were absent of regions 1 and 2 (Supplementary Methods), BPA habitats were first compared to a corresponding conventionally managed field category on a pairwise basis: BPA meadow versus conventional meadow (regions one, two and three, bi-annually from 1999 to 2003, $n = 163$ vs. 71), BPA orchard versus conventional meadow (region one and three, bi-annually from 1999 to 2003, $n = 46$ vs. 56), BPA hedge versus production field (encompasses conventional meadows and crops; region one and three, bi-annually from 1999 to 2003, $n = 38$ vs. 70), and wildflower strip BPA versus crop field (region three, bi-annually from 1997 to 2003, $n = 40$ vs. 82). Paired were defined on the basis of the production fields that would occur in case of conversion of the BPA, e.g., a crop field instead of a BPA hedge. The design of these paired comparisons is a factorial design, each of the habitat type being sampled in each of the regions and every sampling year (see Supplementary Table 2 for details about number of samples per habitat type, region and year). Second, the relative contribution of BPA habitats to the diversity of spider assemblages was investigated at regional scale by analyzing spider assemblages in BPA and production fields within regions.

In each field, spiders were collected using three pitfall traps located in the center of the field and spaced 3 m apart from one another during 5 weeks between May and July in two periods (3 and 2 weeks). In addition to pitfall trapping, sweep net method was used to collect spiders in the vegetation of meadows, BPA orchards, wildflower strips BPA and crop fields, and the beating method in BPA hedges and on trees of BPA orchards. Sweep netting and beating were applied five times from May to August and samples pooled per sampling year for analysis (see Supplementary Methods for details about trapping methods).

Analysis of alpha-, beta-diversity and multivariate dispersion

We defined alpha-diversity of a habitat type as the mean number of species in fields of this habitat type per region and year.

This response variable was based on species density, the total number of species collected in three pitfall traps, in sweep net and in beating samples per field over a sampling year. Measures of species density reflect both the species richness of the community and the number of individuals collected (Gotelli and Colwell, 2001). Catches are affected by - as for example in our study - habitat structure, i.e., individuals more likely fall in pitfall traps placed in habitats with sparse vegetation (e.g., crops at early development stages) than in habitats with dense and diverse vegetation (e.g., meadows; Topping and Luff, 1995). Therefore, we used rarefaction to adjust for differing densities of individuals, i.e., we standardized fields to a common number of 100 individuals (Supplementary Methods) and estimated the species richness of fields using R (R Development Core Team, 2018).

We defined beta-diversity as the variation in species composition among fields per region and year. Differences in the species composition among the spider assemblages of fields were investigated and visualized using non-metric multi-dimensional scaling (MDS). Following Anderson et al. (2006) we used of the following dissimilarity measures that increasingly emphasize the relative abundance of the species: Jaccard dissimilarity index dJ (pure species composition = presence/absence list), the modified Gower's dissimilarity measure (excluding joint absences) with data log10(x) + 1 transformed dMG10, and data log2(x) + 1 transformed dMG2 (Supplementary Methods, formula of dissimilarity measures). Non-metric MDS plots were constructed with dMG10 to allow for an intermediate emphasis of the relative abundance of the species. Polygons enclosing sites of habitat types per region and year were drawn on the plots by connecting the outermost sites. Together with the polygons, line segments linking each site to its centroid plotted per habitat type, region and year were drawn to visualize beta-diversity.

As a measure of the variation in beta-diversity among group of fields we used the multivariate homogeneity of group dispersions following concepts and methods devised by Anderson et al. (2006), and used recently in other studies (Martínez-Núñez et al., 2019). The multivariate dispersion is measured as the average distance (or dissimilarity) from an individual unit (a field) to the group centroid, using a dissimilarity measure. In our case, a group was composed of the sites of a habitat type in a region and a year. Analysis was again performed with dissimilarity matrices based on measures with increasing emphasis on the relative abundance of the species, i.e., dJ, dMG10 and dMG2 (Supplementary Methods). Multivariate dispersion was calculated per habitat type, region and year with R (R Development Core Team, 2018).

Indicator values and species

Characteristic spider species were identified for habitat types, sampling years and regions using the indicator value method (Dufrêne and Legendre, 1997). This method combines measures of specificity and fidelity and provides an indicator value (IndVal) for each species, as a percentage (Supplementary Methods). First, indicator species were searched for pairs of habitat types across regions for each year separately (two groups of sites, i.e., BPA habitats vs. conventionally managed fields, for instance BPA meadows vs. conventional meadows). Indicator fidelity was specified according to the number of sampling years for which the species has a significant indicator value, i.e., regular for 2 years significant, and very regular for 3 years significant. Then, indicator species were identified for the habitats within the three regions to examine whether indicator species for a particular habitat type were specific to a particular region, namely six groups of sites (BPA meadows vs. conventional meadows in the three regions), four groups of sites (BPA orchards vs. conventional meadows and BPA hedges vs. production fields in two regions) and two groups of sites (wildflower strip BPA vs. crop fields in region three). A rarity value for Switzerland was given for indicator species, from one (very common) to six (very rare; comm. Pers., Pozzi et al., 1998). The ecological requirements of species were derived from Maurer and Hänggi (1990).

Habitat specificity in regions

As agri-environmental measures should be implemented according to region-specific goals, BPAs performance as conservation tool has to be assessed at a regional level. This can be approached by investigating the specificity of species for their habitat to derive the contribution of BPA fields to the regional diversity as shown by Wagner and Edwards (2001) with coarse types of land uses, plants and snails. We adapted this concept by summing the specificity of the species for the habitats, which is a component of the indicator value according to Dufrêne and Legendre (1997), in a score per habitat to compare the contribution of each habitat to the regional species richness (see Supplementary Methods for formula). This is a method commonly used to assess the uniqueness or contribution of different habitat types to the meta-community (e.g., García-Navas et al., 2022).

Statistical analysis

Multifactorial mixed-model ANOVAs with permutations (Anderson and Ter Braak, 2010) were performed to test differences between habitat types, regions and years, for alpha-diversity and multivariate dispersion (variation in beta-diversity) using the DISTLM procedure (Anderson, 2001, 2004). This procedure was originally introduced as a distance-based multivariate analysis for a linear model, but can be used for a single response variable (univariate case) by choosing the Euclidean distance to calculate the distance matrix between samples. The beta-diversity among habitat types, regions and years was analyzed with a distance-based multivariate ANOVA (Anderson, 2001; McArdle and Anderson, 2001), using again the DISTLM procedure. Within the analyses, the factors "habitat type" (two levels, BPA habitats versus conventionally managed fields, pooled and pairwise analyzed) and "region" (two or three levels depending on the pairs of habitat types compared, region one to three) were treated as fixed factors and "year" as a random factor (three or four levels depending on the pairs of habitat types compared, year one to three or one to four). Region was a fixed factor because regions were chosen regarding their main agricultural land use, i.e., arable, mixed arable–grassland, grassland. To test particular terms in the permutational analysis of variance, permutation strategies followed a design involving three crossed factors, two of them fixed and one random after Anderson and ter Braak (2010; Supplementary Methods). In case of significant interaction terms, the second order habitat type x region interaction was further considered by analyzing the habitat type effect per region separately, or in region three the habitat type effect in years. Permutational analysis of variance (univariate and multivariate) were performed with 4,999 permutations to calculate the significance of the pseudo-F statistic.

The significance of the indicator values (IndVal) of each species was tested with the random reallocation procedure of sites among site groups (habitat types, regions) according to Dufrêne and Legendre (1997). Each species has a percentage IndVal with an associated measure of significance, with high and significant percentages designating good indicator species. Because several tests of significance are performed simultaneously in this analysis, the Holm's procedure to adjust probability values was performed as proposed by Legendre and Legendre (1998). Furthermore, after calculation of the mean indicator value per species over the sampling years, mean indicator values over all species (mean of the means) were compared among paired habitat types with the Wilcoxon rank sum test per year separately. Multiple comparisons of habitats in regions were done with the Tukey's honest significant difference (Tukey's hsd). Analyses were completed with R (R Development Core Team, 2018).

Pre-analyses

Canonical redundancy analysis (RDA) with principal coordinates of neighbor matrices (PCNM variables) as model for the spatial structure was used to check for independence of samples of pairs or fields compared within regions and years following the procedure of Borcard and Legendre (2002) and Borcard et al. (2004; Supplementary Methods). No significant autocorrelation has been detected in any of the pairs of habitats compared in any region and year at $p = 0.05$ level (RDA, $0.063 < p < 0.91$).

To check for possible influence of sample size on the results, we looked at the linear correlation between dissimilarity matrices calculated with the Chao's abundance-based Jaccard and its bias-corrected version (Chao et al., 2005) as proposed by Anderson et al. (2006). Chao's abundance-based Jaccard and its bias-corrected version were calculated with EstimateS (Colwell and Elsensohn, 2014). The linear correlation between these two matrices ranged between 0.92 and 0.95 depending on the pair of habitats, suggesting that the sample size cannot be responsible for the observed pattern (Supplementary Methods).

Results

The alpha-diversity is usually higher in BPA habitats than in conventionally managed fields

In total, 180,987 individuals were collected and 284 species identified. Pooled BPA habitats (BPA meadows, BPA orchards, BPA hedges and wildflower strip BPAs, n = 276) showed a significantly higher alpha-diversity (rarefied to a common abundance level of 100 individuals per site) than pooled conventionally managed fields (crops and conventional meadows, n = 171) with, respectively, 17.1 ± 0.4 and 13.9 ± 0.3 species (mixed-model ANOVA with permutations, $F = 55.9$, df = 1, $p < 0.05$, see Supplementary Table 4 for the full ANOVA table). Alpha-diversity was significantly influenced by the region (R1 = 17.5 ± 0.5, R2 = 13.1 ± 0.3, R3 = 16.9 ± 0.4, $F = 14.0$, df = 2, $p < 0.05$) but not by the sampling year ($F = 1.2$, df = 2, $p = 0.3$). As the interaction habitat type x region was significant ($p < 0.05$), tests were performed per region separately. They revealed that habitat type was still significant with higher alpha-diversity values in BPA habitats than in conventionally managed fields in region one and three but not in region two (mixed-model ANOVA with permutations, region one, $F = 25.5$, df = 2, $p < 0.05$; region two, $F = 15.4$, df = 2, $p = 0.06$; region three, $F = 64.2$, df = 2, $p < 0.05$).

Furthermore, effects of habitat types, regions and years on alpha-diversity were tested in paired comparisons. Results showed that BPA habitats demonstrated a significantly higher alpha-diversity than conventionally managed fields except for BPA meadow vs. conventional meadow (habitat type effect, Table 1). The largest difference occurred between BPA hedges and production fields and was particularly remarkable in region one (Figure 1). Region showed significant effect excepted for BPA hedge vs. production field (Table 1). On average in meadow habitats, region three demonstrated the highest alpha-diversity (Figure 1, R3 = 17.8 ± 0.8, R1 = 14.4 ± 0.4, R2 = 11.4 ± 0.2) while in region one we found more species than in region two when comparing BPA orchards with conventional meadows (R1 = 17.4 ± 0.7, R2 = 11.9 ± 0.4). In addition, the sampling year had a significant effect in BPA orchard vs. conventional meadow comparison (Table 1), 1999 being on average the most species rich sampling year and 2003 the most species poor (1999 = 16.2 ± 0.8, 2001 = 14.8 ± 0.7, 2003 = 13.6 ± 0.8). Within this comparison, the habitat type, region and sampling year had a significant interaction effect (Table 1); region one showed a higher alpha-diversity than region two in both habitat types (Figure 1); the habitat type effect was stronger in region one (2001 and 2003 showed a significant difference between BPA orchard and conventional meadow, ANOVA with permutations, $p < 0.005$) than in region two (2001 only showed significant difference, $p < 0.05$) and alpha-diversity

TABLE 1 Effects of habitat type (pairwise, BPA habitat types vs. conventionally managed fields), region and sampling year on alpha-diversity of spiders (rarefied at 100 individuals) in three regions of the Swiss plateau (mixed-model ANOVA with permutations).

	BPA meadow ($n = 163$) vs. Conventional meadow ($n = 71$)			**BPA orchard ($n = 46$) vs. Conventional meadow ($n = 56$)**			**BPA hedge ($n = 38$) vs. Production field ($n = 70$)**			**Wildflower strip BPA ($n = 40$) vs. Crop field ($n = 82$)**		
Regions		1, 2, 3			1, 2			1, 2			3	
Sampling years		1999–2003			1999–2003			1999–2003			1997–2003	
Source of variation	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Habitat type	1	3.2	0.21	1	81.7	**0.01**	1	3180.7	**0.00**	1	10.7	**0.05**
Region	2	37.3	**0.00**	1	89.3	**0.01**	1	5.0	0.16	-	-	-
Sampling year	2	0.9	0.39	2	6.3	**0.00**	2	2.1	0.13	3	11.9	**0.00**
Habitat type×Region	2	0.4	0.71	1	5.4	0.15	1	4.7	0.16	-	-	-
Habitat type×Sampling year	2	0.8	0.46	2	0.8	0.45	2	0.1	0.91	3	9.3	**0.00**
Region×Sampling year	4	1.2	0.32	2	1.1	0.35	2	0.3	0.77	-	-	-
Habitat type ×Region×Sampling year	4	2.1	0.08	2	3.4	**0.04**	2	3.7	**0.03**	-	-	-

Underlined stars indicate higher alpha-diversity in BPA habitat types. Significant differences at $p \leq 0.05$ are highlighted in bold type. Production field encompasses crop fields and conventional meadows without pastures.

decreased along the sampling years excepted for BPA orchards in region one (Figure 1). Neither sampling year nor region had a significant effect on alpha-diversity in the BPA hedge vs. production field comparison. The three-way interaction was significant but the differences between BPA hedge and production field were significant each sampling year in region one (ANOVA with permutations, $p < 0.001$) and in region two (1999, $p < 0.05$, 2001, and 2003, $p < 0.005$). The highest alpha-diversity was recorded in BPA hedges in 2003 and in 2001 in region one and two, respectively (Figure 1). The habitat type and the sampling year effects were significant in the wildflower strip BPA vs. crop field comparison as well as the interaction between both (Table 1). Alpha-diversity strongly increased after the first year (1997) for which the difference between both habitat types was not significant (ANOVA with permutations, $p > 0.05$) and then slowly decreased from 1999 to 2003 in wildflower strips BPA while alpha-diversity remained remarkably stable in crops (Figure 1; 1999, 2001, and 2003, $p < 0.001$).

The beta-diversity among BPA habitats and conventionally managed fields differs

Assemblages were characterized by the dominance of a few species as it very often is in arthropod communities in agricultural landscapes. However, crop fields and conventional meadows were more strongly dominated than BPA habitats. The first five most dominant species encompassed 81% of the individuals in conventional meadows, 81% in crop fields, 73% in BPA meadows, 73% in BPA orchards, 44% in BPA hedges, and 63% in wildflower strips BPA (rank-abundance curves, Supplementary Figure 1).

All explanatory factors and interactions except the interaction habitat type x sampling year showed significant effects on the beta-diversity of pooled BPA habitats (BPA meadows, BPA orchards, BPA hedges, and wildflower strip BPAs, $n = 276$) versus pooled conventionally managed fields (crops and conventional meadows, $n = 171$) irrespective of the dissimilarity measure used, i.e., dJ, dMG10 and dMG2 (all $p < 0.001$, see Supplementary Table 5 for the full table of the distance-based multivariate ANOVA with permutations). As the interaction habitat type x region was significant ($p < 0.05$), tests were performed separately per region and revealed that beta-diversity was still significantly different according to the habitat type in the three regions and for the three dissimilarity measures involved (distance-based multivariate ANOVA with permutations, habitat type in all three regions: $p < 0.005$ for dJ, dMG10 and dMG2).

In paired comparisons, non-metric MDS plots indicated effect of region for meadow comparison but also revealed a set of very particular sites for spiders (Figure 2A). The effect of the habitat type was the most obvious by BPA hedge compared to production field (Figure 2C) while a regional effect was clear by BPA orchard compared to conventional meadow (difference not apparent in region two, Figure 2B), and a year effect by wildflower strip BPA compared to crop field (Figure 2D). BPA habitat types and conventionally managed fields revealed significantly different species composition (based on dMG10) for every pair of habitats compared (habitat type effect, Table 2). Regions and sampling years also showed significantly different species composition. As interactions were significant, we further investigated the habitat type effect in regions separately for BPA orchard vs. conventional meadow (sampling year is considered random factor), in regions and sampling years separately for BPA meadow vs. conventional meadow and BPA hedge vs. production field, and according to sampling years for wildflower

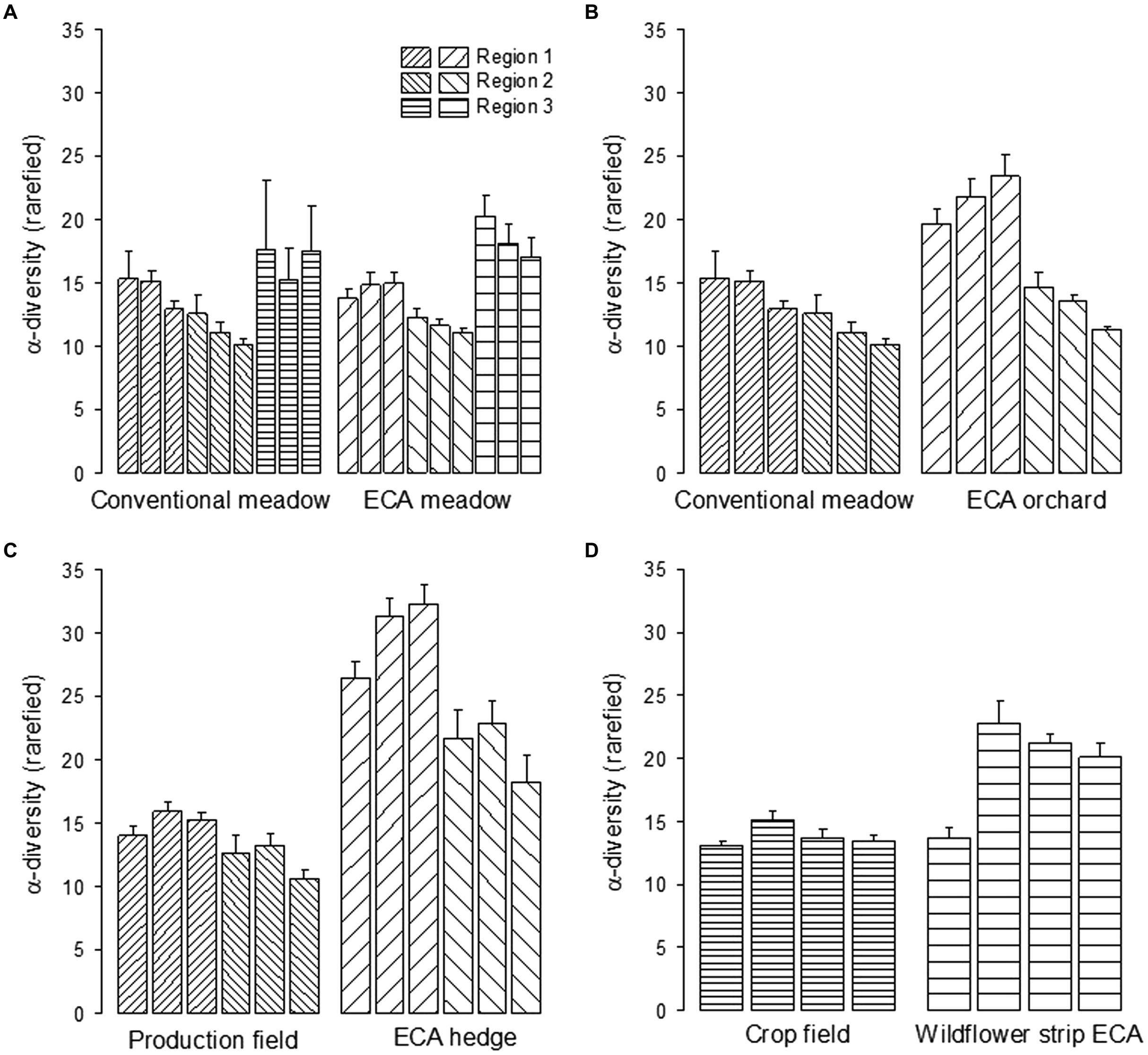

FIGURE 1
Alpha-diversity (mean number of species +1SE) of spider species (rarefied at 100 individuals) in BPAs and conventionally managed fields. Habitat types are compared by pairs, i.e., **(A)** BPA vs. conventional meadow, **(B)** BPA orchard vs. conventional meadow, **(C)** BPA hedge vs. production field (encompasses crop fields and conventional meadows without pastures), **(D)** wildflower strip BPA vs. crop field. The comparison of wildflower strip BPA vs. crop field occurs in region three only. Each bar represents alpha-diversity per year, i.e., 1999, 2001, 2003 from the left to the right (1997 in addition for crop field vs. wild flower strip BPA). The scale among graphs is kept the same to allow vertical comparisons.

strip BPA vs. crop field. In both regions 1 and 2, the species composition was still different among BPA orchards and conventional meadows (distance-based multivariate ANOVA with permutations, $p < 0.001$ and $p < 0.005$ in regions one and two, respectively). The difference between BPA and conventional meadow was not significant in any regions and sampling years ($p > 0.05$). The species composition of BPA hedges was significantly different from production fields in both regions during each sampling year ($p < 0.001$) except 1999 in region three ($p = 0.08$) with a larger dissimilarity in region one as shown on the non-metric MDS (Figure 2C). While the difference in species composition between wildflower strips and crop fields was lower in the first sampling year 1997 than during the subsequent years 1999, 2001, and 2003, as shown on the non-metric MDS (Figure 2D), the differences per year separately were still all significant ($p < 0.005$). Interestingly, among the main explanatory factors, the habitat type had the strongest effect for BPA hedge vs. production field comparison while the difference BPA vs. conventional meadows, and BPA orchards vs. conventional meadows was better explained by the region (Table 2, habitat type and region effects). This was confirmed by the non-metric MDS plots (Figure 2A) which showed a more apparent grouping of BPA meadows according to the region than to the habitat type. In particular, region two revealed similar species composition in BPA and conventional meadows. In contrast, the species composition of BPA hedges was very

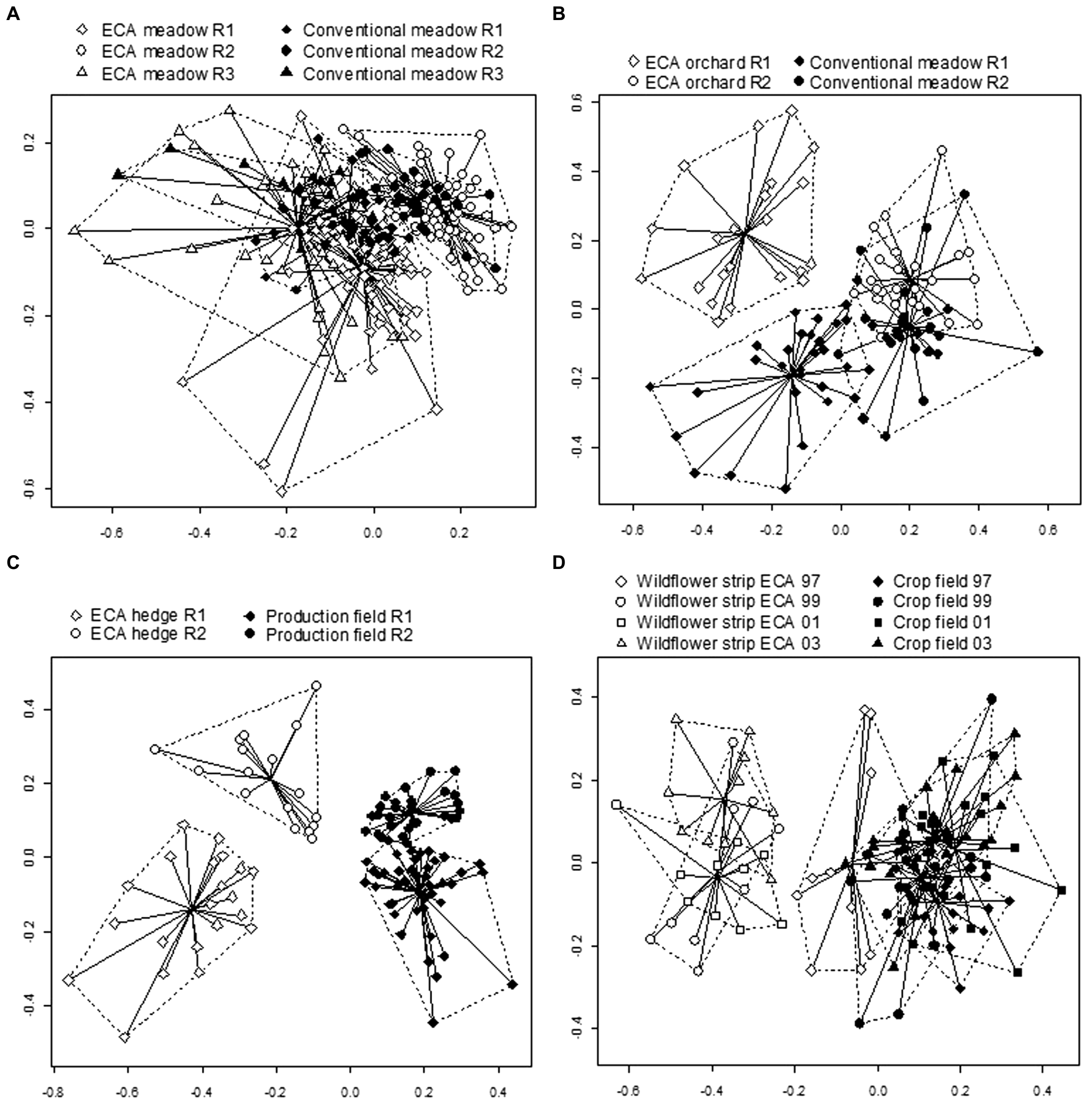

FIGURE 2
Non-metric MDS plots showing the pattern of dissimilarities and distance to centroid (using the Gower dissimilarity measure, excluding double zeros) with log10+1 transformed data among spider assemblages in BPA and conventionally managed fields. Habitat types are compared by pairs, i.e., **(A)** BPA vs. conventional meadow, **(B)** BPA orchard vs. conventional meadow, **(C)** BPA hedge vs. production field (encompasses crop fields and conventional meadows), **(D)** wildflower strip BPA vs. crop field. R1=region one, R2=region two, R3=region three. Centroids, hull envelope and multivariate dispersion (distance between each site and the centroid to which it belongs) are shown for groups combining each habitat type per region. The comparison of wildflower strip BPA vs. crop field occurs in region three only for which centroids, hull envelope and multivariate dispersion combine habitat type and sampling year (1997, 1999, 2001, 2003).

clearly separated from production fields for both regions. Apart from some differences in the significance level, increasing consideration of the species abundance by calculating the dissimilarity matrix with dMG2 and considering the presence/absence list with dJ did not change the results (see Supplementary Table 6, 7 for the full table of the distance-based multivariate ANOVA with permutations).

The multivariate dispersion is higher in BPA habitats than in conventionally managed fields

Pooled BPA habitats (BPA meadows, BPA orchards, BPA hedges and wildflower strip BPAs, n=276) showed a significantly higher multivariate dispersion based on Jaccard's dissimilarity index dJ and

TABLE 2 Effects of habitat type pairwise (BPA habitat types vs. conventionally managed fields), region and sampling year on the species composition (beta-diversity) of spiders in three regions of the Swiss plateau (distance-based multivariate ANOVA with permutations, mixed-model).

	BPA meadow ($n=163$) vs. Conventional meadow ($n=71$)			BPA orchard ($n=46$) vs. Conventional meadow ($n=56$)			BPA hedge ($n=38$) vs. Production field ($n=70$)			Wildflower strip BPA ($n=40$) vs. Crop field ($n=82$)		
Region	1, 2, 3			1, 2			1, 2			3		
Sampling years	1999–2003			1999–2003			1999–2003			1997–2003		
Source of variation	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Habitat type	1	4.6	**0.00**	1	7.2	**0.00**	1	15.0	**0.00**	1	8.5	**0.00**
Region	2	7.8	**0.00**	1	8.0	**0.00**	1	5.3	**0.03**	-	-	-
Sampling year	2	3.2	**0.00**	2	2.6	**0.00**	2	2.3	**0.01**	3	3.7	**0.00**
Habitat type×Region	2	1.0	0.40	1	2.8	**0.00**	1	2.3	**0.00**	-	-	-
Habitat type×Sampling year	2	0.8	0.87	2	0.8	0.73	2	1.8	**0.01**	3	2.3	**0.00**
Region×Sampling year	4	2.4	**0.00**	2	1.8	**0.00**	2	2.0	**0.00**	-	-	-
Habitat type ×Region×Sampling year	4	1.4	**0.00**	2	1.0	0.45	2	2.6	**0.00**	-	-	-

Multivariate multiple regression analysis is calculated on the basis of a Gower's dissimilarity measure with log10(x) + 1 transformed data (dMG10). $p \leq 0.05$ are highlighted in bold type. Results obtained with the Gower's dissimilarity measure with log2(x) + 1 transformed data (dMG2) and the Jaccard's dissimilarity index (dJ), are presented in Supplementary Results. Production field encompasses crop fields and conventional meadows without pastures.

both Gower's dissimilarity measures dMG10 and dMG2 than pooled conventionally managed fields (crops and conventional meadows, $n=171$; $p<0.05$, see Supplementary Table 8 for the full table of the multifactorial mixed-model ANOVA with permutations). By taking more account of relative abundance information, i.e., with dMG2, difference between regions was significant ($p<0.05$), region having the highest multivariate dispersion followed by regions one and two (0.263 ± 0.004, 0.257 ± 0.004, 0.231 ± 0.004, respectively). In contrast, multivariate dispersion values were not significantly different among sampling years with any dissimilarity measure. Furthermore, multivariate dispersion based on dJ was significantly dependent on the interaction term region x sampling year ($p<0.05$), the highest values being recorded in 2003 for region one (0.416 ± 0.008) and three (0.416 ± 0.009) but in 2001 for region two (0.409 ± 0.009). Multivariate dispersion based on dMG2 revealed a significant interaction term habitat type x region x sampling year ($p<0.05$).

In paired comparisons, BPA habitat types demonstrated significantly higher multivariate dispersion based on dMG10 than conventionally managed fields for BPA meadow vs. conventional meadow (0.315 ± 0.004 vs. 0.272 ± 0.006) and BPA hedge vs. production field (0.339 ± 0.006 vs. 0.299 ± 0.005) but neither for BPA orchard vs. conventional meadow (0.321 ± 0.006 vs. 0.310 ± 0.008) nor for wildflower strip BPA vs. crop field (0.325 ± 0.005 vs. 0.300 ± 0.005; habitat type effect, Table 3). This was confirmed by the non-metric MDS plot (Figure 2). A larger multivariate dispersion of the BPA meadows occurred, irrespective of the region, than of the conventional meadows around their centroids. However, two conventional meadows in region three were more distant to their centroid than the average. For BPA meadows, the multivariate dispersion was larger in region one (0.316 ± 0.007) and three (0.343 ± 0.008) than in region two (0.297 ± 0.006). Compared to BPA hedges, production fields were remarkably grouped except two fields in region one (Figure 2C) and had consequently shorter distances to their centroids. As noticeable on the non-metric MDS plot (Figures 2B,C) multivariate dispersion among BPA orchards and conventional meadows, as well as among wildflower strips BPA and crop fields did not obviously differ (no significant habitat type effect, Table 3). In one case, namely BPA orchard vs. conventional meadow, the sampling year had a significant effect on multivariate dispersion, 2001 having the highest multivariate dispersion value and 1999 the lowest (1999: 0.296 ± 0.011, 2001: 0.330 ± 0.007, 2003: 0.313 ± 0.009). Adding abundance information to the pairwise comparisons by calculating the multivariate dispersion based on dMG2 did not qualitatively change the main results but some factors became significant (see Supplementary Table 9 for detailed results), i.e., the region in BPA meadow vs. conventional meadow, the interaction term habitat type x region x sampling year in BPA hedge vs. production field, and interestingly, the habitat type in wildflower strip BPA vs. crop field. In contrast, the habitat type became insignificant by the BPA hedge vs. production field comparison. Comparing multivariate dispersion based on the presence/absence list with dJ (eliminating the relative abundance effect) caused three additional factors to become significant, i.e., the interaction term region x sampling year in BPA meadow vs. conventional meadow, the sampling year in BPA hedge vs. production field, and the interaction term habitat type x sampling year in wildflower strip BPA vs. crop field (see Supplementary Table 10 for detailed results).

Spider species showed higher preferences for BPA habitats than for conventionally managed fields

From 284 species, preference for BPA habitats occurred by 41 species with a significant indicator value for BPA hedge, 22 for wildflower strip BPA and 11 for BPA orchard but less for BPA

TABLE 3 Effects of habitat type pairwise (BPA habitat types vs. conventionally managed fields), region and sampling year on the multivariate dispersion of spiders in three regions of the Swiss plateau (mixed-model ANOVA with permutations).

	BPA meadow ($n = 163$) vs. Conventional meadow ($n = 71$)			BPA orchard ($n = 46$) vs. Conventional meadow ($n = 56$)			BPA hedge ($n = 38$) vs. Production field ($n = 70$)			Wildflower strip BPA ($n = 40$) vs. Crop field ($n = 82$)		
Region		1, 2, 3			1, 2			1, 2			3	
Sampling years		1999–2003			1999–2003			1999–2003			1997–2003	
Source of variation	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df	*F*	*p*
Habitat type	1	186.03	**0.00**	1	0.51	0.60	1	24.36	**0.04**	1	5.36	0.11
Region	2	5.61	0.07	1	6.5	0.12	1	10.22	0.08	-	-	-
Sampling year	2	0.77	0.45	2	3.75	**0.03**	2	2.92	0.06	3	0.33	0.81
Habitat type×Region	2	1.31	0.37	1	5.82	0.14	1	0.59	0.51	-	-	-
Habitat type×Sampling year	2	0.17	0.84	2	2.39	0.10	2	0.89	0.42	3	1.55	0.21
Region×Sampling year	4	1.55	0.20	2	1.13	0.33	2	0.54	0.59	-	-	-
Habitat type ×Region×Sampling year	4	0.67	0.61	2	0.51	0.55	2	3.05	0.06	-	-	-

Notes: Underlined stars indicate higher multivariate dispersion in BPA habitat types. $p \leq 0.05$ are highlighted in bold type. Multivariate dispersion is calculated on the basis of a Gower's dissimilarity measure with log10(x) + 1 transformed data (dMG10). Results obtained with the Gower's dissimilarity measure with log2(x) + 1 transformed data (dMG2) and the Jaccard's dissimilarity index (dJ) are presented in Supplementary Results. Production field encompasses crop fields and conventional meadows without pastures.

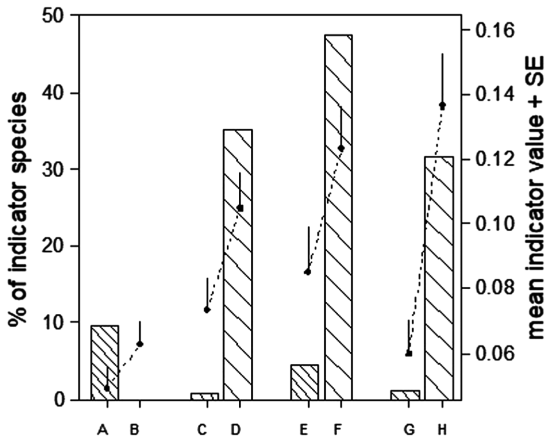

FIGURE 3
Percentage of significant indicator species per habitat type across regions and years (bars), and mean indicator value (+ 1SE) of all species (black dots). A: conventional meadow, B: BPA meadow, C: conventional meadow, D: BPA orchard, E: production field, F: BPA hedge, G: crop field, H: wildflower strip BPA.

meadow (three species) while four and two species had significant values for crop fields and conventional meadow, respectively (list of species with indicator value IndVal in Supplementary Table 11).

Across regions and over the three sampling years, 22 of 231 species (about 10%, Figure 3) had a significant indicator value ($p < 0.05$, Holm-corrected) for conventional meadows while none had a significant indicator value for BPA meadows. In all other pairwise comparisons, the mean indicator value of species was significantly higher in BPA habitats than in non-BPA controls. For BPA meadow, though, the difference was relatively modest (Wilcoxon rank sum test, $p < 0.05$, over the years and every year; Figure 3). Some indicator species were also detected when considering different regions, years and sampling methods (Supplementary Results).

Habitat specificity in regions

Altogether, region one was the region with the highest number of species in this study, being slightly ahead of region three while region two had ¼ less species.

At the regional level, BPA hedges showed the highest numbers of characteristic species with significant indicator values in regions one and two(about 25 and 23% of the total regional number of species, respectively, Figure 4). Low numbers of indicator species were counted in crop field sites (4 and 5.5% in regions one and two, respectively). The conventional meadow was the poorest indicator species habitat over the regions one and two (1 and 0%, respectively) but not in region three where it was the richest (2.8%). However, in region three where no BPA hedge sites had been sampled, the number of species with significant indicator value was low, i.e., under 3%. Further interesting information was provided by the mean indicator value over all the species per habitat type. BPA hedge had the significantly highest mean indicator value in regions one and two (Tuckey's hsd, $p < 0.05$, Figure 4). Although a similar number of species with significant indicator values were found in conventional meadows, crop fields and BPA orchards of region one, the mean indicator value over all species of the later habitat was significantly higher (Tuckey's hsd, $p < 0.05$, Figure 4).

The sum of the species specificity for habitats was the highest in region one, followed by regions three and two (Figure 5). BPA habitats had higher specificity values than production fields in the

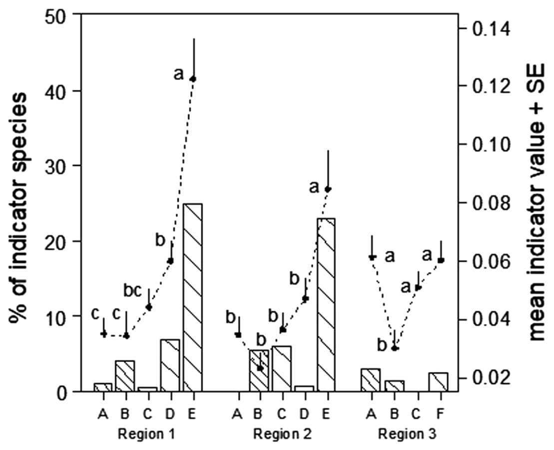

FIGURE 4
Percentage of significant indicator species per habitat type in three regions across years (bars), and mean indicator value of all species (black dots). A: conventional meadow, B: crop field, C: BPA meadow, D: BPA orchard, E: BPA hedge, F: wildflower strip BPA. Points with the same letter are not significantly different (Tukey's hsd, $p<0.05$).

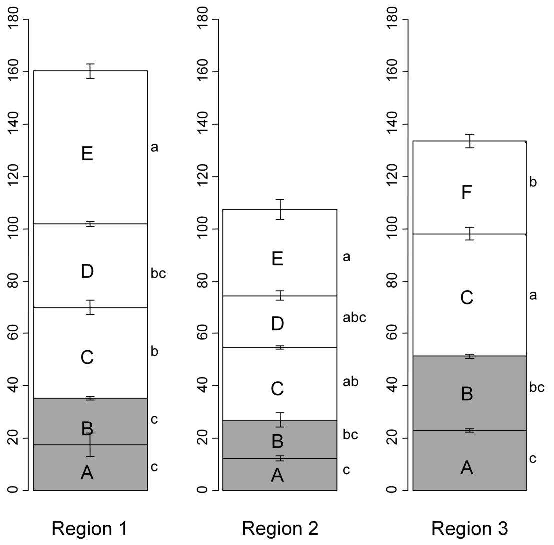

FIGURE 5
Mean specificity score of habitats and standard error over three (four for region three) years in the three regions (comparative contribution to regional diversity). **(A)** conventional meadow, **(B)** crop field, **(C)** BPA meadow, **(D)** BPA orchard, **(E)** BPA hedge, **(F)** wildflower strip BPA. Specificity values with the same letter are not significantly different (Tukey's hsd, $p<0.05$).

three regions although not always significant. BPA hedges in regions one and two (Figure 5E), and BPA meadows in region three showed the highest values (Figure 5C). In the three regions, conventional meadows exhibited the lowest specificity value.

Discussion

Effects of agri-environment schemes on diversity components are modulated by environmental heterogeneity

Studies assessing the effectiveness of AES have traditionally concentrated on the number of species found in fields under the scheme compared to conventional production fields. However, the number of species (α-diversity) represents only a part of the diversity of a given species group. Indeed, as we show, agricultural intensification can have more pernicious and hidden effects on other diversity components such as beta-diversity, conducting to (meta-) community homogenization (also shown by Gabriel et al., 2006 and Tarifa et al., 2021). Indeed, alpha- and beta-diversity can be decoupled in their response to environmental perturbation (Smart et al., 2006). Simplification of species diversity to the number of species or to a composite diversity index leads to a substantial loss of information that may be crucial in examining the effect of environmental factors as shown in this study. This might be especially important in a context where the anthropic-driven simplification of ecosystems leads to biotic homogenization and the preponderance of generalist species (McKinney and Lockwood, 1999; Kehinde and Samways, 2014; García-Navas et al., 2022).

Comparison of diversity patterns of spider assemblages in BPA habitats and corresponding production fields showed that farmers act in different ways on diversity components by implementing BPA habitats. While alpha-diversity was on average higher in BPA hedges, BPA orchards and wildflower strip BPA compared to the corresponding production fields, alpha-diversity of BPA meadows did not significantly differ from conventional ones. Similar results were obtained by Knop et al. (2006) but not by Albrecht et al. (2010), who also compared the number of spider species between BPA meadows and conventional ones. Contrasting results likely reflect the longer survey period by Albrecht et al. (2010) including autumn assessment while both other studies were capturing spider during springtime and early summer. Vascular plants, bees and grasshoppers responded positively to the scheme in both investigations. Comparing several AES across Europe and their impact on biodiversity, Kleijn et al. (2006) found a low effectiveness of schemes on the species density of arthropods. A more recent study showed an overall positive effect of AES on multiple taxa (Boetzl et al., 2021). In a recent study, Gayer et al. (2021) showed that promoting diverse habitats such as flower strips, hedgerows and organic farms promotes the diversity of plants and arthropods in croplands. Our results show that conclusions may differ if, in addition to species richness, species composition is taken into account (see also Smart et al., 2006; Clough et al., 2007). Furthermore, our study revealed that traditional woody elements of the agricultural landscape, i.e., hedgerows and orchards with high-stem trees, differ most from production fields, with higher species richness and particular species composition. The value of these hedgerows and orchards in supporting arthropod diversity has been emphasized recently by other authors (Garratt et al., 2017; Kolb et al., 2020).

Analyzing the diversity components of spider assemblages in meadows in more detail allows deeper insights. The main effect (BPA vs. conventional) was observed on the species composition and this is probably due to a large number of particular BPA meadows which strongly differed from a group gathering BPA and conventional ones as shown on the MDS plot (Figure 2) and quantified by the multivariate dispersion (Table 3). This dispersion is very likely to be the consequence of the environmental heterogeneity across BPA meadows and not of the management intensity because all BPA meadows were managed similarly, according to the same – BPA – rules. Then, it can be concluded that extensively managed BPA meadows have the potential to support many different species and the specific assemblage found in this habitat will be more strongly determined by the environmental heterogeneity around them. Spiders may then profit from the choice of farmers, deciding to allocate BPA meadows in diverse environments related to low productivity and/or plots which are more difficult to manage (e.g., steep slope, forest shadow) as shown by Herzog et al. (2005). Nonetheless, benefits for spider communities have been reported after the implementation of uncut meadow strips AES management (Řezáč and Heneberg, 2018). No indicator species were found (according to IndVal) for BPA meadows while 10% of the species were indicators of conventional meadows. This is due, in one side, to particular species occurring in some BPA meadows but not in others (fidelity component of IndVal was low), and on the other side, to the particular species assemblages of conventional meadows in region three. In this region, one site was characterized by a concentration of rare species for Switzerland (rarity values from three to five according to Pozzi and Borcard, 2016). Again, this demonstrates the need to focus on multiple components on diversity beyond species richness. In this situation, farmers of region three should be encouraged to maintain the current management of the particular meadows to sustain species diversity at regional level.

On average more species were found in BPA orchards than in conventional meadows, and this had an effect on the species composition, which was also significantly different. In region one, however, beta-diversity of both habitat types was highly different in that region while less preeminent in region two, where assemblages of conventional meadows can be considered as a subset of the BPA orchards. The similar multivariate dispersion of both habitat types suggests that the environmental heterogeneity acted similarly on spider assemblages among fields in both habitat types and regions. Furthermore, BPA orchards are particularly interesting habitats for spiders because they combine both trees and a meadow. Although 1/3 of the 54 indicator species were characteristic species of tree canopy (caught by beating), a similar proportion of species were captured simultaneously in the tree canopy and in the vegetation (sweep net) or on the ground (pitfall trap) of BPA orchards. For the major part of these species which were not caught at all in conventional meadows, BPA orchards represent a unique habitat combination in the landscape that even BPA hedges do not provide. Published studies relating effect of orchards with high stem fruit trees on biodiversity have been traditionally underrepresented (e.g., but see Bailey et al., 2010; Samnegård et al., 2019; Martínez-Núñez et al., 2021), although the role of such agro-ecosystems in providing particular environmental conditions by combining trees with meadows or crops has for long been recognized in the agroforestry literature (Herzog, 1998; Jose, 2009).

BPA hedges had the most characteristic spider assemblages of BPA habitats compared to corresponding production fields in the agricultural landscape. This is reflected in the higher number of species, a highly distinctive assemblage of species and a high number of regular indicator species showing high specificity and fidelity, irrespective of the region and the sampling year (47% of species are indicators of BPA hedges compared to production fields). However, the species composition of BPA hedges in regions one and two largely differed. The multivariate dispersion was higher among BPA hedges than among production fields, reflecting both different environmental conditions of the BPA hedges and a possible difference of management within them. Investigations have emphasized the important role of hedgerows as conservation element for biodiversity in the agricultural landscape (e.g., Forman and Baudry, 1984; Rey et al., 2021) and also for biocontrol purposes (e.g., Marino and Landis, 1996; Kolb et al., 2020; Martínez-Núñez et al., 2021).

In our study, hedgerows were demonstrated to play a significant role for spider conservation by providing habitat for a number of species that did not occur neither in production fields nor in other BPA habitats (see below effect at region level). Further investigations would be necessary to know whether these species spillover to adjacent fields to their hunt territory, and then provide biocontrol ecosystem services (Tscharntke et al., 2016; Růžičková et al., 2020). A recent study has shown that spiders are more abundant and species-rich in set-aside fields, but their dispersal capacity is limited and their pest-control service might depend importantly on habitat proximity to the crop fields (Růžičková et al., 2020).

Wildflower strip BPA are inserted in landscapes dominated by crops in Switzerland, and are part of the crop rotation (may move every 6 years). This condition strongly influenced the spider assemblages occurring there because most of the species were typical crop species during the first 2 years after the strip has been sown. However, after 2 years in our study, spider assemblages of wildflower strip BPA were highly distinct from those of the crops with about 32% of significant indicator species. After the colonization period, species assemblages remained stable. This suggests, that the role played by the wildflower strip BPA as refuge in the landscape for typical crop species during unsuitable periods of cropping operations, and as reservoir for further re-colonization of the crop, which should deliver biological control effects. Such species are *Diplostyla concolor* (Wider, 1834) and *Mangora acalypha* (Walckenaer, 1802; see Supplement for the IndVal values of species). On the other side, wildflower strip BPA provide suitable habitat for particular species that can permanently benefit after rapid colonization (corroborated by Frank and Nentwig, 1995). Such species are *Argiope bruennichi* (Scopoli, 1772) and *Aulonia albimana* (Walckenaer, 1805). Similarly, fallow vineyards grown in steep slopes have also been identified as important habitats and reservoirs for rare species (Wersebeckmann et al., 2021).

Region effect

Together with the comparative contribution of habitats to the regional diversity, results on alpha- and beta-diversity, and the multivariate dispersion suggest that AES were more effective in the region where the spider diversity was higher as a rule. This might be due to the fact that species-diverse regions have a higher potential to allocate species with contrasting habitat requirements in different BPAs while simplified regions probably host a pool of species that has already been strongly filtered, minimizing the potential impact of AES (Smart et al., 2006; Gámez-Virués et al., 2015). Indeed, observed regional differences in spider diversity can be explained by three factors: regional land use, management intensity and the farming history. Region two is characterized by a high production level of its dominating land use, namely grassland, with about 42% more hay produced on average by conventional meadows and about twice more livestock units per ha, compared to the mixed farming region one, and the crops' region three (Table 4). This production level can only be reached by intensive management of the grasslands, also reflected by the number of cuts which averaged 4.6 in region two compared to 3.1 and 3.0 cuts per year in regions one and three, respectively. As a consequence, differences between BPA habitats and production fields, as well as habitat specificity by spider species were lower in region two. Land use history can be a main driver of current diversity levels of multiple taxa (Le Provost et al., 2020). Therefore, it is not surprising that it also plays an important role when it comes to BPA effectiveness. For instance, the high importance of BPA meadows for spiders in region three can be explained by land-use history during the last century. In this region, meadows were gradually replaced by more productive arable crops on the plateau, but some of them were kept and are still extensively managed on slopes around the plateau, some of which were now declared as BPA (on average 27.2 dt/ha hay yield). Apparently, spider assemblages in these meadows were quite characteristic and remarkably enriched the regional diversity.

Comparison of BPA habitats with production fields, together with land use and production data of the regions suggests that management intensity of the main land use and of particular BPA are the most important drivers for spider diversity at regional scale. A high proportion of BPA in the landscape does not ensure high species diversity depending on the BPA type and its management, as shown in region two which encompassed the higher proportion of BPA (16.2% against 8, 5 and 2.9% in regions one and three, respectively) but with a large area covered by BPA orchards (11.1%) where the undergrowth meadow is unfortunately very often intensively managed.

Ecological theory predicts that AES demonstrate higher effectiveness for biodiversity in simple landscape types compared to cleared and complex one's when complexity is measured as the proportion of non-crop areas (Tscharntke et al., 2005). In our opinion, this is not true in all circumstances. Indeed, our data showed that the effectiveness of the scheme, i.e., the difference between BPA habitats and production fields in terms of diversity (alpha and beta) is the most effective for spiders in a landscape with primarily less intensively managed fields (that can also be grasslands) and a balanced proportion of crops, grasslands and BPA (i.e., region one). Furthermore, regions with high percentage of crops but with valuable BPA (e.g., wildflower strip BPA) as well as less intensively managed grasslands (region three in this study) may also demonstrate high diversity value. In our study, region two had the lowest proportion of crops but grasslands including meadows of BPA orchards were intensively managed preventing then a high spider diversity to occur even in BPA habitats. Investigations have shown that many factors may confound the effect of AES on biodiversity. One of them is the landscape configureuration and the proportion of non-crop habitats in the surrounding of AES fields versus conventionally managed fields (Clough et al., 2005; Schmidt et al., 2005, 2008; Gámez-Virués et al., 2015). However, effects were demonstrated for large gradients in these studies, i.e., from 0 to 80% of non-crop area. In our study, gradient was comparatively very low with values of 0 to 20% and consequently landscape effects were minor (Jeanneret et al., 2003b).

Indicator species

BPA habitats were characterized by significantly higher mean indicator values of all species calculated with IndVal (Dufrêne and Legendre, 1997) than production fields. The mean indicator value indicates how unique the habitat in the farmland landscape is for spiders. In paired comparison of BPA habitats vs. production fields, significant differences in favor of BPA habitats demonstrated their importance for a majority of species. The results of the regional analysis point into the same direction, BPA hedges showing in particular highly characteristic species assemblages which were not found in other habitat types. Remarkably, a large part of the species indicators for BPA hedges came from pitfall trapping. This indicates that not only species of shrubs and trees were characteristic but also soil dwelling species, due to particular micro-climatic conditions. Compared to conventional meadows, BPA orchards showed a high percentage of species preferably occurring there, but their value as particular habitat for spiders decreased in the regional analysis. This is due to the other habitat types, BPA meadows, BPA hedges and crop fields, which were part of the analysis, and where species of BPA orchards also occurred. However, detailed analysis of the species distribution revealed that

TABLE 4 Major land use categories, agricultural characteristics of BPA, and production fields in the 3 regions.

		Region		
		1	2	3
Land use in regions (% area)				
Forest		26.8	17.1	25.1
Agricultural land use (% UAA)		66.8	75.8	60.1
Crops		57.5	23.6	74.9
Grassland		33.6	59.9	9.9
Total BPA		8.5	16.2	2.9
BPA meadow		5.8	4.6	1.8
BPA orchard		2.2	11.1	0.0
BPA hedges		0.4	0.5	0.1
Wildflower strip BPA		0.1	0.0	1.0
Average Utilized Agricultural Area (UAA)(ha) of farms in region		29.1	18.0	21.2
Average Livestock Unit /ha		1.2	2.3	0.5
Average yield (dt/ha)				
BPA meadow		40.9	35.1	27.2
BPA orchard		59.0	101.5	-
Conventional meadow		60.0	101.4	58.8
Crop field	Corn	138.5	-	120.6
	Winter wheat	66.6	64.3	58.3
	Sugar beet	-	-	650.0

Notes: Average yield for BPA orchard refers to the grass use of the underlying meadow. Average Livestock Unit/ha of UAA is calculated for meadows when pastured. UAA: Utilized Agricultural Area.

the combination of a meadow with traditional high-stem trees favored two rare species of Switzerland known to live on the trunk of trees and which were exclusively caught in BPA orchards in the branches of the trees as well as in the low vegetation. Despite being isolated in a crop dominated landscape and regularly re-sown, wildflower strip BPA were rapidly colonized by species that were then recorded as indicator species because they were not occurring in the other habitats of region three. Crops, meadows and wildflower strip BPA are highly contrasted habitats resulting in a high specificity of spiders for them. Similarly, Rischen et al. (2021) reported positive effects of non-crop habitats in ground-dwelling beetle diversity, showing also that different habitat types contributed particular species assemblages to the landscape. These results might explain, at least partially, why schemes aimed at set aside areas (non-productive habitats) seem to be more effective (Ekroos et al., 2014; Batáry et al., 2015) and a mosaic of different habitat types in the landscape increase regional diversity of arthropod predators (Mader et al., 2017).

Spatial and temporal design of evaluation

To investigate the effectiveness of agri-environmental measures on biodiversity, replicated spatial and temporal designs are necessary. Temporal and spatial variability in experimental designs may affect conclusion as results may depend on sampling years and regions. Indeed, in our study alpha-diversity of BPA orchards and conventional meadows were not significantly different for one and two sampling years in regions one and two, respectively. Based on those findings, it is difficult to draw conclusions about the success of the AES with respect to BPA orchards compared to conventional meadows for spiders. Analyzing beta-diversity in addition to alpha-diversity revealed significant differences in species composition among habitat types in both regions and across all years. This emphasizes the importance of investigating effects at several diversity levels and across several years. In 1997, the recently sown wild flower strip BPA harbored the same number of species on average and a similar species composition than the crop fields but were clearly more species-rich and with specific species composition in the following years. Those results suggest succession processes with a somewhat delayed response of spider assemblages, representing a clear case supporting the recovery debt hypothesis (Moreno-Mateos et al., 2017). If succession processes had not been considered in the evaluation design, wrong conclusions would have been drawn, i.e., no success in 1997. When time is replaced by space in snapshot studies, evaluation should take place once the effects can be expected to be established. This is actually very challenging. Indeed, even if targets of AES are precisely defined, e.g., the occurrence of a target species, goal achievement can only be recorded by monitoring data over time in both control and treatment fields following a before-after-control-impact design, as recommended by Kleijn et al. (2006). To our knowledge, our investigation is the first one to assess the effectiveness of an AES across a 7-years period of time, and covering all habitat types belonging to the scheme in a common analysis. In addition, data used in this study were collected ca. two decades ago, at the beginning of the implementation of these measures. This adds value to the work since it can be used as a baseline over which to compare more recently collected data.

Recommendations and conclusions

This study highlights that, schemes aimed at promoting set-aside habitats (areas out of production) interspersed in farmland landscapes increase spider diversity at multiple levels. In addition, focusing only on α-diversity may bias conclusions about the benefits delivered by AES. To properly assess the efficacy of AES and derive useful recommendations, a comprehensive analysis of the species composition and the indicator species is necessary. Our findings on spider assemblages support that the conservation of semi-natural elements in the agricultural landscape such as hedgerows and traditional orchards with high-stem trees may be of crucial importance for biodiversity. The key role AES play in preserving species diversity in cultivated landscapes not only relies on harboring particular assemblages related to the habitat itself (e.g., hedgerows, wild flower strips) but also on increasing the range of environmental conditions in which these habitats usually occur (grasslands). The range of environmental conditions encountered even in regions of about 7 to 8 km^2 can increase the regional diversity by acting on the β-diversity component and its variation. This has important implications because it is easier to maximize environmental heterogeneity by establishing less or non-productive habitats like BPA in the farmland landscape compared to changing local crop conditions or management. Restoring intensively managed agricultural fields for biodiversity conservation purposes proved to be difficult under certain circumstances and may even fail (grassland restoration). Therefore, maintaining and conserving present small fragments of semi-natural habitats in the agricultural landscape ("preserving what is still occurring"), and planting or sowing new elements should be the first priority of AES.

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.

Author contributions

PJ participated to the elaboration of the study, performed the analysis, and wrote the manuscript. SP identified the spiders. CM reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.866947/full#supplementary-material

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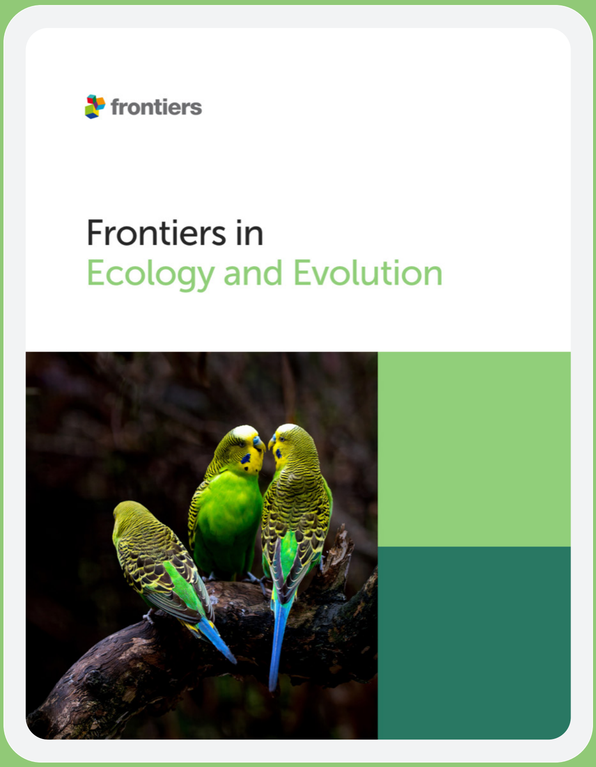

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