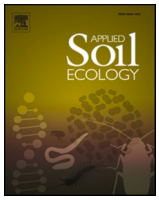
Applied Soil Ecology 197 (2024) 105326

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Soil invertebrate biodiversity and functionality within the intensively farmed areas of the Po Valley

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ARTICLE INFO

Keywords:
Soil eDNA metabarcoding
Soil invertebrate communities
Soil invertebrate functional diversity
Agroecosystems

ABSTRACT

Although agricultural activities can strongly affect soil biodiversity and health, with consequences on the provisioning of soil biota-mediated functions, their specific impact on soil invertebrate communities is far from being fully elucidated. In this study, the invertebrate communities associated with the soils of six habitat types, including both semi-natural and cropping systems, of one of the most intensively farmed areas in Europe, the Po Valley (North Italy), were characterized using the eDNA metabarcoding approach. The aims were to examine the variation in the taxonomic and functional diversity among the habitats and evaluate the relation between the disturbance caused by the main agronomic practices adopted in the area and the community diversity. Overall, the invertebrate communities were found to substantially differ in terms of taxonomic and functional diversity between the six habitats considered. For example, cornfield and rice paddy showed the highest diversity of annelids and the lowest one of nematodes. Woodland was found to host the most unique soil fauna, while grassland shared the majority of its soil taxa with almost all the other habitat types. The trophic groups had significantly lower diversity in specific habitats (e.g., carnivores, herbivores, microbivores in cornfield) suggesting that biological soil quality and ecosystem services provision may vary among them. Concerning agronomic practices, it was not observed an inverse relation between diversity and the disturbance they cause. In detail, while tillage and insecticide use negatively affected invertebrate diversity as a whole, specific soil taxa and trophic groups were idiosyncratically affected by the different agronomic practices (e.g., pesticide and fertilizer use was related to an increase of annelid and bacterivore diversity). In this regard, the peak of diversity observed for specific taxonomic and functional groups might be attributed to an impaired community balance. Altogether, the results obtained contribute to a more comprehensive understanding of the intricate interplay between agricultural practices and soil invertebrate communities, with implications for the awareness of soil health, ecosystem services provision and biodiversity conservation in agroecosystems.

1. Introduction

One of the main present and future challenges of our society, also formalized within the EU Green Deal, is to find a compromise between the rising demands for primary production and the parallel need for reducing the impact of agricultural practices on biodiversity and its capacity for providing ecosystem services. Soil is a complex system and represents one of the most important substrates for life on Earth,

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https://doi.org/10.1016/j.apsoil.2024.105326
Received 8 May 2023; Received in revised form 2 February 2024; Accepted 5 February 2024
Available online 10 February 2024

harbouring a wide variety of organisms (Bardgett and Van Der Putten, 2014) and providing essential ecosystem services (e.g., biogeochemical and water cycles, carbon storage, support to the primary production; Jónsson and Davíðsdóttir, 2016; Pereira et al., 2018). The crucial importance of soil health for humans was recognized by the Food and Agriculture Organization, which launched the Global Soil Partnership to spread awareness of the fundamental role of soil resources in adapting to and mitigating climate change and guaranteeing the provision of essential ecosystem services (Montanarella and Vargas, 2012). At present, being able to assess the impact of agricultural practices on soil biodiversity, from the organismal and population level to the community level and at different spatial scales, is crucial (de Graaff et al., 2019). This knowledge would allow us to adopt sustainable soil management practices and eco-schemes promoting soil biodiversity and functionality, and therefore the associated ecosystem services (Thiele-Bruhn et al., 2012; Bach et al., 2020; FAO et al., 2020; Creamer et al., 2022).

Soil communities are highly diversified both in terms of composition and structure, with hundreds of thousands of species comprising Prokaryota, Fungi, Protozoa and Metazoa (Anthony et al., 2023). The scientific literature on soil prokaryotes and fungi is considerable (e.g., Anderson and Cairney, 2004; Philippot et al., 2007; Morrissey et al., 2008; Tedersoo et al., 2014), whereas soil metazoans are less investigated. Even though the knowledge of below-ground invertebrate communities is still limited, in recent years, various studies have started to shed some light on their distribution, their possible reaction to climate change, their trophic interactions and their impact on biogeochemical cycles (e.g., Wu, 2013; Bardgett and Van Der Putten, 2014; Briones, 2014; Coyle et al., 2017). Moreover, soil invertebrate diversity, activity and distribution were found to be regulated by several abiotic factors that vary in different habitat types, such as temperature (Haimi et al., 2005), soil moisture (Tan et al., 2021), soil texture and structure (Mikhail, 1993), pH (Curry, 2004) and availability of micro and macronutrients (Huhta et al., 1986; Callaham Jr et al., 2003; Ball et al., 2018), but also by the disturbance due to human activities (Montagna et al., 2018; Köninger et al., 2023). However, since soil invertebrates tend to form aggregates at the micro-scale, resulting in a patchy distribution (Nielsen et al., 2010), it is quite difficult to study the specific influence of different factors on them. For this reason, most of the studies published so far on the topic were focused on a few taxonomic groups and considered a limited number of factors. The target taxa, usually sampled with traditional techniques (e.g., Berlese funnel, Baermann funnel, wet extraction), were morphologically identified at different taxonomic levels, usually above species (e.g., Sabu et al., 2011; Barberena-Arias et al., 2012; Gill and McSorley, 2012). All these constraints have so far prevented the achievement of a comprehensive overview of the drivers of invertebrate soil community diversity and composition and the identification of the main stressors affecting them.

DNA-based surveys could represent a way to expand the knowledge of soil communities, giving the possibility to process in parallel multiple samples collected across space and time and perform large-scale investigations (Porter and Hajibabaei, 2018; Young and Hebert, 2022). Among the DNA-based methods, amplicon-based ones like DNA metabarcoding are the most used for biodiversity surveys, even if in recent years, PCR-free methods were also developed (Andrews et al., 2018). Starting from bulk samples (organisms isolated from the soil) or soil samples in which the DNA of soil taxa is present, DNA metabarcoding can thus be used for characterizing soil communities (Orgiazzi et al., 2016). This method can potentially overcome the need for the morphological identification of organisms (Taberlet et al., 2012), even if coupled with the morphology, it can give a more complete picture of the community (e.g., information on organisms' sex and development stage from DNA based analyses only cannot be retrieved). A deeper characterization of soil communities can give an insight into their structure and specific groups functions (Arribas et al., 2016) and consequently allow to address multiple biological questions related to soil biodiversity at different spatial scales, from the microscale of the soil aggregates to the macroscale of the landscape (e.g., Ettema and Wardle, 2002; Treonis et al., 2018; Lin et al., 2021; Le Provost et al., 2021).

In this study, the environmental DNA (eDNA) metabarcoding was employed to characterize the invertebrate communities associated with the soils of one of the most intensively farmed areas in Europe, the Po Valley (North Italy). The results obtained with this approach were used to i) examine the variation in the invertebrate communities diversity and structure, both from the taxonomic and the functional point of view, among the six of the most widespread and economically important habitat types present within the area, accounting also for their soil chemical and physical properties and ii) evaluate the relation between the disturbance due to the agronomic practices adopted in the area and the soil invertebrate diversity.

2. Materials and methods

2.1. Sampling

Within the study area represented by the Po Valley – Italy (covering the majority of Northern Italy, extending from the Western Alps to the Adriatic Sea, and bordered to the South by the Northern Apennines), the selection of the sampling sites was made using georeferenced data obtained from the Geoportale della Regione Lombardia (https://www.geoportale.regione.lombardia.it/) and processed with the QGIS software v.3.10.0 (http://www.qgis.org). Three layers were overlaid to select homogeneous sites: *i.* soil texture (fine, medium, coarse); *ii.* pH (acidic, neutral, alkaline); and *iii.* Land use (by choosing among the most widespread and economically important habitat types present within the landscape). The adopted selection procedure led to the identification of a total of 79 sampling sites: 10 deciduous woodlands, 14 semi-natural grasslands, 10 alfalfa fields, 13 vineyards, 22 cornfields and 10 rice paddies (Fig. 1; Supplementary Table S1). The selected habitat types are located in the central-south and north parts of the Po Valley at an altitude spanning from 100 m a.s.l. to 800 m a.s.l. Woodland here is a seminatural habitat characterized by deciduous forests occasionally subjected to silvicultural activities (mostly coppicing with a turnover of 20–30 years). According to the habitat directive (92/43/EEC), this habitat type can be ascribed to subalpine beech woods with *Acer* and *Rumex arifolius* (habitat code 41.15), Tilio-Acerion ravine forests (41.4) and mixed oak-elm-ash forests of great rivers (44.4). Also grassland is a semi-natural habitat that is characterized by a relatively high diversity of herbaceous plants used for hay production. Grasslands are not irrigated and once or twice a year they are fertilized, usually with sewage. According to 92/43/EEC, this habitat type can be ascribed to semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco Brometalia; codes 34.31 to 34.34), mesophile grasslands of lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*; code 38.2) and of mountain hay meadows (British types with *Geranium sylvaticum*; code 38.3). Vineyard is a perennial non-irrigated agricultural system mainly present on the slope of the Po Valley hilly regions. In the vineyards of Po Valley different agroecological strategies are adopted, such as the use of inter-row cover cropping. The remaining habitats (alfalfa, cornfield and rice paddy) are irrigated arable lands delimited by drainage ditches, regularly ploughed and under crop rotation.

The sampling sites selected for each habitat type corresponds to a field of at least 5000 m^2 where four bulk soil cores were collected along a transect, at a distance of two meters from each other. Each bulk soil core was obtained using a sterile Dutch soil auger (diameter 6 cm) at a depth of 20 cm, through the O and A soil horizons. Soil cores were frozen at −20 °C within a few hours from their collection. Along each transect, two to three soil core samples were also collected to determine the bulk density using stainless steel rings. While the absence of temporal replicates in this study may provide only a snapshot of the actual biological communities present in the soil throughout the entire year, the enduring presence of eDNA in the soil for an extended period (e.g., Foucher et al., 2020) should enable the acquisition of a reasonably comprehensive

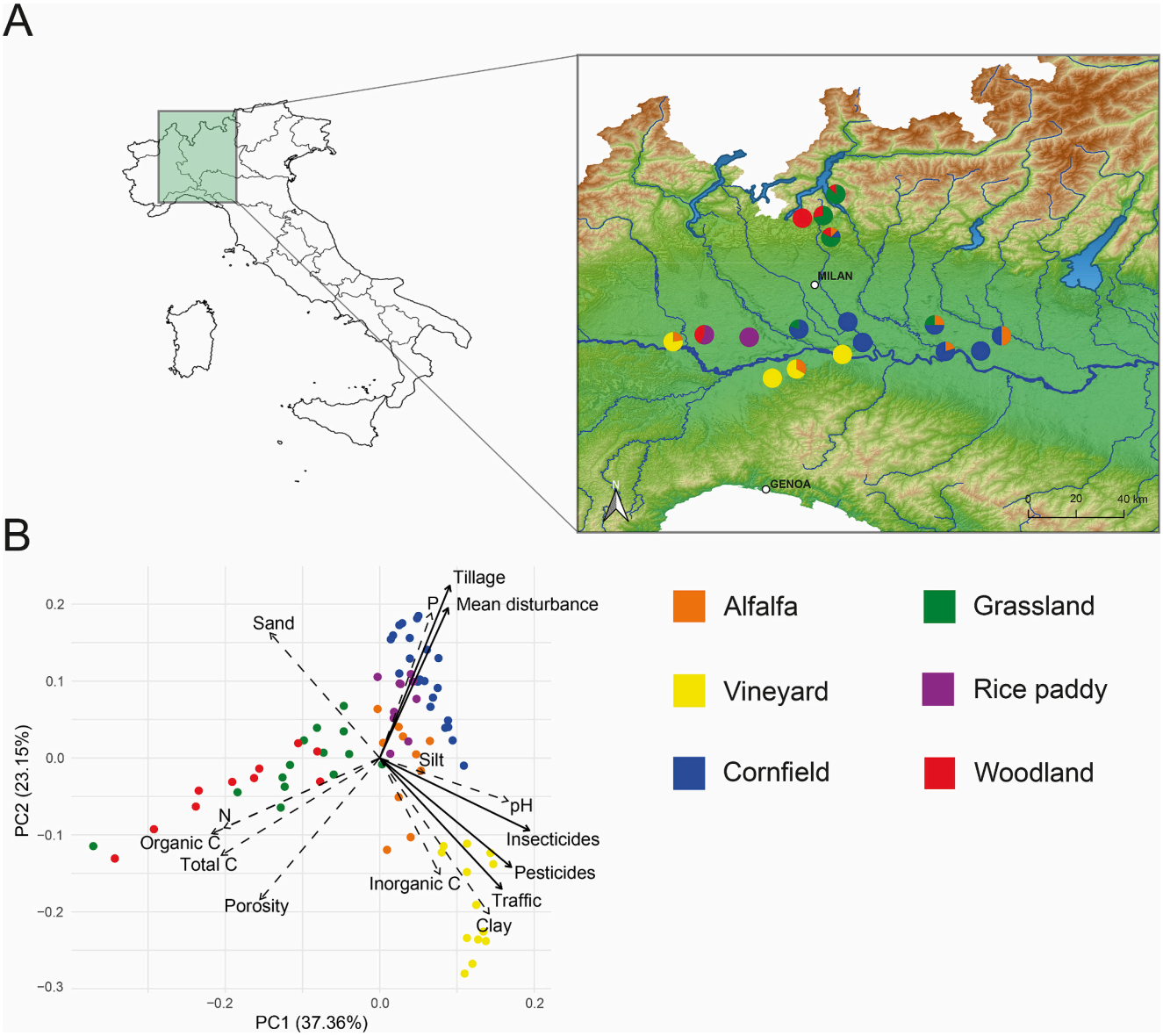

Fig. 1. Geographic location and characteristics of the habitats analysed in this study. Colors correspond to the different habitat types considered. (A) The maps show the area of the Po Valley (Italy) in which the study took place and the position of the sampling sites (pie charts) within it. Map layers were downloaded from http://www.pcn.minambiente.it/mattm/ and elaborated with the software QGIS v.3.22.6. (B) Principal component analysis (PCA) of soil physical-chemical properties (dashed line arrows) and agronomic practices (solid line arrows). Total/Inorganic/Organic C: total/inorganic/organic carbon (%); N: nitrogen (%); P: phosphorous (mg/kg); Porosity: soil porosity derived from bulk density; Sand/Silt/Clay: percentage of soil particles belonging to the classes of sand/silt/clay.

portrayal of these communities.

2.2. Measurement of soil physical-chemical properties and agronomic disturbance intensity

Part of the soil collected from each transect was air dried for 10–14 days and sieved using a 2 mm stainless mesh sieve. From each transect, 300–400 g of soil was obtained and used to measure soil physical and chemical properties (measured as described in Supplementary Material S1; results in Supplementary Table S1).

The agronomic practices adopted in the sampling sites were summarized in five categories: tillage, insecticide use, pesticide use, field traffic by farm machines and fertilizer use. The level of intensity at which each practice was adopted was estimated. The tillage intensity of each crop was calculated by multiplying the average number of interventions by the relative disturbance weights estimated for each type of tillage (field leveling, primary and secondary tillage operations, mechanical weeding and rolling) and summing all the contributions. Pesticide intensity value considered the number of fungicide, insecticide, acaricide, and herbicide applications per crop and year. Field trafficking intensity value was estimated by quantifying the travelled infield distance during each crop cycle, considering the equipment width and the number of interventions. Fertilizer application intensity was quantified by summing the amount of each element (N, P, K) applied to crops per cultivation cycle. A value for the disturbance intensity (DI) in each habitat type was then calculated by dividing the average value of intensity for each of the five agronomic practice categories by the highest average value obtained for that category. All details on the agronomic practice-related disturbance estimation and the results obtained are reported in Supplementary Material S2 and Supplementary Table S2. Finally, a total value of disturbance intensity per habitat was also calculated averaging the DIs of all the agronomic practices.

2.3. eDNA isolation, libraries preparation and sequencing

The four soil cores collected at each site were pooled, homogenized and then sieved with a sterilized sieve (mesh 5 mm^2) to remove coarse material. Three aliquots (50 ml each) of the sieved soil were collected in vials and stored at −80 °C until the DNA extraction. From each vial, 10 g of soil was ground in liquid nitrogen using a sterilized mortar and pestle; the DNA was isolated from 0.5 g of ground soil using the Qiagen DNeasy PowerSoil Kit (Qiagen, Germany) following the standard protocol, including mechanical lysis with TissueLyser II (Qiagen, Germany). Negative samples were processed together with soil samples to control possible contaminations. A mock community was also prepared combining equimolar quantities of DNA from representatives of the following groups: Araneae, Annelida, Isopoda, Diplopoda, Carabidae and Collembola. An efficiency test for identifying the most efficient primers combination to be used in this study was performed (details are reported in Supplementary Material S3). Thus, the primer pairs COI-L (Leray et al., 2013) for the COI gene and 18S-fw (Capra et al., 2016) for the 18S rRNA were selected for library preparation. For each of the three DNA extraction replicates, PCRs were performed in a volume of 50 µl each by using HotStartTaq PCR Master Mix (Qiagen, Germany), 0.2 mM of each dNTP, 0.5 pmol of each primer and 20 ng of DNA. PCR conditions employed: 15 min at 95 °C, followed by 25 cycles of 1 min of denaturation at 95 °C, 1 min of annealing at 58 °C and 1 min of extension at 72 °C, with a final single extra extension step of 7 min at 72 °C. The amplicons obtained from the three DNA extraction replicates were then pooled at equimolar concentration (for a total of 80 amplicon DNA samples) and used for the further step of the library preparation. Amplicons were cleaned up with Agencourt AMPure XP (Beckman, USA) and the sizes were checked with a Bioanalyzer 2100 (Agilent Technologies, USA). Libraries were prepared following 16S Metagenomic Sequencing Library Preparation protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). The libraries obtained were quantified by Real Time PCR with KAPA Library Quantification Kits (Kapa Biosystems, USA) pooled in equimolar proportions and sequenced with MiSeq sequencer (Illumina, USA) using reagent kit v3 with paired-end reads of 250 bp.

2.4. Raw data analyses and taxonomic assignment

All data analyses were performed with the QIIME2 platform (Bolyen et al., 2019) and the R software v.4.2.2 (R Core Team, 2022) by using various libraries as specified below. Raw sequences were denoised, filtered and checked for chimera presence using the dada2 pipeline (Callahan et al., 2016) to obtain the Amplicon Sequence Variants (ASVs). COI sequences, as suggested in the best practices for metabarcoding studies using this marker (Antich et al., 2021; Creedy et al., 2022), were also filtered to include only sequences with a length of 313 bp (region amplified by the selected primers) and presenting an open reading frame using custom R scripts and the R library coil (Nugent et al., 2020). The taxonomic identification of the ASVs was made by training a machine learning naïve Bayes classifier (QIIME2 feature-classifier plugin) on the reference database and then using the fitted classifier to identify the ASVs' sequences (Bokulich et al., 2018). To improve the accuracy of the taxonomic classification, the QIIME2 clawback plugin (Kaehler et al., 2019) was used to compute a weight matrix that incorporates habitat-specific taxonomic abundance information in the classification. The reference databases used for the taxonomic classifications were SILVA v.132 (Quast et al., 2012; Glöckner et al., 2017) for the 18S rRNA sequences and a custom-made database obtained from the data deposited in BOLD (Ratnasingham and Hebert, 2007) using the QIIME2 rescript plugin (Robeson et al., 2021) for the COI sequences. Only sequences assigned to Metazoa were kept for subsequent analyses.

2.5. Development of taxonomical and functional datasets

The two markers adopted in this study showed a differential efficiency in detecting the different groups of Metazoa, with COI having a lower taxonomic coverage and a stronger bias toward some groups (Arachnida and Annelida), and 18S rRNA with a wider taxonomic coverage but a lower taxonomic depth (Table 1, Supplementary Material S3). For this reason, the taxonomic diversity analyses were performed on the whole 18S rRNA dataset, as representative of Metazoa, and on sub-datasets generated extracting the ASVs assigned to the most abundant metazoan clades present in each marker dataset. These sub-datasets are Nematoda and Arthropoda extracted from the 18S rRNA dataset; Arachnida and Annelida extracted from the COI dataset. The Metazoa dataset was also used to classify the taxonomically identified ASVs in seven trophic guilds (mostly following the categories defined by Potapov et al., 2022): bacterivores, fungivores, microbivores (unspecialized, feeding on different microorganisms like bacteria, fungi, algae), herbivores, carnivores, omnivores (feeding on plant and animal sources), detritivores (feeding on plant, microbial or animal origin dead organic matter). Guild assignment to each taxon was performed based on bibliographic research (guild assignments and related references are reported in Supplementary Table S3).

2.6. Estimation of community diversity and structure

To identify the taxa characterizing the invertebrate fauna of each specific habitat, an indicator species analysis was performed on the Metazoa dataset collapsed to the genus level with the R library indicspecies by using the indicator value index (hereafter IndVal, Dufrêne and Legendre, 1997; De Cáceres and Legendre, 2009; De Cáceres et al., 2012). To normalize all the datasets prior to proceed with the following

Table 1
Relative abundance of the main taxonomic groups identified by 18S rRNA and COI markers.

Taxon		18S rRNA		COI	
		Sequences (%)	ASVs (%)	Sequences (%)	ASVs (%)
Nematoda		49.2	45.6	n.p.	n.p.
	Dorylaimida⁻	32.2	10.8	n.p.	n.p.
	Tylenchida⁻	29.1	52.8	n.p.	n.p.
	Rhabditida⁻	15.2	14.9	n.p.	n.p.
	Triplonchida⁻	6.5	4.9	n.p.	n.p.
	Araeolaimida⁻	5.5	5.7	n.p.	n.p.
	Mononchida⁻	5.4	1.2	n.p.	n.p.
	Enoplida⁻	2.6	3.4	n.p.	n.p.
	unassigned⁻	3.5	6.3	n.p.	n.p.
Arthropoda		17.8	25.8	73.2	91.2
	Arachnida†	48.0	38.9	58.2	61.5
	Collembola†	28.1	21.8	1.3	0.4
	Insecta†	15.9	23.6	4.2	1.3
	Diplopoda†	2.6	1.5	0.01	0.04
	Chilopoda†	1.1	1.8	0.2	0.06
	Diplura†	n.p.	n.p.	0.06	0.03
	Protura†	0.1	1.2	n.p.	n.p.
	others†	1.3	1.1	0.03	0.04
	unassigned†	2.9	10.1	36.0	36.6
Annelida		9.3	2.2	21.6	1.9
	Enchytraeidae‡	5.1	5.9	80.2	74.1
	Lumbricidae‡	19.8	7.8	15.9	9.2
	others‡	1.9	6.9	2.7	0.01
	unassigned‡	73.2	79.4	1.2	16.7
Rotifera		11.9	13.5	n.p.	n.p.
Tardigrada		8.2	2.1	0.08	0.04
Unassigned Metazoa		2.1	7.4	5.1	6.8

Notes. ⁻percentage refers to the total number of sequences/ASVs assigned to Nematoda; †percentage refers to the total number of sequences/ASVs assigned to Arthropoda; ‡percentage refers to the total number of sequences/ASVs assigned to Annelida; n.p.: not present.

diversity analyses, averaged rarefied tables were generated randomly subsampling the dataset (100 iterations) at a sampling depth selected based on rarefaction curves and computing with the QIIME2 repeat-rarefy plugin (Yao, 2021) the average count of each ASVs (sampling depth of each dataset: Metazoa, 8607 sequences; Nematoda, 3059 sequences; Arthropoda, 1303 sequences; Annelida, 2514 sequences; Arachnida, 2012 sequences). On the rarefied datasets and on the trophic guild dataset obtained from the rarefied metazoan dataset, diversity analyses were performed within the statistical framework of Hill numbers that estimates the number of species equivalents (SEs) in a community (i.e., the number of equally abundant species in a community with the same diversity as the investigated one) allowing the consideration of the influence of ASVs' abundance on the diversity estimates, using the order parameter q (Hill, 1973; Jost, 2006; Alberdi and Gilbert, 2019a; Roswell et al., 2021). Differences in the diversity estimated for habitats were tested with the Kruskal-Wallis test (Kruskal and Wallis, 1952). Hill numbers diversity estimates per-sample and dissimilarity measures of beta diversity were computed with the R library hilldiv (Alberdi and Gilbert, 2019b) using the averaged rarefied ASVs tables. In order to look for differences in the composition of soil invertebrate fauna of the different habitats the PERMANOVA (Anderson, 2014) and ANOSIM (Clarke, 1993) tests were performed on dissimilarity measurements of beta diversity; since these methods may confound location and dispersion effects (Anderson, 2001), the PERMDISP test (Anderson, 2006) was also performed to specifically test for differences in the dispersion between groups. To assess the role of agronomic practices in driving invertebrate diversity, a linear model for each of the considered taxonomic and trophic groups was fitted and the results compared using the standardized regression coefficients. Finally, to compare the relative abundances of different groups taking into account the sparse compositional nature of metabarcoding data, a robust Aitchison PCA was applied using the tool DEICODE on QIIME2 (Martino et al., 2019) to calculate log-ratios and differential ranks of ASVs assigned to different trophic guilds.

3. Results

3.1. Taxonomic composition of the communities

A total of 7,896,662 (mean per sample = 98,709) and 10,198,450 (mean per sample = 125,907) raw reads were obtained from the sequencing of 18S rRNA and COI gene, respectively (deposited on the NCBI SRA database, project PRJNA899435). After the denoising, filtering and chimera removal steps, the 18S rRNA dataset consisted of 13,032 amplicon sequence variants (ASVs) (total reads = 4,012,717, average reads per sample = 50,159) and the COI dataset consisted of 74,356 ASVs (total reads = 6,952,983, average reads per sample = 85,839). In the length filtering step, 17,091 COI ASVs were filtered out and 903 more ASVs were eliminated since lacking the open reading frame, leaving the COI dataset composed of 56,362 ASVs (total reads = 6,107,264, average reads per sample = 76,340).

Most of the obtained 18S rRNA sequences belong to Metazoa, while only a few sequences belong to non-target taxa or cannot be assigned to a specific taxon (Table 1). With this marker, the most abundant Metazoa phylum detected was Nematoda, with the majority of the ASVs assigned to the orders Dorylaimida, Tylenchida and Rhabditida. The second most abundant phylum was Arthropoda (prevalently represented by Arachnida, Collembola and Insecta), while Rotifera, Annelida and Tardigrada were detected only with low relative abundance. Regarding COI, about half of the sequences obtained were assigned to Metazoa, whereas the others were assigned to Fungi or remained unassigned (Table 1). The most abundant Metazoa phylum was Arthropoda, mostly Arachnida, only a few representatives of Insecta, Collembola, Chilopoda, Diplopoda, and Diplura were detected. The second most abundant phylum was Annelida, mainly represented by Enchytraeidae and Lumbricidae.

Several invertebrates genera strictly associated with specific habitats were identified by performing the indicator species analysis on the 18S rRNA Metazoa dataset (indicator genera associated with an IndVal-A $\geq$ 0.95 and p-value $\leq$ 0.05 are reported in Table 2, while all the assigned genera with a p-value $\leq$ 0.05 are in the Supplementary Table S4).

3.2. Diversity and composition of the invertebrate communities in the different habitats

The habitat type in which the highest values of metazoan diversity were registered is the grassland, but with diversity levels that are not significantly different from those of woodland and alfalfa (Fig. 2A). Intermediate diversity values were recorded for the vineyard, followed by rice paddy and finally cornfield which had the lowest diversity estimate (Fig. 2A). When considering the main invertebrate groups detected within these habitats, Nematoda diversity was higher in the grassland, and lower in the cornfield, together with rice paddy (Fig. 2C), while Arthropoda diversity does not vary much among habitats but the higher values were recorded in the grassland and vineyard, and lower in the cornfield (Fig. 2D). Arachnida diversity does not change significantly among habitats but rice paddy had the higher diversity (Fig. 2E). Finally, the highest diversity levels for Annelida were registered in the rice paddy and cornfield, and the lowest in the alfalfa and woodland

Table 2
Indicator genera for each habitat/group of habitats. Only genera with IndVal-A > 0.95 and p-value < 0.05 are shown (see Supplementary Table S4 for the full results).

Habitat/s	Indicator genera
Woodland	Annelida: *Mesenchytraeus*, *Achaeta*, Acari: *Trachytes*, *Xenillus*. Chilopoda: *Cryptops*. Insecta: *Cephennium*, *Oreogeton*. Nematoda: *Domorganus*, *Baldwinema*, *Cephalenchus*, *Tylolaimophorus*
Grassland	Nematoda: *Globodera*, *Gracilacus*, *Nygolaimus*, *Tripylina*. Tardigrada: *Acutuncus*, *Macrobiotus*.
Alfalfa	Acari: *Phorytocarpais*
Rice paddy	Annelida: *Bothrioneurum*. Branchiopoda: *Wlassicsia*. Insecta: *Stenolophus*, *Carpelimus*, *Culicoides*, *Ochlerotatus*, *Laodelphax*. Maxillopoda: *Microcyclops*. Gastropoda: *Physella*. Nematoda: *Propanagrolaimus*, *Rhabdolaimus*, *Epitobrilus*. Nemertea: *Prostoma*. Rotifera: *Limnias*, *Ascomorpha*
Vineyard	Pseudoscorpiones: *Chthonius*. Collembola: *Protaphorura*. Insecta: *Ectopsocopsis*. Nematoda: *Xiphinema*
Woodland + Grassland	Annelida: *Hrabeiella*. Nematoda: *Bunonema*. Rotifera: *Didymodactylos*. Tardigrada: *Mesobiotus*.
Woodland + Alfalfa	Nematoda: *Steinernema*
Woodland + Rice paddy	Acari: *Naiadacarus*. Diplopoda: *Polydesmus*
Grassland + Rice paddy	Nematoda: *Meloidogyne*
Grassland + Vineyard	Nematoda: *Paraphelenchus*, *Tylocephalus*, *Ogma*, *Cervidellus*
Alfalfa + Rice paddy	Nematoda: *Psilenchus*
Alfalfa + Cornfield	Annelida: *Hemienchytraeus*
Alfalfa + Vineyard	Collembola: *Folsomides*. Acari: *Terpnacarus*. Nematoda: *Zygotylenchus*
Rice paddy + Cornfield	Nematoda: *Distolabrellus*
Woodland + Grassland + Vineyard	Nematoda: *Mesocriconema*
Woodland + Alfalfa + Vineyard	Acari: *Protoribates*
Alfalfa + Rice paddy + Cornfield	Collembola: *Isotomurus*
Woodland + Grassland + Alfalfa + Vineyard	Nematoda: *Prismatolaimus*
Woodland + Grassland + Rice paddy + Vineyard	Nematoda: *Microdorylaimus*
Alfalfa + Rice paddy + Cornfield + Vineyard	Nematoda: *Irantylenchus*
Woodland + Grassland + Alfalfa + Cornfield + Vineyard	Nematoda: *Aphelenchoides*

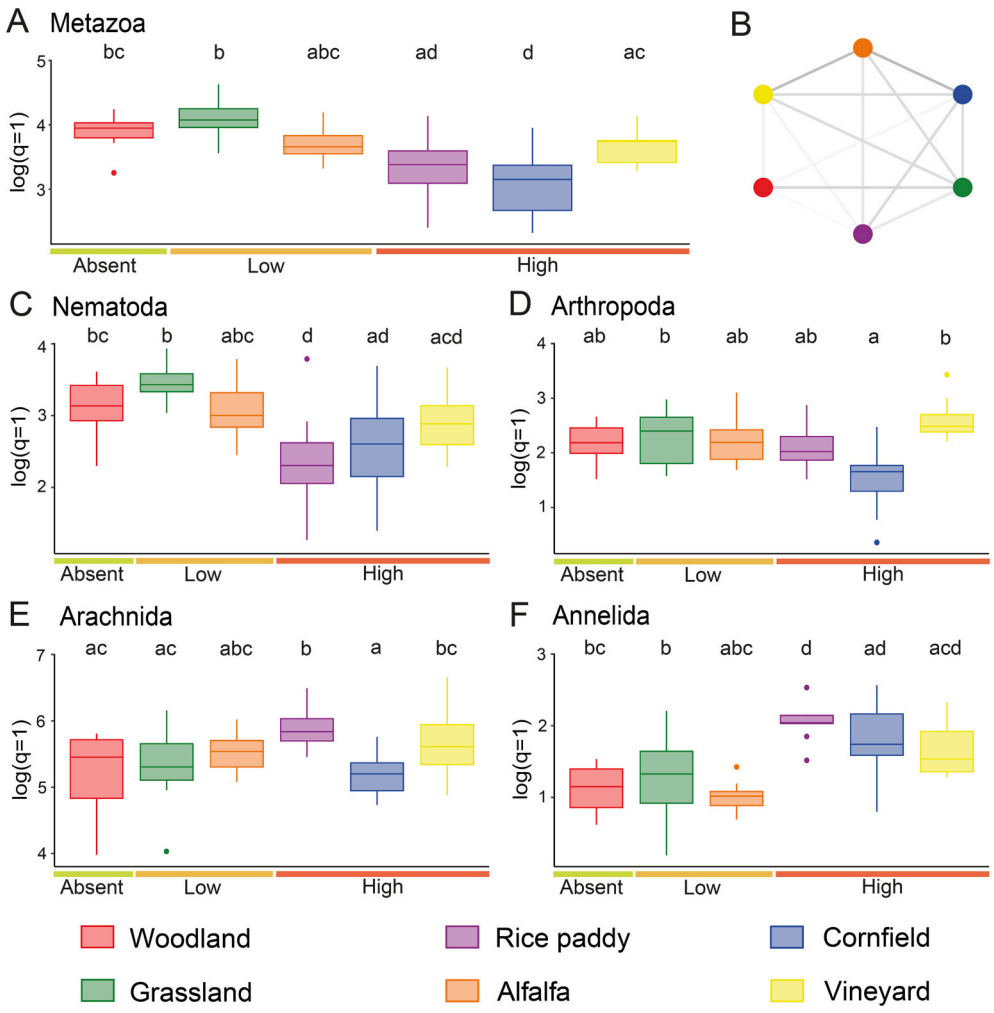

Fig. 2. Taxonomic diversity estimates. Each group of boxplots shows the diversity of a single taxonomic group in different habitats considered; (A) = Metazoa, (C) = Nematoda, (D) = Arthropoda, (E) = Arachnida, (F) = Annelida. Habitats are identified by different colors and ordered along the x axis based on the average disturbance intensity they are subjected to, i.e., absent (DI = 0); medium (DI < 0.5); high (DI > 0.5). The y axis represents the logarithm of the alpha diversity estimated using Hill's numbers with the order parameter q = 1 (log(q = 1)). Letters over the boxplots are used to indicate the differences between groups (estimated through Kruskal-Wallis, Dunn's post-hoc test).
(B) Network of the community composition of the habitats. The width of the links corresponds to the similarity (Sørensen-type overlap) between the different habitat communities.

(Fig. 2F).

PERMANOVA, ANOSIM and PERMDISP on the Metazoa dataset (Supplementary Table S5) showed that the soil communities' composition differs among the habitats (Fig. 2B). Considering only the presence-absence of ASVs (q = 0), the most similar communities were those of alfalfa and cornfield (1-C_{qN} = 0.70, ANOSIM R = 0.36), while woodland hosted the most unique fauna, sharing few ASVs with the majority of the habitats (1-C_{qN} > 0.86, ANOSIM R > 0.87), but having ~22 % of the ASVs in common with the grassland (1-C_{qN} = 0.78, ANOSIM R = 0.51). Increasing the q parameter value (q = 2), the differences among habitats were reduced but remained significant (0.53 < 1-C_{qN} < 0.82). In this case, the most unique fauna was the cornfield one (1-C_{qN} > 0.71, ANOSIM R > 0.54) that shared a significant proportion of dominant ASVs with the alfalfa only (1-C_{qN} = 0.55, ANOSIM R = 0.3).

3.3. Diversity of the trophic guilds in the different habitats

The lowest carnivore diversity was recorded in the cornfield, while all the other habitats (except alfalfa) had significantly higher diversity levels of this trophic guild (Fig. 3A). Cornfield also had the lowest diversity of herbivores, while a significantly higher diversity for this trophic guild was recorded in grassland (Fig. 3B). Bacterivore diversity was significantly higher in the semi-natural habitats (woodland and grassland) than the others (Fig. 3C). Microbivore diversity reach the highest levels in woodland and the lowest in cornfield (Fig. 3D). The diversity of detritivores, omnivores and fungivores did not significantly change in different habitats (Fig. 3E, F, G, respectively).

3.4. Influence of agronomic practices on the communities' diversity

Fertilization showed a positive significant impact on Annelida diversity (Fig. 4) while regarding the trophic guilds, it was negatively related to the diversity of microbivores and positively to that of bacterivores and omnivores (in the latter case with the nearly significant *p*-value of 0.055; Fig. 5). An opposite pattern was recorded for these trophic guilds when considering tillage, in fact, it had a positive impact on microbivore diversity and a negative one on bacterivore and omnivore diversity, in the last case again almost significant (p-value = 0.065; Fig. 5). Tillage was also negatively related to the diversity of Metazoa in general, but positively related to the diversity of Arachnida alone (Fig. 4). The use of insecticides had a strong negative relation with the diversity of Metazoa and specifically with Annelida (Fig. 4). A strong

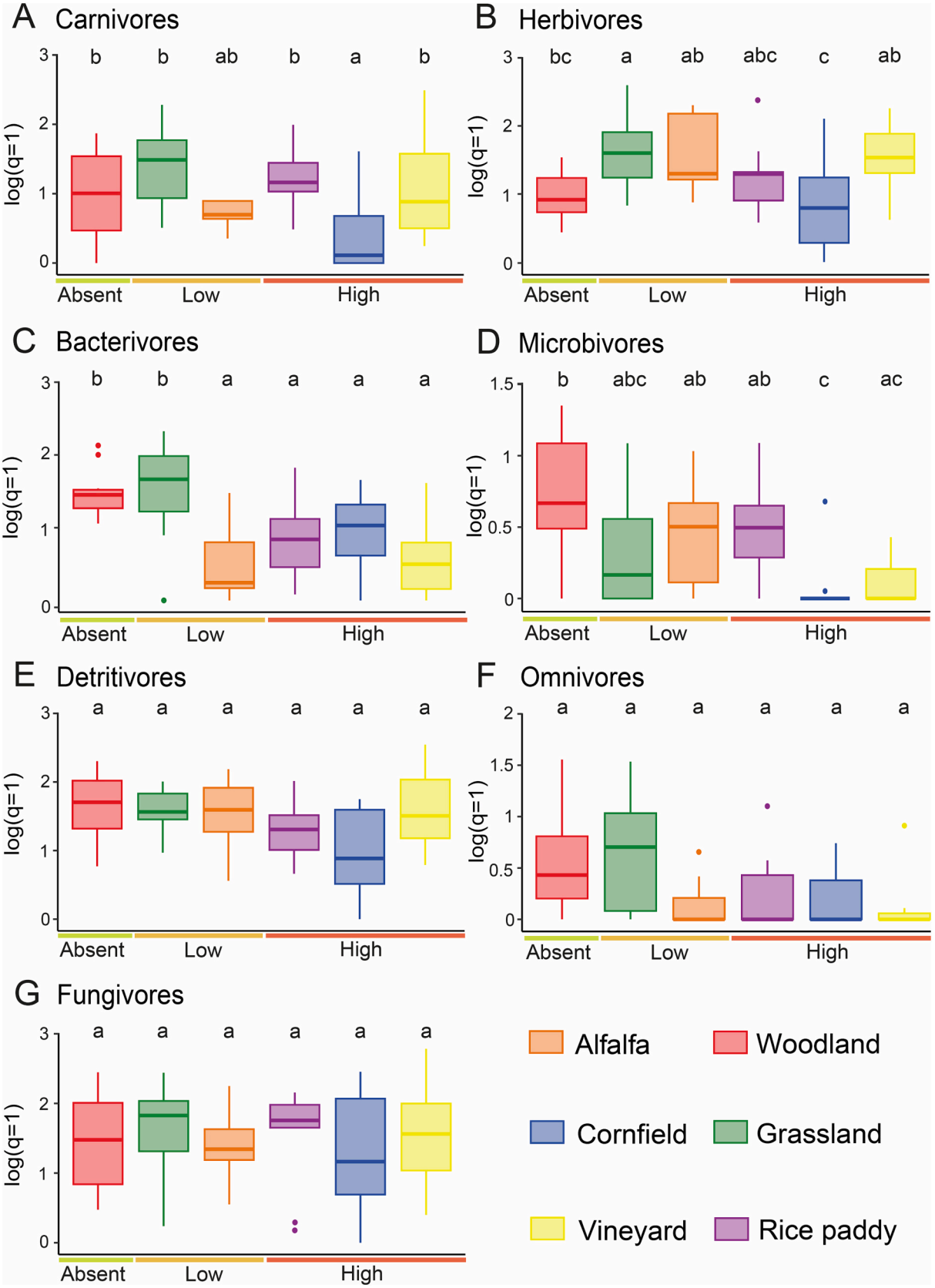

Fig. 3. Functional diversity estimates. Each group of boxplots shows the diversity of a single trophic guild in different habitats considered; (A) = carnivores, (B) = herbivores, (C) = bacterivores, (D) = microbiovores, (E) = detritivores, (F) = omnivores, (G) = fungivores. Habitats are identified by different colors and ordered along the x axis based on the average disturbance intensity they are subjected to, i.e., absent (DI = 0); medium (DI < 0.5); high (DI > 0.5). The y axis represents the logarithm of the alpha diversity estimated using Hill's numbers with the order parameter q = 1 (log(q = 1)). Letters over the boxplots are used to indicate the differences between groups (estimated through Kruskal-Wallis, Dunn's post-hoc test).

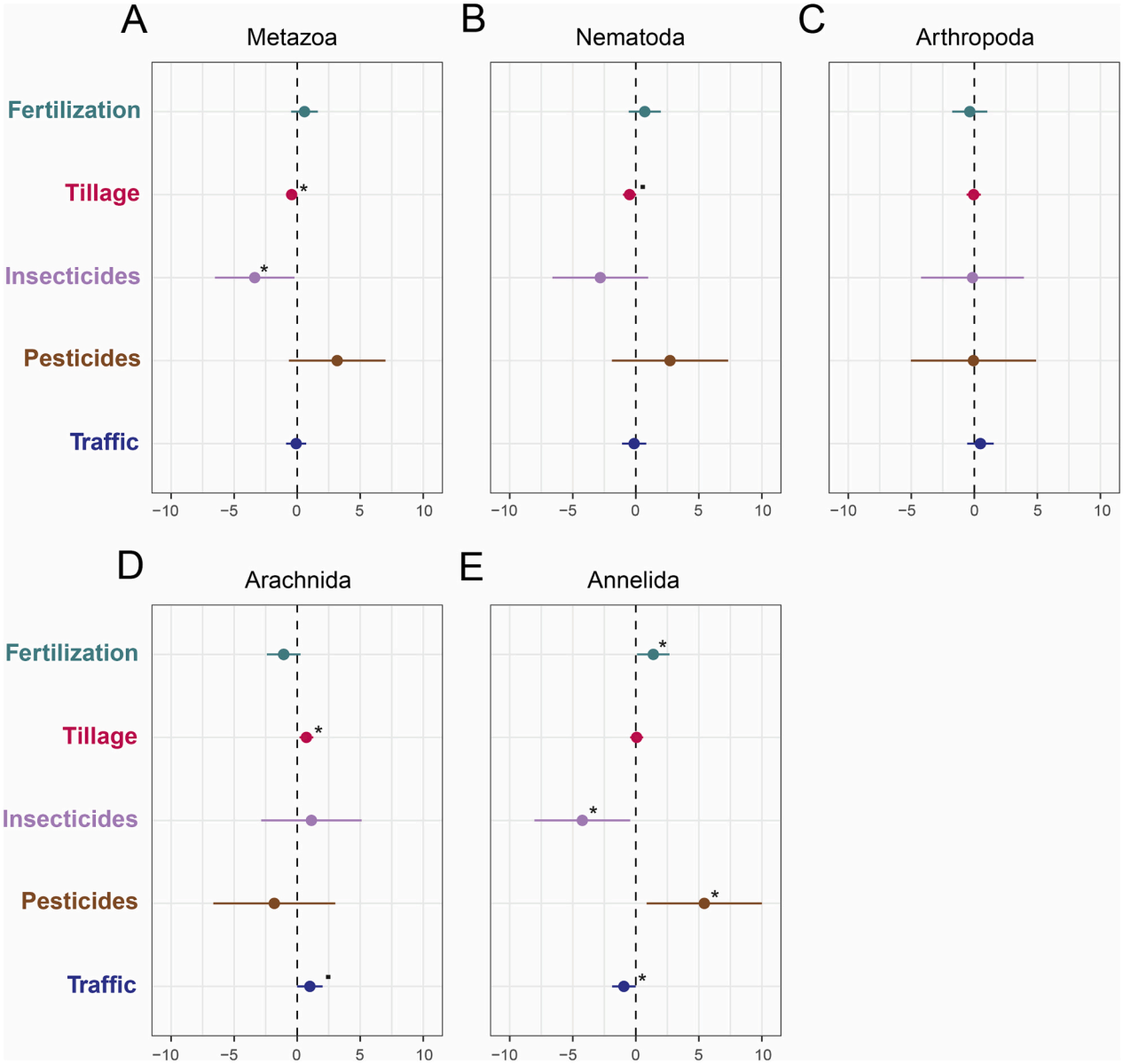

Fig. 4. Influence of agronomic practices on the taxonomic diversity. The forest plots show the parameter estimates (standardized regression coefficients) and 95 % confidence intervals obtained from linear models explaining the effect of agronomic practices on taxonomic diversity. All predictors were scaled to make the estimates comparable. The p-values for each model parameter are given as: $^{\bullet}p < 0.10$; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

negative relation with insecticides was also recorded for the diversity of the trophic guilds, specifically the one of carnivores, omnivores, and bacterivores (Fig. 5). On the other hand, the same taxonomic and functional groups showed an opposite pattern when considering the disturbance due to pesticides, in fact, their diversity was positively related to such kind of disturbance (Fig. 5). The diversity of Annelida was also negatively related to the traffic of machineries, while this kind of disturbance had a positive relation with Arachnida diversity (Fig. 4). The traffic of machineries had also a positive effect on herbivores and microbivore diversity, and a negative one on bacterivore diversity (Fig. 5).

4. Discussion

4.1. eDNA metabarcoding for the soil invertebrate characterization

In this study, the diversity of soil invertebrate communities in different habitats of the intensively farmed area of the Po Valley (Italy) and its relation with adopted agronomic practices was investigated by using an eDNA based approach targeting two commonly adopted genetic markers (i.e., the mitochondrial COI gene and the nuclear 18S rRNA). The results obtained, besides enhancing the knowledge of soil biodiversity of both the semi-natural and farmed components of the area, can be of help for designing future studies aimed at exploring soil invertebrate diversity by using the eDNA metabarcoding approach. In general, the 18S rRNA gene proved to be preferable to COI as a molecular marker for this purpose due to its wider taxonomic coverage with respect to soil taxa, but also because it is less prone to bacterial contamination (Horton et al., 2017). More importantly, it gives the possibility to identify at low taxonomic levels some important soil taxa such as nematodes (Porazinska et al., 2009; Macheriotou et al., 2019), which instead (at least in this study) were not detected using the COI marker. However, the high variability of the COI gene (Andújar et al., 2018; Giebner et al., 2020) coupled with the availability of a large number of reference sequences, proved that this marker is more suitable for the low taxonomic level characterization of other soil groups, e.g.,

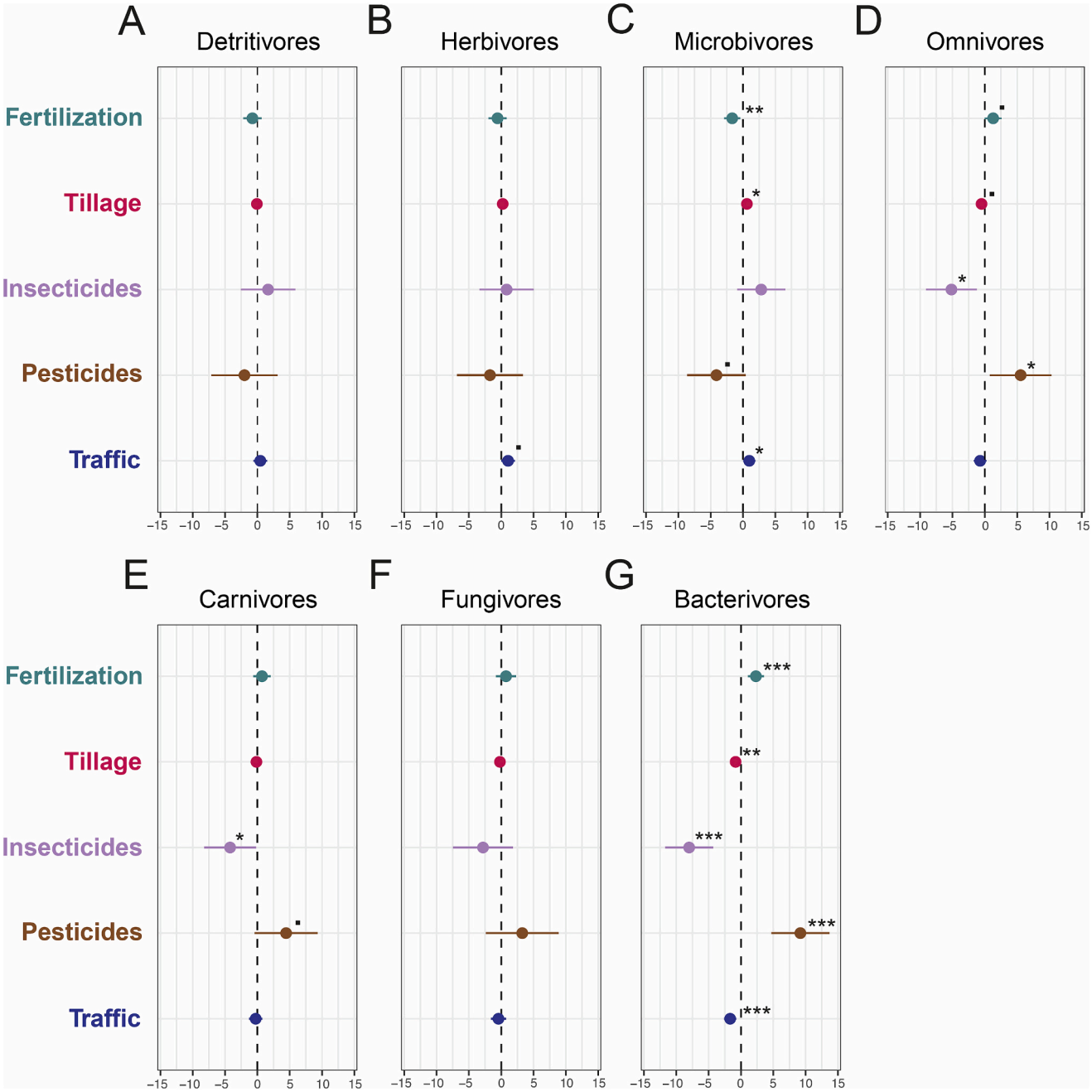

Fig. 5. Influence of agronomic practices on the functional diversity. The forest plots show the parameter estimates (standardized regression coefficients) and 95 % confidence intervals obtained from linear models explaining the effect of agronomic practices on functional diversity. All predictors were scaled to make the estimates comparable. The p-values for each model parameter are given as: •p < 0.10; *p < 0.05; **p < 0.01; ***p < 0.001.

earthworms (Lumbricidae in particular) and insects. Despite the use of two molecular markers, it is likely that a fraction of the soil communities of the considered habitats was uncharacterized, indeed none of the two revealed the presence of isopods (Supplementary Material S3). Besides these results, the need for improving DNA sequence reference databases for soil taxa was evident, due to the impossibility of reaching genus or species level identifications for some groups (e.g., some mites). This lack is not only due to the absence of DNA reference sequences for these animals but also to the fact that only a proportion of all the species living in soils have been described until now (Briones, 2018). Accordingly, morphological taxonomy-based studies on soil invertebrates are the solution for improving the current state.

4.2. Taxonomical and functional invertebrate diversity variation among habitats

The intensification of land use due to human activities is one of the causes of the reduction of soil biodiversity (Tsiafouli et al., 2015; Buhk et al., 2017; Archidona-Yuste et al., 2021; Le Provost et al., 2021). Since soil invertebrate fauna is crucial to soil formation, litter decomposition, nutrient cycling and plant growth and health, understanding the variation of invertebrate taxonomical and functional groups among habitats within intensively farmed landscapes is of importance for assessing soil degradation and related consequences (e.g., ecosystem services losses) (Briones, 2018). In this study, it was found that the soil invertebrate diversity differs among the main habitats in Po Valley, confirming that habitat heterogeneity is an important factor for preventing biotic

homogenization within intensively farmed areas (Vanbergen et al., 2007; Gossner et al., 2016; Maskell et al., 2019). However, some taxonomical and functional groups reached significantly higher diversity in specific habitats suggesting biological soil quality and ecosystem services provision may vary among these habitats (Fig. 2, Fig. 3). Hereafter, the main findings per habitat type are discussed in the context of the investigated area.

Grassland is one of the least human disturbed habitats within the Po Valley, in which human intervention is restricted to mowing, usually two times a year. In this habitat, mainly dedicated to hay production for cattle maintenance, a high variety of herbaceous plants is commonly present especially compared to the surroundings, mainly dominated by a few plant species of agricultural importance (Gardi et al., 2002). This plant diversity is known to enhance the above ground invertebrate diversity, especially the one of insects and other invertebrates interacting with plants (Perner et al., 2005; Hertzog et al., 2016; Petermann and Buzhdygan, 2021). This habitat can be thus considered an above ground biodiversity reservoir within intensive agricultural areas. In contrast, less is known about the soil invertebrate diversity, in particular for the Po Valley which is poorly studied from this perspective (Gardi et al., 2002; Menta et al., 2011). In this study, grassland was found to host the highest invertebrate diversity among the habitats considered, even if not significantly differing from woodland and alfalfa (Fig. 2A). When considering the diversity estimates for specific groups, the highest diversity of nematodes and one of the highest of arthropods were found, while arachnids and annelids were less diverse with respect to other habitats (Fig. 2C, D, E, F). Anyway, this pattern was not mirrored from the functional point of view. In fact, grassland was always among the habitats with the highest diversity for each trophic guild, thus indicating a general high level of resources and/or microhabitats available to soil invertebrates, possibly linked to the high plant diversity (in accordance with Petermann and Buzhdygan, 2021; Fig. 3). Moreover, contrary to what happens in other areas (Hilpold et al., 2018), grassland resulted to host the less unique community with several components shared with the other habitats, thus emphasizing its possible role as a biodiversity reservoir within the investigated area (Fig. 2B).

Woodlands of the Po Valley are usually deciduous forests, fragmented within the agricultural landscape (Stefanelli et al., 2014). Despite the small area they cover, woodlands can play a fundamental role in sustaining both the above and belowground invertebrate biodiversity (Menta et al., 2011), especially allowing preserving some taxa with specific ecological needs. Accordingly, in the present study, woodland was the habitat with the most unique fauna with several taxa exclusively detected in it (e.g., the annelids of the genera *Mesenchytraeus* and *Achaeta*, the mites of the genera *Trachytes* and *Xenillus*, the nematodes of the genera *Domorganus*, *Baldwinema*, *Cephalenchus* and *Tylolaimophorus*; Table 2). Woodland was also one of the habitats with the highest diversity of nematodes, together with grasslands and alfalfa (Fig. 2C), a result that is in contrast with a recent study where a higher diversity of nematodes in the croplands compared to woodland and grassland European soil was detected (Köninger et al., 2023), but in accordance with studies reporting that nematode diversity negatively correlates with the intensification of land use (e.g., Kimenju et al., 2009). Moreover, previous studies found a positive relation between the C/N ratio and nematode diversity (Renčo et al., 2020, Ilieva-Makulec et al., 2015; Mulder and Maas, 2017), similarly to what was found in this study (woodland and grassland were in fact the two habitats with the highest C/N ratio). This high diversity was also reflected in the trophic guilds, in fact both woodland and grasslands had a significantly higher diversity of bacterivores (mainly driven by nematodes) (Fig. 3C). A high abundance of bacterivorous nematodes is known to enhance microbial turnover and nitrogen mineralisation (Forge and Simard, 2001; Djigal et al., 2004), however, considering the results of this study (woodlands and grasslands soil had the highest nitrogen concentrations), it is likely that also a high diversity of this group can promote them.

Legumes are used for the traditional crop rotations in the Po Valley area, a practice adopted for preventing soil degradation, against pests and plant diseases and for enhancing biodiversity (Cortignani and Dono, 2020; Samaddar et al., 2021). Among the legumes used for this aim, one of the most widespread is the multiannual alfalfa that allows preserving simultaneously soil quality and biodiversity in the cropping areas, thanks to its high capacity to increase the nitrogen available in soil (due to the radical symbiosis it establishes with nitrogen-fixing bacteria), but also for the limited management needed for its maintenance (low mechanical disturbance and chemical input) (Song et al., 2021; del Portillo et al., 2022). In the present study, within alfalfa fields, an intermediate level of soil invertebrate diversity compared to the other habitats was found (Fig. 2A). Moreover, a community composition mainly similar to the one of vineyards and cornfields and, to a lesser extent, of grasslands was observed, suggesting that in alfalfa a transitional soil invertebrate diversity between cropping and semi-natural systems is present. This result is in line with the alfalfa role of soil restoration system after the agricultural intensive use.

Italy is among the leading wine producer in the world (Costantini et al., 2016), and the Po Valley significantly contribute to this primacy. Similarly to alfalfa, also in vineyard an intermediate value of invertebrate diversity was registered (Fig. 2A). However, it was one of the habitats with the highest diversity of arthropods (Fig. 2D). Since within arthropods numerous species associated with plants are present, this result suggests that vineyards, that hosts both herbaceous (wild herbs or cultivated ones used as cover crops in the inter-rows) and shrubby/arboreal vegetation (*Vitis vinifera*), could be seen as a sort of transition environment where several microhabitat and ecological niches available for plant associated organisms living in soil are present.

Besides the results of this study that refers to vineyards in general, further studies aimed at improving the knowledge on the soil invertebrates taxonomical and functional composition of each specific wine cultivar are required since soil characteristics they contribute to maintain are one of the most important factors supporting wine quality and unicity (Giffard et al., 2022; Gobbi et al., 2022).

Po Valley is the main area of rice production in Europe, that also represents its most profitable cultivation (Zampieri et al., 2019). However, the agronomic practices adopted in this cropping system are particularly invasive with respect to the environment, having a high impact on soil properties and consequently soil fauna (Blengini and Busto, 2009; Korobushkin et al., 2019). In this study, rice paddy was one of the habitats with the lowest soil invertebrate diversity followed only by cornfield (Fig. 2A). When considering the diversity of specific taxa, the less diverse nematodes fauna was observed, while in contrast, it hosted one of the highest of arachnids (higher abundance of Araneae over Acari) and annelid diversity (Fig. 2C, E, F). The community composition of this habitat mainly differs from the others considered in this study because of the presence of a fauna typically associated with humid environments (e.g., Brachiopoda, Maxillopoda and Ostracoda). While another consistent part of the soil community is shared with the most intensively farmed sites (vineyards and cornfields). Likely both the taxonomic diversity and community composition observed in this habitat are affected by flooding that is known to strongly shape soil fauna making a significant environmental selection for invertebrates with certain traits (e.g., large body size, flooding resistance, high mobility allowing to re-colonize the environment after drying; Ausden et al., 2001). In accordance with the results of the present study, among taxa known for being resistant to flooding are Araneae and Annelida (Ausden et al., 2001; Steward et al., 2017), while some nematodes are strongly affected by it (Cesarz et al., 2017; Galeng-Lawilao et al., 2018).

Almost all the corn of Italian origin is produced in the Po Valley, and it has mainly a zootechnical use (Kayad et al., 2021). However, in the last years, corn production in the area has been strongly reduced, for many reasons including policies for the mitigation of soil degradation. Po Valley in fact is one of the European areas more severely affected by soil erosion, and cornfield is the crop that mostly contributes to this type of degradation (Panagos et al., 2016). As mentioned, cornfield resulted

to be the habitat with the lowest soil invertebrate diversity, but not significantly differing from rice paddy (Fig. 2A). Together with the latter habitat, it was one with the highest diversity of annelids (Fig. 2F) that in general are considered beneficial to ecosystems since they enhance the nutrient contents and improve microbial activity in the soil (Sharma et al., 2017), but on the other hand in specific context these invertebrates can also enhance soil erosion (Orgiazzi and Panagos, 2018). Interestingly, concerning trophic guilds, cornfield was also the habitat associated with the lowest carnivore and herbivore diversity (Fig. 3A, B), but with the highest and significantly different herbivores/carnivores ratio with respect to the other habitats investigated. Altogether, the latter results let us hypothesize that pest control ecosystem service mediated by invertebrates in this habitat is reduced with respect to the others (Supplementary Fig. S1).

4.3. Influence of agronomic practices on the community diversity

Besides the habitat characteristics, soil community composition and diversity within a specific habitat are influenced also by the level of disturbance to which it is subjected (Montagna et al., 2018; Köninger et al., 2023). In previous studies contrasting patterns of soil biodiversity variation in response to disturbance were observed (Tsiafouli et al., 2015; Orgiazzi and Panagos, 2018; Köninger et al., 2023). This evidence together with the results of many experimental studies focused on specific soil organisms (e.g., those rewired in Menta, 2012) suggest that the disturbance influence is taxon-dependant and, more specifically, different soil taxa respond idiosyncratically to the various types of disturbances. In the present study, the influence of the disturbance related to agricultural activities (considered as the sum of the disturbances caused by each single agronomic practice adopted in a site) on soil invertebrates was investigated and a direct relation between diversity and disturbance severity was not observed, neither in general for invertebrates nor for lower taxa (Fig. 2A). However, tillage, insecticides, pesticides, machinery traffic and fertilizers taken individually were found to affect the diversity of specific taxa (Fig. 4) as well as the one of at least one group within the trophic network (Fig. 5). This result suggests each of the considered practices has the potential to change the trophic network equilibrium within the habitat.

Among those tested, only two agronomic practices have been found to significantly impact the diversity of invertebrates as a whole, i.e. tillage and insecticides use, both negatively (Fig. 4). However, previous studies found that tillage does not always have a detrimental impact on soil invertebrates, but its influence varies based on the organism body size (bigger size organisms are more sensitive to tillage) and ecology and, depending on its frequency and intensity (Li-Li et al., 2013; Coulibaly et al., 2002; Jacobsen et al., 2022). While intensive tillage can physically damage the organisms, disrupt their habitat or expose them to harsh environmental conditions and predators (Giller et al., 1997), low levels of tillage can enhance soil taxa diversity by alleviating soil compaction and allowing the incorporation of crop residues in soil which serve as food resource for many soil taxa (Chan, 2001; Ricci et al., 2015; Coulibaly et al., 2002). Besides the negative relation with invertebrate diversity (Fig. 4A), in the present study tillage was also found to enhance arachnid diversity (Fig. 4D). This result is in accordance with previous studies reporting that some taxa within this class are favoured by tillage, but also in contrast with others referring the opposite pattern (Jorrín and González-Fernández, 2016; Khan et al., 2017; Kladivko, 2001). This discrepancy is likely related to the high heterogeneity of ecological traits of the species included in arachnids, and also to the different effects that tillage can have on the different soil types, making difficult to draw general conclusions (Capelle et al., 2012). A further result of this study is that microbivore diversity significantly increases and bacterivores one significantly decreases following tillage intensity (Fig. 5C, G). It is known that tillage by physically mixing soil causes a shift in the composition of microbial communities, favouring bacterial growth to the detriment of fungal one (Hendrix et al., 1986). In accordance, our results suggest that tillage can change the food web composition by favouring the presence of generalist microbial feeders (i.e., the microbivores) that can adapt to the shift in the microbial community periodically caused by tillage, rather than specialist microbial feeders (i.e., the bacterivores). Besides tillage, also traffic disturbance revealed the same pattern, suggesting that also other physical disturbances to soil cause the same alterations of the microbial feeding taxa diversity within the trophic network (Fig. 5C, G). However, in the latter case, a stronger reduction of bacterivore diversity is observed, possibly due to the additional impact of soil compaction on the bacterial community (Weisskopf et al., 2010). Regarding insecticides, the results of the present study are in accordance with current knowledge considering them, among pesticides, those with the higher impact on soil invertebrate abundance and diversity (Beaumelle et al., 2023). Nevertheless, we also observed a tendency toward a diversity increase when the combined effect of different pesticides on soil taxa diversity was evaluated (Fig. 4). Noteworthy is the case of annelids, being among the taxa considered in this study the one for which the strongest diversity decrease in relation to insecticides use was registered, but whose diversity was found to significantly increased in relation to pesticides use (Fig. 4E). The latter result may be related to the increased dead and decaying organic matter in the litter, on which annelids feed, after herbicide and fungicide application. Previous studies report a boost of soil bacterial biomass and activity following some pesticides application (Lo, 2010; Arora and Sahni, 2016), which may be due again to an increase in dead and decaying organic matter lying on the soil. Congruently, in this study, an increase in bacterivore diversity was observed in relation to pesticide use (Fig. 5G).

Even the use of fertilizer was found to significantly affect some taxonomic and functional group diversity. In detail, annelid diversity was enhanced by the use of this practice, likely because fertilizers can be directly exploited by them as food (Tiwari, 1993; Curry and Schmidt, 2007; Fig. 4E), and also the one of bacterivores that possibly follow the increase of bacterial abundance and growth stimulated by fertilization (Niu et al., 2023; Fig. 5G). A further finding of interest regards the herbivorous-carnivorous ratio that significantly increased in relation to fertilizers application, especially nitrogen-based ones, suggesting that fertilization, besides enhancing plant growth can favour plant enemies' abundance to the detriment of their predators one.

5. Conclusions

The results obtained in this study suggest that, despite the protracted intensive land use that the Po Valley soils experienced over decades, a homogenization of the soil fauna within the area has not occurred. In fact, significant differences in the composition of soil communities were detected not only between the semi-natural habitats and the cropping systems but also between the different cropping systems. Interestingly, significantly higher diversity of some taxonomical and functional groups was observed in specific habitats suggesting biological soil quality and ecosystem services provision may vary among them, but the highest diversity levels registered were not always related to the lowest disturbance by agronomic activities. Nevertheless, the disturbance related to the most commonly adopted agricultural practices in the area (tillage; insecticides, pesticides and fertilizers use; machinery traffic induced disturbance) were found to affect the diversity of at least one among the main soil taxonomical and functional groups present there, sometimes also leading to a diversity increase. However, this increase in the diversity of some groups should be not seen as a positive consequence of agronomic activities on soil invertebrates, but indeed attributed to the impaired community balance that they cause within the habitat.

Finally, the results obtained in the present work further highlighted the usefulness of eDNA metabarcoding in characterizing the biodiversity associated with agricultural soils and its effectiveness in describing the relation between biodiversity, habitat features, and agronomic practices. The strengths and weaknesses of this approach highlighted by the

present study can also help to delineate the effort needed for using it as an innovative monitoring technology for agroecosystem biodiversity aimed at achieving sustainable production.

CRediT authorship contribution statement

Matteo Brunetti: Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing, Data curation, Validation, Visualization. **Giulia Magoga:** Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing, Validation, Investigation. **Alex Cussigh:** Validation. **Sumer Alali:** Formal analysis, Investigation, Methodology. **Flavia Pizzi:** Formal analysis, Validation. **Paola Cremonesi:** Formal analysis, Validation. **Ilaria Di Lelio:** Validation. **Andrea Becchimanzi:** Validation. **Roberto Comolli:** Formal analysis, Methodology, Validation. **Pietro Marino Gallina:** Formal analysis, Methodology, Validation. **Gustavo Gandini:** Methodology. **Alberto Spada:** Formal analysis, Methodology, Investigation. **Matteo Montagna:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interest.

Data availability

I have reported the link where the data are available in the manuscript

Acknowledgments

Authors would like to thank Alessandro Ferri and Matteo Piombino (Corteva Agriscience – IT) for the local support with farmers, Alessandro Valiani and Alessandro Heinzel for their contributions in fieldwork activities. This work was partially supported by the following: i) the Research Support Plan 2015-2017 of the University of Milan (Molecular Biological Index of Soil Quality–MoBioS); and ii) the European Union Next-GenerationEU [PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022]. This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105326.

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