



Land use intensity differently influences soil communities across a range of arable fields and grasslands

Chenguang Gao^{a,1,*}, Johanna E.M. Schild^{b,1}, Gabriel Y.K. Moinet^c,
T. Martijn Bezemer^d, Franciska T. de Vries^e, Jan Hassink^f, Nick van Eekeren^g,
Kevin Beentjes^h, Peter M. van Bodegom^a

^a Environmental Biology, Institute of Environmental Sciences, Leiden University, Einsteinweg 2 2333 CC Leiden, the Netherlands

^b PBL Netherlands Environmental Assessment Agency, Bezuidehousweg 30 2594 AV The Hague, the Netherlands

^c Soil Biology Group, Wageningen University, Droevendaalsesteeg 3 6708PB Wageningen, the Netherlands

^d Institute of Biology, Above-Belowground Interactions Group, Leiden University, Sylviusweg 72 2333 BE Leiden, the Netherlands

^e Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904 1090 GE Amsterdam, the Netherlands

^f Wageningen Plant Research, Wageningen University & Research, Droevendaalsesteeg 1 6708PB Wageningen, the Netherlands

^g Louis Bolk Institute, Kosterijland 3-5 3981 AJ Bunnik, the Netherlands

^h Naturalis Biodiversity Center, Darwinweg 2 2333 CR Leiden, the Netherlands

ARTICLE INFO

Handling Editor: Y. Capowiez

Keywords:

Agricultural practices
Co-occurrence networks
Regional study
Soil biodiversity
Soil microorganisms

ABSTRACT

While there is an emerging body of research showing the consequences of land use intensity on soil biodiversity, most studies focus on biodiversity responses to a single or a limited number of agricultural practices in controlled settings or at a single field site, neglecting that multiple practices are simultaneously applied by farmers in real agroecosystems. The combined effects of various agricultural practices have, until now, been largely overlooked in agroecosystems.

Here, we conducted a field soil sampling campaign on 87 farms with two land use types (39 arable fields and 48 grasslands) to investigate the relationship between land use intensity, determined by various agricultural practices, and multiple soil communities (bacteria, fungi, protists and invertebrates) at regional scales.

We found that land use intensity influenced the diversity and community composition of various soil taxa differently, and these impacts strongly depended on land use type. Soil fungi were most susceptible to land use intensity in both arable fields and grasslands. Specifically, irrigation and pest control were the main practices shaping soil communities in arable fields, while phosphorus and nitrogen fertilization were the main practices structuring soil communities in grasslands. Furthermore, an increase in land use intensity led to greater soil network complexity in arable fields.

Our findings reveal regional on-farm patterns of land use intensity effects on various soil communities and identified key agricultural practices that structure soil communities. A key strength of this study is that these patterns can be generalized because the samples were collected from 87 farmlands where multiple agricultural practices were implemented simultaneously. Overall, this work provides a comprehensive perspective on the different responses of multiple soil communities and their associations to land use intensity in agroecosystems.

1. Introduction

Soil, where our food begins, represents one of the largest reservoirs of biodiversity in terrestrial ecosystems and contain various taxonomic groups, such as bacteria, fungi, protists, and invertebrates (Anthony et al., 2023; Delgado-Baquerizo et al., 2020; Orgiazzi et al., 2016). Land

use intensity, including soil tillage, intensive fertilization and application of pesticides, has been shown to improve crop yields and boost agricultural production to support a continuously growing world population (Hartman et al., 2018), but it also has detrimental impacts, notably on soil health by inducing soil biodiversity loss (Geisen et al., 2019b; Le Provost et al., 2021; Peng et al., 2024). Since soil biodiversity

* Corresponding author.

E-mail address: c.gao@cml.leidenuniv.nl (C. Gao).

¹ Shared first authorship.

is fundamental in supporting multiple ecosystem functions and services (Delgado-Baquerizo et al., 2020; Soliveres et al., 2016; Wagg et al., 2014), understanding the effects of land use intensity on soil communities is crucial for preserving and restoring biodiversity in agricultural soils and enhancing their role in agroecosystem functioning (de Vries et al., 2013; Labouyrie et al., 2023; Postma-Blaauw et al., 2012).

Land use intensity is often accompanied with soil compaction, salinization, acidification, organic matter loss and nutrient imbalance (Hartmann and Six, 2022). These unfavorable changes consequently influence the diversity, community composition and structure of soil communities (Peng et al., 2024; Tsiafouli et al., 2015). An emerging body of studies has indicated that an increase in land use intensity can lead to a decline in soil community diversity and that it can significantly alter their community structure and composition (Jia et al., 2022; Sünemann et al., 2021). For instance, it has been shown that intensive use of pesticide can lead to a decline in community diversity of arbuscular mycorrhizal fungi (Edlinger et al., 2022) and nitrogen-fixing bacteria (Walder et al., 2022). Inorganic fertilization (Betancur-Corredor et al., 2022; Lian et al., 2022; Sabir et al., 2021; Zhao et al., 2020) and conventional tillage (Tsiafouli et al., 2015; van Capelle et al., 2012) can significantly alter soil community structure and composition. In addition, it has been demonstrated that responses to land use intensity can differ greatly among soil organisms (Allan et al., 2014; Guo et al., 2023). Soil bacteria and fungi have been found to respond differently to crop rotation (Ai et al., 2018; Zhang et al., 2014). Fungi are more sensitive to crop management than bacteria in arable fields. Larger organisms are also expected to be affected more strongly by environmental changes than small-sized microbial taxa (Postma-Blaauw et al., 2012).

Although there is an emerging body of research showing the consequences of land use intensity on soil biodiversity, most studies focus on their response to a single or limited number of agricultural practices in controlled experiments or at a single field site (Guo et al., 2020; Legrand et al., 2018), neglecting that multiple practices are simultaneously applied by farmers in commercial agroecosystems. The interaction effect of various agricultural practices has, until now, been largely overlooked. Moreover, given that responses of the belowground biodiversity depend on the prevailing environmental conditions (Feng et al., 2022; Hueso et al., 2012), results examining the impacts of land use intensity at a single site with specific agricultural practices cannot be generalized to a larger scale. This limitation prevents a systematic assessment of the relationship between land use intensity and soil biodiversity across real agroecosystems.

To better understand the changes in soil biodiversity across a gradient of land use intensity, more detailed studies across different land use types in agricultural fields are required that reflect the changes taking place across a variety of landscapes. In that context, it is important to distinguish among land use types (e.g., arable fields or grasslands) that can vary greatly in how diversity and community composition of soil communities are structured due to great differences in the agricultural management, environmental conditions and social-economic factors (Aslani et al., 2024; Labouyrie et al., 2023; Peng et al., 2024; Romdhane et al., 2022). For instance, Szoboszlay et al. (2017) indicated that across Europe, croplands exhibit higher bacterial diversity than grasslands. Studies have also shown that conversion between arable fields and grassland can lead to significant change in the composition and activity of soil community (French et al., 2017; Turley et al., 2020; van Eekeren et al., 2008). Therefore, examining the extent and nature of how land use intensity influence the soil community across different land use types at regional scales can help us to accurately quantify its impacts and adjust sustainable agricultural management tailored to each land use type, thereby mitigating the adverse effects of land use intensity on soil biodiversity.

In addition to its well-known impacts on the diversity and structure of soil communities, land use intensity can also affect the interrelationships among soil organisms and change their associations (Banerjee et al., 2019; Morriën et al., 2017). For instance, long-term

organic fertilization influences soil microbial networks by increasing the diversity of keystone species and network complexity (Ji et al., 2020; Zhao et al., 2023). Importantly, impacts of land use intensity on a specific taxon can initiate cascading effects throughout the entire meta-community, facilitated by species co-variation within these multitrophic associations. More associations are assumed to lead to greater ecosystem functionality because of a more efficient flow of energy and materials through the entire soil community (Chen et al., 2024; de Vries and Wallenstein, 2017). Understanding these multitrophic associations is therefore prerequisite for revealing community assembly and nutrient cycling within the belowground metacommunity. However, most studies have focused on the networks of specific biotic groups (e.g., bacteria), neglecting potential interrelationships among co-evolved domains, such as protists and invertebrates, potentially resulting in incomplete characterizations of belowground community patterns in response to land use intensity and their implications in agroecosystems functionality.

In this study, our aim was to investigate the relationship between land use intensity and soil biodiversity (bacteria, fungi, protists and invertebrates) across different land use types and geographic locations. For this purpose, we examined 39 arable sites and 48 grassland sites embedded in a landscape with various agricultural practices in the east of the Netherlands. Land use intensity was assessed using an integrated land use intensity index (LUI) to quantify the intensity of multiple agricultural practices which were applied by farmers simultaneously. We assessed: (1) how do different soil groups respond to land use intensity in arable fields and grasslands? (2) how does land use intensity influence the network complexity of the soil metacommunity? Our main hypothesis is that different soil taxa will show distinct response patterns to land use intensity, and specifically that land use intensity may exert stronger impacts on higher trophic-level soil organisms (such as invertebrates and protists) (Chassain et al., 2024) compared to bacteria and fungi which have highly flexible metabolism (Liang et al., 2024). Second, we hypothesize that the responses of different soil taxa to land use intensity are not consistent in arable fields and grasslands due to their differences in agricultural practices. We also expect that land use intensity will may inhibit multitrophic interactions (Banerjee et al., 2019; Bloor et al., 2021) and thus reduce the network complexity of soil communities.

2. Materials and methods

2.1. Study design and soil sampling

A total of 87 farms was selected, including 39 arable sites and 48 managed grasslands, in the east of the Netherlands (Fig. S1). At each farm, one site was selected for soil sampling during a 5-week period in May and June 2022. At each site, a sampling area of 10 × 10 m was identified for soil sampling. Each sampling area was located in the field with a buffer zone of at least 20 m from the field edges. The 10 × 10 m square was divided into 100 1 × 1 m plots which were numbered from 1 to 100. Three plots were randomly selected for soil sampling in each sampling area. In each plot, four soil cores were collected (0–25 cm depth), resulting in 12 cores per site which were homogenized and stored together on ice for transport to the laboratory. The soil cores were homogenized again in the laboratory and sieved to 2 mm to remove large roots and stones. Then each soil sample was divided into three parts: one subsample was stored at –20 °C for soil DNA extraction, one subsample was used for measuring soil physical parameters, and another subsample was air-dried for soil chemical analysis (more details in Supplementary methods and Table S1). The results of soil nutrients indicated that the nutrient differences observed are likely linked to differences in fertilization practices rather than intrinsic site characteristics (Table S1).

2.2. Land use intensity index

A detailed outline for determining the land use intensity index was provided in our previous publication (Gao et al., 2024). Briefly, the land use intensity index (LUI) was assessed for each site by means of questionnaires about land management practices at each site. These questionnaires were used to conduct farmers interviews during the field sampling campaign and collect information about specific management practices including the amount of mineral and organic fertilizer use of nitrogen and phosphorus, liming, tillage, irrigation, and pest control for both arable fields and grasslands (Supplementary methods). These practices were common to grasslands and arable fields and were recorded as categories: mineral and organic N application in 2022 (for both: 0; 1–25; 26–75; 76–125; 176; 225; >225 kgN/ha), mineral and organic P fertilization in 2022 (for both: 0; 1–30; 31–60; >60 kgP₂O₅/ha), tillage frequency in 2022 (0; 1; 2; 3; 4; >4 times/season), irrigation (0; 1–40; 41–80; >80 mm/season), pest control (yes; no) and liming (yes; no) in 2022. The variables were converted into normalized indices by assigning numerical values ranging from 0 to 1, where 0 corresponded to the lowest ordinal category and 1 to the highest. In addition, information were collected about crop diversity for arable fields, and about grazing intensity, mowing intensity and plant community biodiversity for grasslands (see more details in Supplementary methods). The land use intensity index was calculated as the sum of individual indices of different practices normalized to the maximum of the sum as detailed in Supplementary methods. The relationships between different variable practices were analyzed (Tables S2–S3). Furthermore, the full variation in LUI values for arable fields and grasslands is presented in Fig. S2.

2.3. Assessment of microbial community composition and diversity

The diversity of soil bacteria, fungi, protists and invertebrates was determined via amplicon sequencing using the Illumina NovaSeq platform (BaseClear, Leiden). As this study aims to analyze macrofauna next to meso- and microfauna, a large soil sample of 15 g was used for extracting genomic DNA. This 15 g soil sample was homogenized with 15 ml of saturated phosphate buffer (Donald et al., 2021; Zinger et al., 2016). A 2 ml sample was taken from this mixture and centrifuged. 0.5 ml of the sample's supernatant was taken to use in the further extraction steps of the QIAGEN DNeasy PowerSoil Pro Kit (Qiagen, Venlo, Netherlands) following the manufacturer's instructions.

The bacterial 16S rRNA and fungal ITS genes were amplified with primer 515F/806R with the updated sequences for the 515-forward primer (Parada) (Apprill et al., 2015; Caporaso et al., 2011; Parada et al., 2016) and ITS3/ITS4 (Tedersoo et al., 2015, 2014; White et al., 1990), respectively. Eukaryotic 18S rRNA were amplified for protists and invertebrates using forward primer IDT_V4f and reverse primer IDT_TAREukREV3 (Bass et al., 2016). 18S was selected as a marker for eukaryotes, since it captures relatively more diversity especially when using the phosphate buffer extraction method (Kirse et al., 2021; Taberlet et al., 2012). The V4-region of 18S is nowadays most commonly used for eukaryotes (Geisen et al., 2019a). PCR products were attached with IDT indexes, followed by beads cleaning and pooling into equimolarity. The end pool was sequenced with an Illumina NovaSeq platform (BaseClear, Leiden) producing 250-bp paired-end sequences. Three extraction blanks and one amplification blank were taken along.

Quality filtering and clustering of raw sequences was performed in a custom pipeline on the OpenStack environment of Naturalis Biodiversity Center through a Galaxy instance (Afgan et al., 2018). First, raw sequences were merged using FLASH v1.2.11 (Magoč and Salzberg, 2011) (minimum overlap 10, mismatch ratio 0.25); non-merged reads were discarded for further analyses. Next, primers were trimmed from both ends of the merged reads using Cutadapt v1.16 (Martin, 2011) (minimum match 5, mismatch ratio 0.2). Then, sequences were dereplicated and clustered into Amplicon Sequence Variants (ASVs) using UNOISE2

(Edgar, 2016), with $\alpha = 2$ and a minimal accepted abundance before clustering of 8 reads.

For taxonomic annotation, ASV sequences were blasted against a custom reference database using an extended BLAST + script (Beentjes et al., 2019) (query coverage cutoff 85 %, identity percentage cutoff 85 %), and processed in a custom lowest common ancestor (LCA) script to identify ASVs to genus level (Beentjes et al., 2019). The LCA script was performed on the top 100 hits, with bit-score > 170, a minimum identity of 85 % and a minimum coverage of 85 %. ITS data was blasted against a local UNITE dataset (Abarenkov et al., 2022). 16S and 18S were compared against local copies of the respective NCBI GenBank (Benson et al., 2005) datasets (downloaded 16–03-2022). Protists are defined as all eukaryotic taxa, except for fungi, invertebrates (Metazoa) and vascular plants (Streptophyta). All ASVs that contributed less than 0.05 % to the total reads of the sample were removed for each sample for downstream analyses. ASVs only occurring in arable fields or grasslands were identified as unique species.

2.4. Statistical analysis

All statistical analyses were conducted using packages in R (v.4.2.3). We selected ASV richness (observed ASV numbers per sample) and Shannon diversity index to investigate changes in alpha diversity of soil organisms. For the overall soil biodiversity, a quantitative index for each site was calculated. Following an approach outlined elsewhere (Delgado-Baquerizo et al., 2019), the diversity of bacteria, fungi, protists, and invertebrates was standardized using the equation: $(\text{rawDiversity} - \text{min}(\text{rawDiversity})) / (\text{max}(\text{rawDiversity}) - \text{min}(\text{rawDiversity}))$, where min = minimum diversity value and max = maximum diversity (richness) value across all samples. The standardized samples were averaged across organism groups.

We used generalized linear models to analyze the relationship between LUI and the Shannon diversity in arable fields and grasslands. Generalized linear models with negative binomial residuals (glmer.nb) were used to analyze the relationship between LUI and the richness of soil organisms because richness values exhibited overdispersion. The relative abundance of each phylum in the different soil groups was calculated based on the total reads of ASVs. The relative abundance data of soil biota were Hellinger pre-transformed to avoid overemphasizing the impacts of rare species (Legendre and Gallagher, 2001). Soil community differences were represented on a two-dimensional ordination plot following NMDS analysis, using the metaMDS function in the “vegan” package (Oksanen et al., 2013). The effects of LUI on the composition of the soil community in arable fields and grasslands were compared using PERMANOVA (function “adonis”) based on a Bray-Curtis dissimilarity matrix (999 permutations) in the “vegan” package. In order to examine the response of soil biotic composition to individual agricultural practices, distance-based redundancy analysis (db-RDA) was performed with Bray-Curtis dissimilarities in the “vegan” package (Oksanen et al., 2013). To statistically evaluate the effects of individual agricultural practices in real farming systems, hierarchical and variation partitioning analysis was applied using “rdacca.hp” package (Lai et al., 2022).

Co-occurrence networks have been increasingly used to unravel relationships between soil organisms (Barberán et al., 2012; Morrién et al., 2017). Although co-occurrence networks might not reflect the true complexity of microbial interactions (Blanchet et al., 2020), this integrative approach can provide complementary insights into the consequences of land use on soil community structure (Banerjee et al., 2019; Hu et al., 2021) and their related functions (Chen et al., 2024; Jiao et al., 2021). Intra- and inter-kingdom co-occurrence networks were constructed using ASVs from bacteria, fungi, protists and invertebrates under different LUI in arable fields and grasslands. To construct the co-occurrence networks with the same number of samples, 39 samples were randomly selected from samples in grasslands (in total 48 samples). A moving average approach was used to ensure robustness, selecting ten

samples each time after ranking all samples by LUI values for network analysis. Only ASVs that summed to a relative abundance $> 0.5\%$ in all samples were retained for network analysis. ASVs numbers present in more than 5 sites were retained and all soil invertebrates given their limited number of ASVs. Co-occurrence networks were established based on Spearman's correlation matrices using the "igraph" package. We focused on ASVs that strongly co-occurred in the network with adjusted cutoffs of $P < 0.05$ and $r > 0.6$. The networks were visualized using a Fruchterman-Reingold layout with Gephi software. The topological properties of each network under two land use types were calculated in Gephi. A highly complex network has a great number of nodes, edges, average degree, clustering coefficient and graph density, and lower values of average path length and betweenness centrality (Jiao et al., 2022). The linear model was applied to evaluate the relationships between land use intensity and the topological properties of network complexity.

3. Results

3.1. Land use type and intensity, and overall soil biodiversity

Alpha diversity of the overall soil community did not vary significantly in response to LUI in both arable fields and grasslands (Fig. S3, Table S4). Among the soil samples collected on the 87 sites, most of the sequences were found to belong to bacteria (50 %), followed by soil fungi (25 %) and soil protists (23 %). Invertebrates (3 %) had the lowest richness across all samples (Fig. S4a). These soil groups showed different occurrences across different land use types. In general, arable soils harbored relatively fewer bacteria and fungi than grasslands, whereas there were more protists in arable fields (Fig. S4b). In addition, 29 % of these ASVs were shared among the two land use types, with the grasslands harboring a higher number of unique species (39 % ASVs)

than arable fields (32 % ASVs). When separated between the soil taxa, grassland soils contained more unique species of bacteria, fungi and invertebrates, except for protists which had more unique species in arable fields (Fig. S4c).

3.2. LUI and the diversity and composition of soil communities

Relationships between the belowground diversity and LUI were not consistent across distinct soil groups and were dependent on land use type (Fig. 1 and Fig. S5). Alpha diversity of soil bacteria and protists communities did not vary significantly in response to changes in LUI in both arable fields and grasslands (Table 1 & Table S5). In contrast, significant effects of LUI were observed for the alpha diversity of the fungi and invertebrates in both arable fields and grasslands, even though the associated R^2 values were relatively low. An increase in LUI significantly reduced the diversity of soil fungi, especially in arable fields. The response of invertebrates diversity to LUI depended on land use type (Fig. 1 & Fig. S5). A significant increase with LUI was shown in the richness of invertebrates in arable fields, whereas increasing LUI led to a reduction in the richness of invertebrates in grasslands (Fig. 1).

In both arable fields and grasslands, multivariate tests using Bray-Curtis dissimilarity matrices revealed that soil bacterial, fungal, protistan and invertebrate community composition differed significantly along LUI gradients in arable fields and grasslands (Table 2 & Fig. S6). In general, soil communities clustered according to land use types and LUI values (Fig. S6). Grassland bacterial, fungal and protistan communities were more divergent compared to arable communities, whereas arable invertebrate communities were more distinct than grassland communities (Fig. S6).

Distinct differences in the relative abundance of the phyla within the four soil groups were observed. Bacterial phyla Proteobacteria, Actinobacteria and Planctomycetes were the most abundant phyla in both

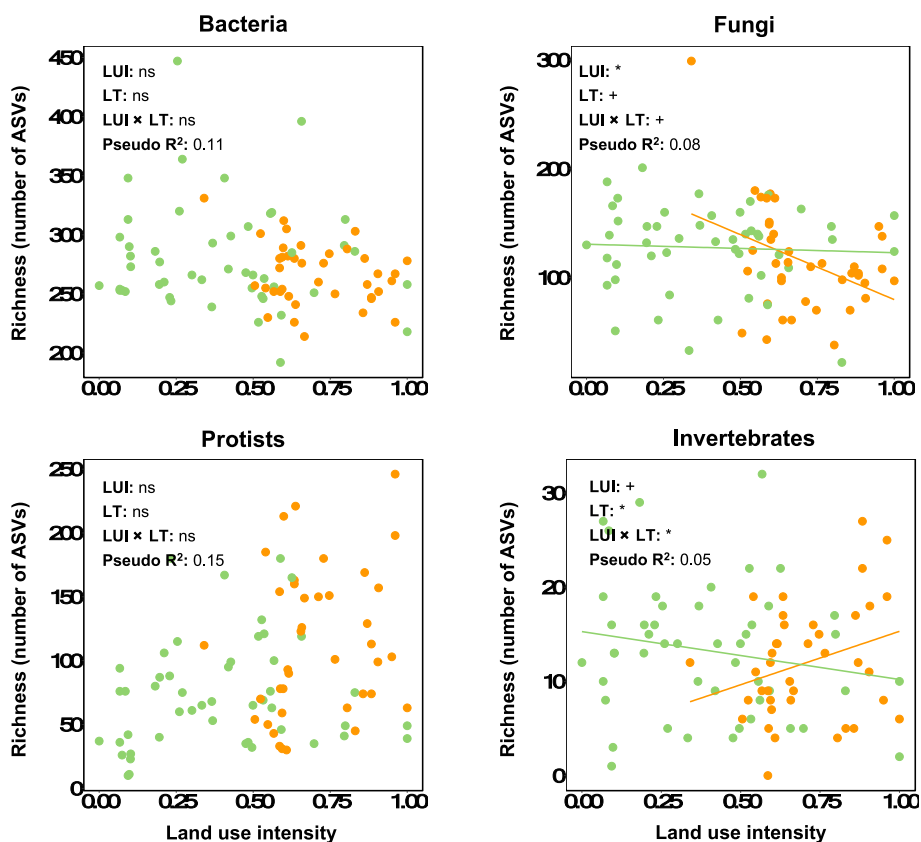


Fig. 1. Effects of land use intensity (LUI) and land use type (LT) on the richness of soil communities. Orange and green dots denote sites in arable fields and grasslands, respectively. "*" and "+" indicate a significant correlation at $p < 0.05$ and $p < 0.10$, respectively.

Table 1
Effects of land use intensity (LUI index, 0–1) and land use type (LT) on the richness of the soil community.

Soil community	Land use intensity (LUI)			Land use type (LT)			LUI × LT		
	Est.	z	p	Est.	z	p	Est.	z	p
Bacteria	−0.09	−0.75	0.456	0.04	0.44	0.661	−0.10	−0.71	0.481
Fungi	−0.89	−2.33	0.020	−0.49	−1.67	0.095	0.83	1.91	0.057
Protists	0.68	1.21	0.227	−0.12	−0.28	0.779	−0.25	−0.38	0.701
Invertebrates	0.92	1.68	0.094	0.92	2.18	0.030	−1.33	−2.13	0.033

Note: Values in bold denote (marginally) significant correlations at $p < 0.10$.

Table 2
Summary statistics of a PERMANOVA testing the effects of land use intensity (LUI), land use type (LT) and their interactions on the community composition of soil bacteria, fungi, protists and invertebrates.

Soil biota	Treatments	df1,df2	F-value	R ²	p-value
Bacteria	LUI	1,83	6.23	0.07	0.001
	LT	1,83	2.07	0.02	0.006
	LUI × LT	1,83	1.70	0.02	0.018
Fungi	LUI	1,83	5.23	0.06	0.001
	LT	1,83	3.44	0.04	0.001
	LUI × LT	1,83	2.08	0.02	0.002
Protists	LUI	1,83	3.69	0.04	0.001
	LT	1,83	2.16	0.02	0.002
	LUI × LT	1,83	1.95	0.02	0.003
Invertebrates	LUI	1,81	4.37	0.05	0.001
	LT	1,81	3.08	0.03	0.001
	LUI × LT	1,81	1.76	0.02	0.032

Note: Presented are degrees of freedom, variance explained (R²), F-values and p-values. Significant effects ($p < 0.05$) are presented in bold.

arable and grassland soils (Fig. 2). Higher LUI values coincided with a significantly lower relative abundance of Verrucomicrobia at both arable fields ($p < 0.01$) and grasslands ($p < 0.10$) (Fig. 3). A total of 8 fungal phyla was detected in all samples and Ascomycota, Basidiomycota and Mortierellomycota were the most abundant phyla (Fig. 2). The abundance of Basidiomycota ($p < 0.05$) and Glomeromycota ($p < 0.01$) were significantly negatively correlated with LUI, whereas the abundance of Mortierellomycota ($p < 0.01$) was significantly positively correlated with LUI in arable fields. In grasslands, the abundance of Mortierellomycota ($p < 0.05$) and Chytridiomycota ($p < 0.05$) was significantly positively correlated with LUI, whereas Glomeromycota ($p < 0.05$) were significantly negatively correlated with LUI (Fig. 3). Regarding soil protists, Cercozoa, Ciliphora and Apicomplexa were the dominant phyla in all samples (Fig. 2). There was a significant negative relationship between the relative abundance of Apicomplexa and LUI ($p < 0.05$), and a positive relationship between Tubulinea and LUI ($p < 0.05$) at arable fields. Increasing LUI values coincided with decreased relative abundances of Ciliphora ($p < 0.05$) and Perkinsozoa ($p < 0.05$), but an increased abundance of Endomyxa ($p < 0.05$) (Fig. 3). For the invertebrate phyla, Annelida, Nematoda and Arthropoda were most abundant invertebrate phyla in all samples (Fig. 2). There was no significant correlation between the relative abundance of different phyla of invertebrates and LUI in arable fields. In grasslands, the abundance of Nematoda ($p < 0.01$) was significantly positively correlated with LUI while the abundance of Arthropoda ($p < 0.05$) was significantly negatively correlated with LUI (Fig. 3).

3.3. Individual agricultural practices and the composition of soil communities

A redundancy analysis and variation partitioning analysis showed the major agricultural management practices determining the community composition of soil biota (Fig. 4, Fig. S7). Irrigation and pest control practices were two most important agricultural practices determining the composition of bacterial, fungal and protistan communities in arable soils (Fig. 4, Fig. S7). For invertebrate communities, crop diversity was another practice driving their composition in addition to irrigation. In grasslands, different soil communities were shaped by different practices (Fig. 4, Fig. S7). Phosphorus fertilization intensity (PFI) and liming were the most important practices in shaping the soil bacterial community. The composition of the fungal community was mainly affected by PFI and Nitrogen fertilization intensity (NFI) and grassland diversity. PFI and NFI played a major role in shaping the soil protistan community composition. Soil invertebrates were primarily structured by liming, PFI and NFI in grassland soils.

3.4. Associations across soil organism groups differ among land use type and intensity

The co-occurrence network analysis showed how land use intensity affects the associations within and among different soil groups in arable and grassland soils. We found that an increase in LUI leads to an increase in the network complexity in arable fields but decline at grasslands (Fig. 5). There was no relationship between bacterial network complexity and LUI at arable fields, but a negative relationship at grasslands (Fig. S8, S9). We found negative relationships between LUI and fungal network complexity at both arable fields and grasslands (Fig. S8, S9), similar to the responses of their diversity to LUI. In contrast, an increase in LUI increased the network complexity of invertebrates community in arable fields, but reduced their complexity in grasslands (Fig. S8, S9). In general, the soil organisms that participated in network constructions were noticeably different at arable fields and grasslands (Fig. 6, Table S6). Furthermore, we found that higher complexity of interkingdom networks in grasslands with more nodes ($p < 0.01$) and links ($p < 0.01$) than in arable fields, and most associations were positive. This pattern is similar to the networks within individual soil groups, except for soil protists which had more complex networks in arable soils (Fig. 7, Table S7).

4. Discussion

Our regional on-farm study showed the response of soil bacteria, fungi, protist and invertebrate communities to land use intensity based on multiple agricultural practices at 39 arable fields and 48 grasslands. The diversity, composition and interactions of bacteria, fungi, protists and invertebrates varied in response to land use intensity, with a strong

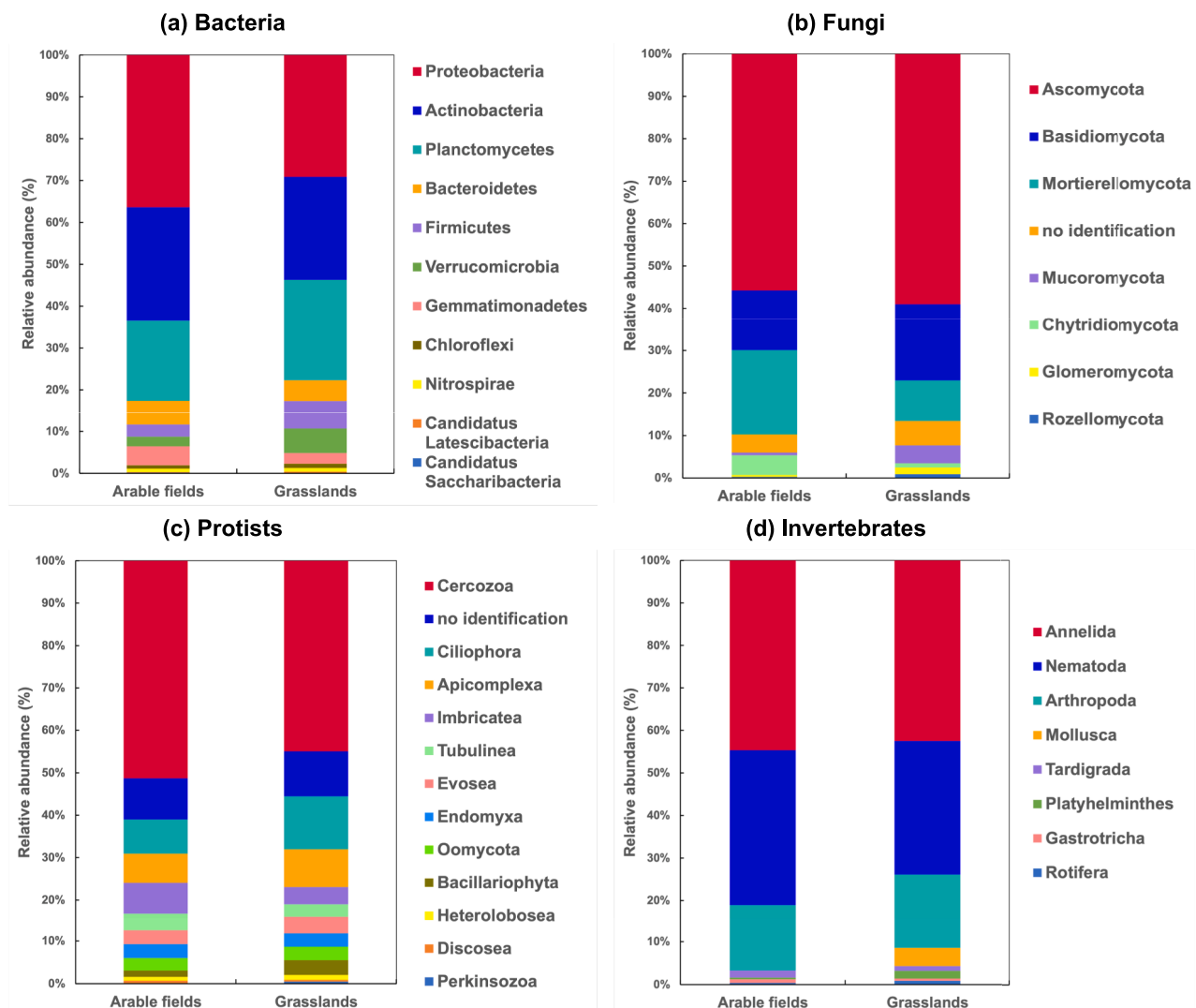


Fig. 2. Differences in community structure of soil bacteria (relative abundance > 0.1 %), fungi (relative abundance > 0.1 %), protists (relative abundance > 0.1 %), and invertebrates at phylum level in arable fields and grasslands.

dependence on land use types. Uniquely, this study details the response of multiple soil communities to various agricultural practices across a range of field sites in real agroecosystems consisting of both grasslands and arable fields. These findings improve our understanding of anthropogenic management impacts on soil biodiversity and will help identify measures to conserve their diversity for agricultural sustainability.

4.1. Differential responses to land use intensity in arable fields and grasslands

We observed that soil organisms were differentially influenced by LUI (Fig. 1), which is in agreement with our first hypothesis. LUI had no impacts on the diversity and composition of soil bacteria in both arable fields and grasslands. Compared to other soil organisms (e.g., fungi), the rapid growth rates (Allison and Martiny, 2008), and flexible metabolism (Chen et al., 2022; Jiao et al., 2020) of soil bacteria can increase their rate of adaptation and evolution. This allows bacteria to have greater environmental tolerance and adapt to the disturbance induced by LUI,

which consequently also leads to their dominance among soil organisms in frequently disturbed ecosystems (Chen et al., 2021; Liang et al., 2024). Consistent with recent studies (Clocchiatti et al., 2020; de Vries and Bardgett, 2012; Pölme et al., 2020), our results showed that increases in LUI lead to decline in the diversity of soil fungi. Moreover, we noticed a stronger reduction of fungal diversity in arable fields, highlighting that fungal communities at arable sites were more susceptible to LUI than fungal communities in grasslands (Fig. 1). This could be explained by the greater frequency of applied agricultural practices in arable soils, such as intensive tillage which can damage fungal mycelial networks directly and reduced fungal abundance (Bowles et al., 2017; Frey et al., 1999). Contrasting to our first expectation that higher-trophic-level soil groups are more sensitive to land use intensity, we did not find a significant response of soil protists to differences in LUI at both arable fields and grasslands. This could be explained by the variable responses of the distinct protist phyla to LUI (Fig. 3), resulting from niche differentiation, resource competition, and cross-kingdom interactions (Aslani et al., 2024). The decrease in the relative abundance

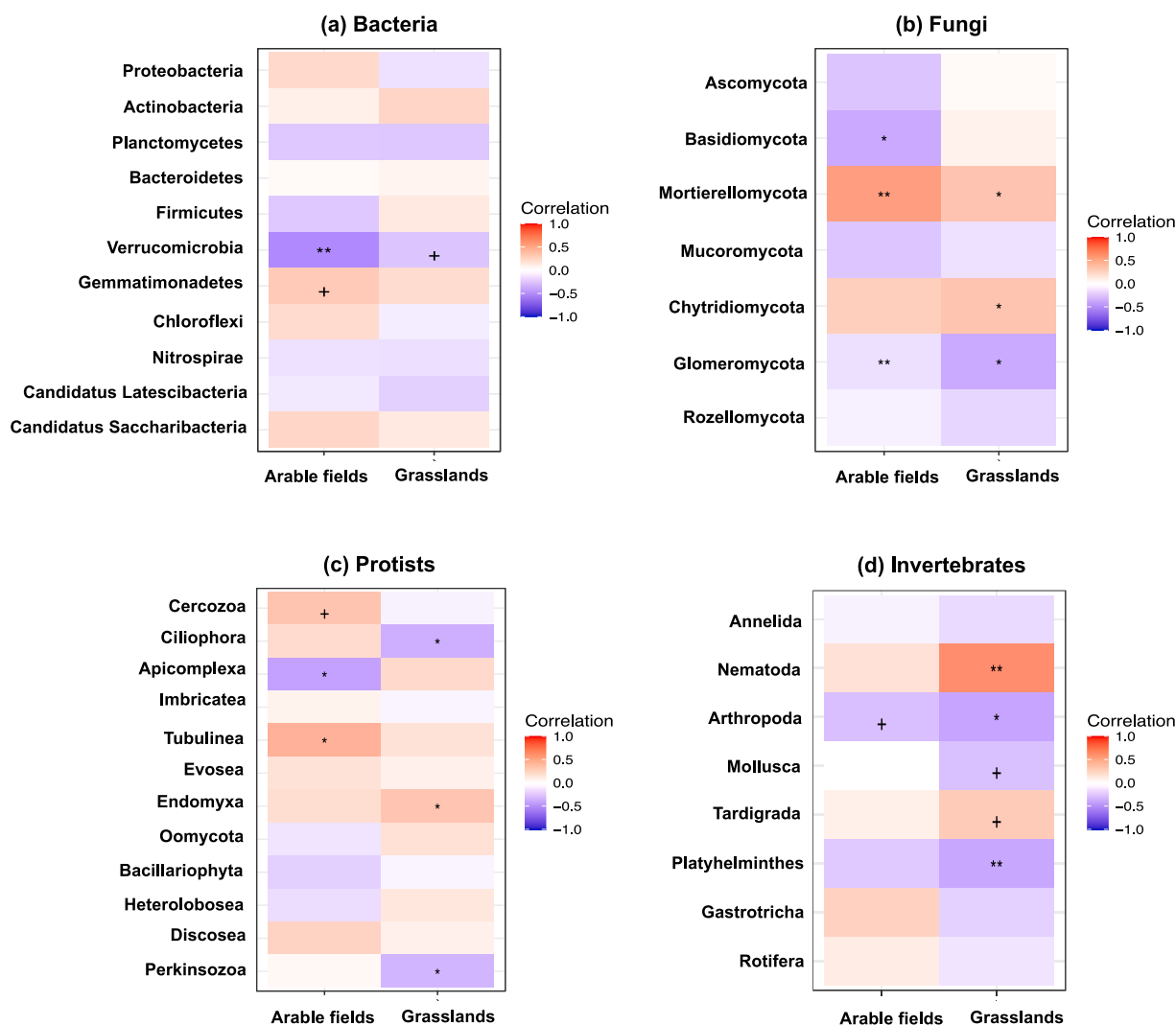


Fig. 3. Heatmaps summarizing Spearman's correlations of LUI and the relative abundance of soil bacteria, fungi, protist and invertebrate phyla in arable fields and grasslands. “***”, “**” and “+” indicate a significant correlation at $p < 0.01$, $p < 0.05$ and $p < 0.10$, respectively.

of some phyla was complemented by the increase in other phyla. For instance, we observed that increasing LUI led to an increase in the relative abundance of Tubulinea, but decreases in the relative abundance of Apicomplexa and Imbricatea at arable fields. In addition, the non-significant response of soil protists may be attributed to their adaptability. Like bacteria, soil protist can rapidly adjust their activity and abundance in response to changes in environmental factors, such as soil moisture and nutrient availability (Foissner, 1997; Geisen et al., 2018).

An increase in LUI led to an increase in the diversity of invertebrates in arable soils, whereas it reduced their diversity in grasslands. This can be explained by the greater nutrient availability in grasslands with more frequent application of fertilizers (Pothula et al., 2019). After addition of nutrients, soil invertebrates, such as nematodes, respond immediately and flourish due to the newly available nutrients. Moreover, intensive agricultural practices may erase environmental constraints (such as temperature and resources availability) on arthropoda communities (Li et al., 2022; van den Hoogen et al., 2019; Vazquez et al.,

2019). However, it is important to note that our results were based on DNA sequencing data. While sequencing data is widely used and provided valuable insights into soil communities, such as community composition, it has limitations in capturing actual biomass or the absolute abundance of soil communities. To better understand the responses of soil communities to LUI, integrating sequencing data with absolute abundance measures (e.g., phospholipid fatty acids, direct cell counts) and employing advanced techniques such as quantitative sequencing and ddPCR (droplet digital polymerase chain reaction) is recommended to achieve a more comprehensive understanding of complex soil communities (Barlow et al., 2020; Wang et al., 2024). Taken together, these results highlights the variable responses of different soil groups to LUI in arable fields and grasslands. It is essential to survey multiple soil groups to draw holistic conclusions on the effects of land use on soil communities, and to allow regulating soil functioning for sustainable management in agroecosystems (Allan et al., 2014; Chauvat et al., 2007; Cozim-Melges et al., 2025).

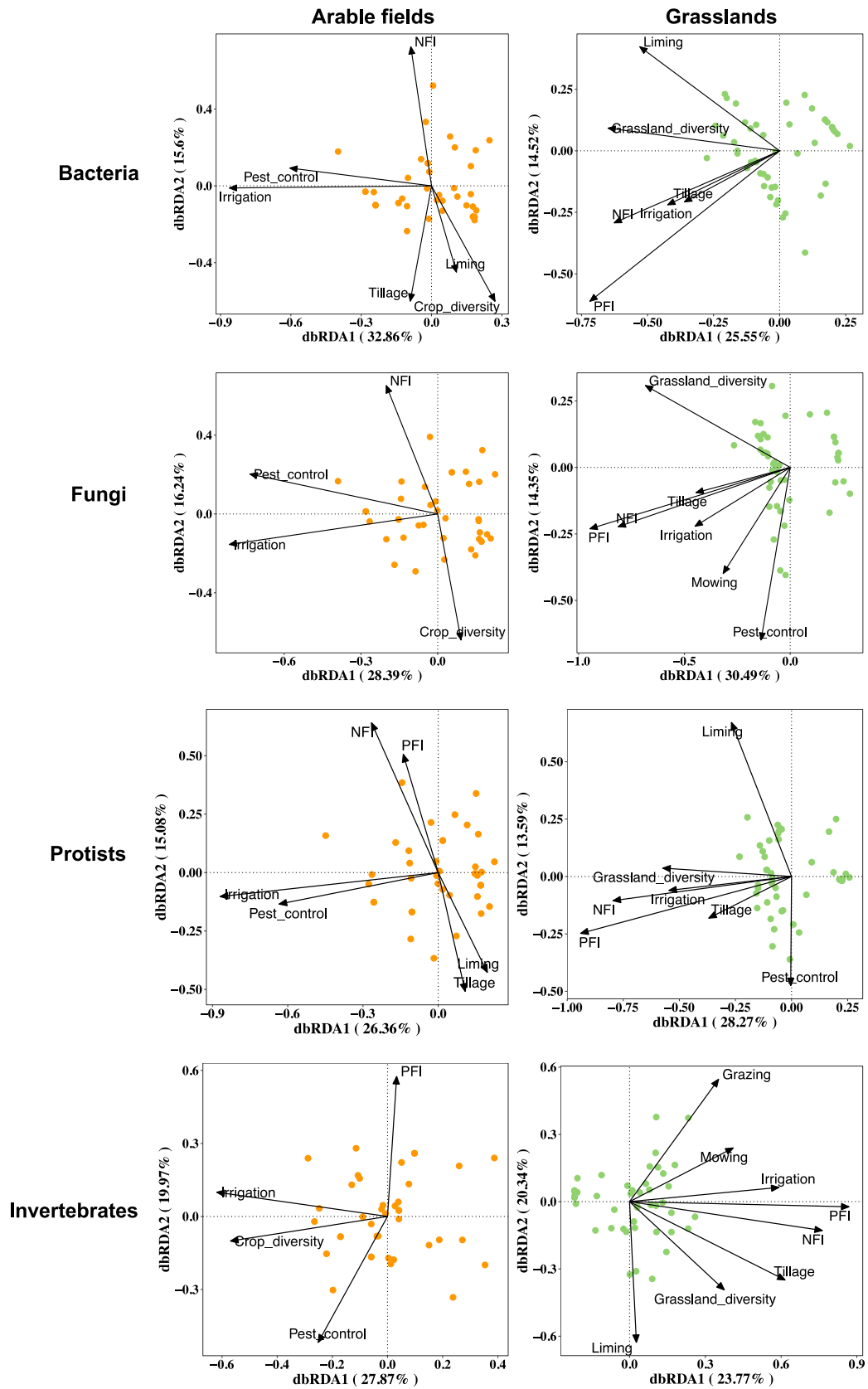


Fig. 4. Redundancy analysis of soil bacteria, fungi, protist and invertebrate communities as affected by different agricultural practices. Only significant agricultural practices are shown in the figure. The arrow represents different agricultural practices, the angle represents the correlation between the two practices, and the ray length of agricultural practices represents the influence degree of the influencing factors. Note: PFI, phosphorus fertilization intensity; NFI, nitrogen fertilization intensity.

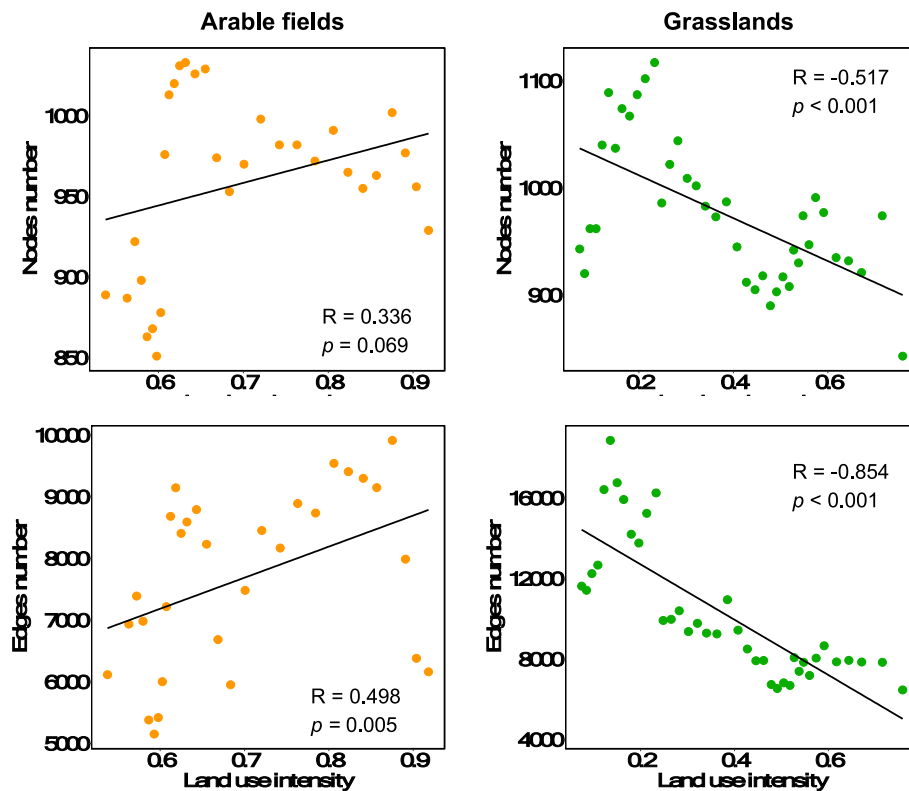


Fig. 5. The relationship between the network complexity of soil communities (nodes number and edges number) and land use intensity at arable fields ($n = 30$) and grasslands ($n = 39$).

4.2. Individual agricultural practices underly responses in community composition to land use intensity

Compared to the diversity of soil communities, the community composition was more susceptible to land use intensity. The significant responses of some soil phyla (Fig. 3), probably translate into significant differences in the community composition along LUI gradients (Xu et al., 2021). In line with our second hypothesis, we found that the impacts of LUI on the diversity and community composition of multiple soil communities strongly depended on the practices applied at the different land use types. In arable soils, irrigation was the most important practice shaping soil bacterial, fungal, protistan and invertebrates communities. Considering the summer drought in 2022 (Fig. S10), mediating water stress via irrigation is vital in shaping soil communities in arable soils (Li et al., 2021; Morugán-Coronado et al., 2019). Pest control was another main practice influencing the community structure of soil communities. This supports recent studies demonstrating that the intensive application of pesticides has deleterious impacts on soil communities and reduction of pesticide applications can promote the soil biodiversity (Riedo et al., 2021; Vahter et al., 2022; Wolejko et al., 2020). We also noted that crop diversity played an important role in structuring soil invertebrates composition. This is in line with recent studies showing that crop management significantly shapes soil invertebrates communities (e.g., mesofauna, earthworms and nematodes) (Chassain et al., 2024; Tsiafouli et al., 2015; Yin et al., 2022). Additionally, we noticed that there were few correlations between individual practices (Table S2), suggesting independent influences of individual practices on soil communities in arable fields.

Contrastingly to arable fields, phosphorus and nitrogen fertilization were the main practices in driving soil communities composition in grasslands. The effects of phosphorus and nitrogen fertilization were strongly correlated (Table S3), indicating that it is difficult to decipher whether it is phosphorus or nitrogen fertilization that influences the soil communities. The generally crucial impact of fertilizer application on

soil communities is in line with numerous studies (Cruz et al., 2009; Guo et al., 2018; Liang et al., 2020; Wang et al., 2021). Besides fertilization intensity, we found that liming practice strongly structured bacterial, protistan and invertebrate communities. Liming, as a common practices in agriculturally managed land, can mediate soil pH against soil acidification and improve nutrient cycling, which consequently affects soil communities (Heyburn et al., 2017; Holland et al., 2018). This result suggests that there is potential for utilizing liming practices for mediating soil biodiversity for sustainable agriculture. Overall, our results show that different agricultural practices contribute differently to soil communities in arable fields and grasslands and that an increasing intensity of these practices enhances the differences among soil communities. Understanding the impacts of specific practices may help avoiding adverse effects and inform on sustainable agricultural management.

4.3. LUI influences the associations among soil organisms

Interkingdom co-occurrence networks encompassing all four taxa provided an integrated and holistic view of the associations between soil organisms along the LUI gradient in arable fields and grasslands. In contrast to our expectation, we observed that increased LUI coincided with an increased network complexity of soil communities in arable soils (Fig. 5), which might result from the increased network complexity of soil invertebrate communities (Fig. S8). This result contradicts with previous observations that microbial network complexity decreases as the agricultural practices intensify (Banerjee et al., 2019; Creamer et al., 2016; Xue et al., 2020). Increased network complexity in arable soils may be attributed to the increase in soil nutrient availability (Morrién et al., 2017). Intensive agricultural practices, such as nitrogen and phosphorus fertilization, may enhance trophic interactions and cooperation within or between soil communities. Moreover, Zhu et al. found that the proportion of potential interkingdom associations increases in microcosms with sufficient nutrient supply (Zhu et al., 2023).

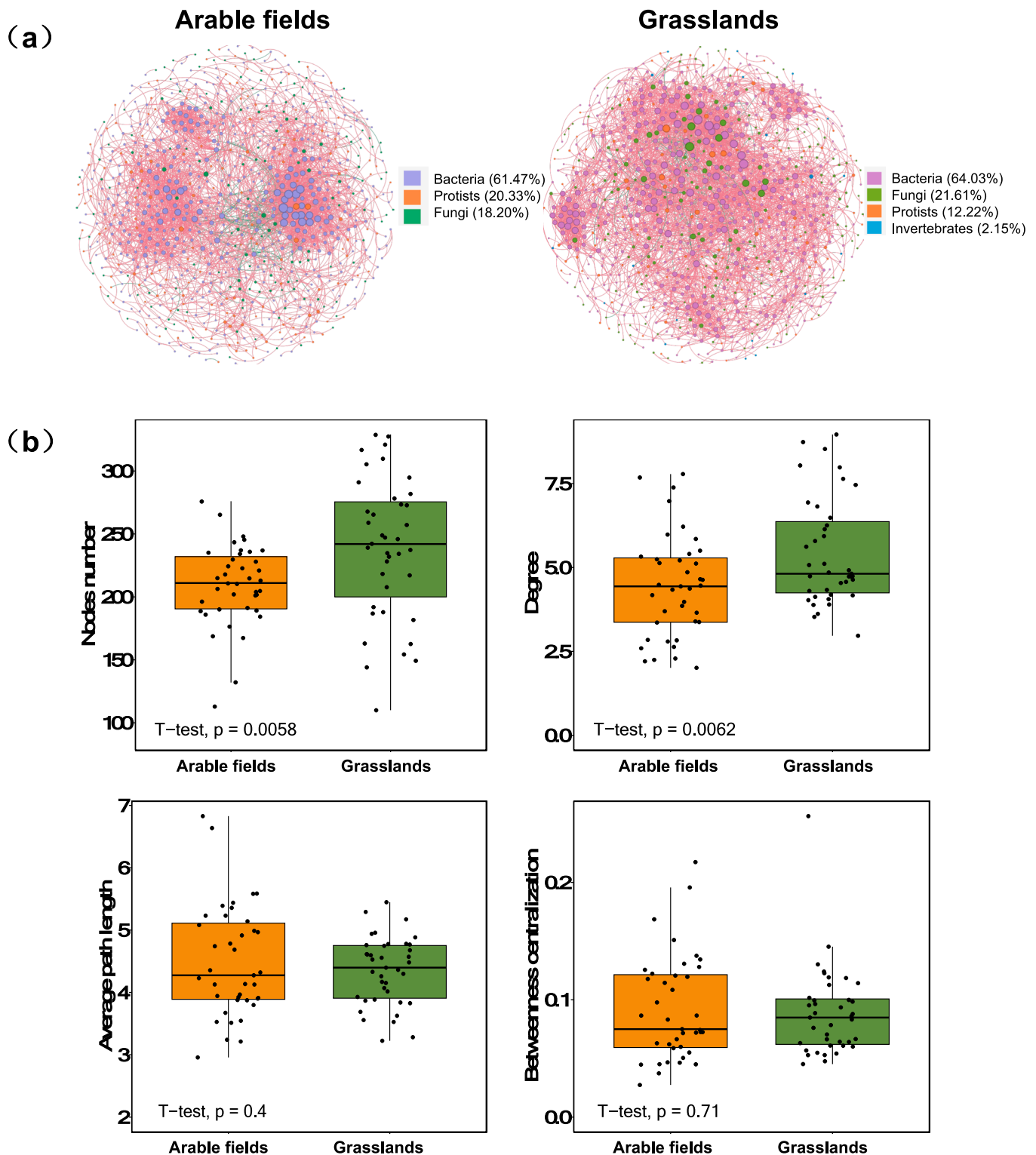


Fig. 6. Interkingdom co-occurrence networks across bacteria, fungi, protist and invertebrate groups in arable fields and grasslands (a). Nodes are colored according to their taxonomic affiliation at kingdom levels. The size of nodes is proportional to the number of links per node (i.e. degree) and the thickness of each edges (the lines) is proportional to the values of Spearman's correlation coefficients. The edges (the lines) indicated significant correlations between the corresponding soil organisms, with positive interactions in red lines and negative interactions in green lines. Network topological features in arable fields and grasslands (b).

We also noted that relatively more protists with a greater number of nodes occurred in the interkingdom network of arable communities than of grasslands. This is consistent with our finding that arable soils contained a greater number of unique species, suggesting that protists might play more important roles in arable soils than grasslands. This is likely to

be explained by the greater soil moisture with intensive irrigation in arable soils (Table S1, Fig. S11). Previous studies have demonstrated that soil water availability strongly affect the diversity and community composition of soil protists, given that most protistan lineages require water to move, feed and reproduce (Clarholm, 1981; Oliverio et al.,

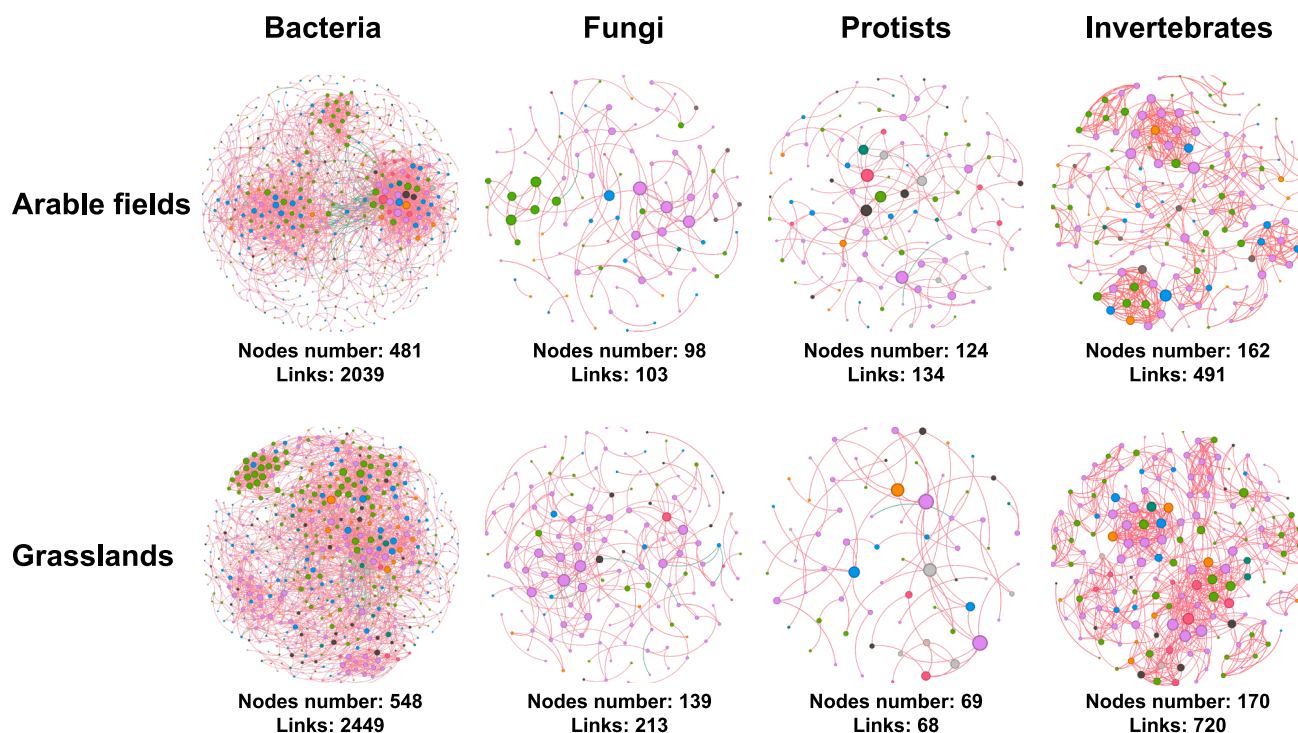


Fig. 7. Co-occurrence networks of bacteria, fungi, protist and invertebrate in arable fields and grasslands. Nodes are colored according to their taxonomic affiliation at phylum levels. The size of nodes is proportional to the number of links per node (i.e. degree) and the thickness of each edges (the lines) is proportional to the values of Spearman's correlation coefficients. The edges (the lines) indicated significant correlation relationships between the corresponding soil organisms, with positive interactions in red lines and negative interactions in green lines. Different cut-off parameters were used for analyzing the network of invertebrates (all species were included even though some of them were only present in one sample) due to their low ASV richness.

2020; Stefan et al., 2014). Therefore, intensive irrigation in arable soils may widen their habitat niche availability with higher soil moisture content and consequently promote their associations with other soil communities. Altogether, these results highlight the importance of protists as key elements of soil multitrophic networks in arable soils that should be considered in future studies (Romdhane et al., 2022).

5. Conclusions

Our study reveals distinct responses of various soil communities to land use intensity. Fungi and invertebrates responded most strongly to land use intensity, in particular in grasslands. A key strength of this study is that these reported effects can be generalized because the samples were collected from 87 farms where multiple agricultural practices were applied simultaneously. Overall, this study provides regional-scale evidence of land use intensity impacts on various soil communities in real commercial agroecosystems. We showed that irrigation and pesticide practices have most impact on arable land soil biodiversity, while fertilization practices have most impact on grasslands. These findings provide valuable insights into soil management strategies to maintain or improve the diversity of soil organisms, a critical component of ecosystem functioning.

CRedit authorship contribution statement

Chenguang Gao: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Johanna E.M. Schild:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Gabriel Y.K. Moinet:** Writing – review & editing, Resources, Methodology, Investigation, Funding acquisition, Data curation. **T. Martijn Bezemer:** Writing – review & editing, Methodology. **Franciska T. de Vries:** Writing – review & editing, Methodology. **Jan Hassink:** Writing –

review & editing, Resources, Methodology, Investigation, Funding acquisition, Data curation. **Nick van Eekeren:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Kevin Beentjes:** Writing – review & editing, Methodology. **Peter M. van Bodegom:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank all the farmers for allowing sampling in their fields and for providing us with detailed field management information. We also thank Howard Koster (for farmers' interviews, scheduling, farmer contact), Marta Loreggian (for field sampling and lab samples preparations), Giulia Vultaggio (for field sampling), Joris Hijmans (for taking interviews), Erik Engelbrecht (for field sampling), Henk Martens (for field sampling, laboratory analyses and sampling design), Rachel Creamer (for sampling design) and Kornelia Serwatowska (for laboratory analyses) for their help with the sampling design, field sampling, taking farmers' interviews and lab preparations and measurements. We thank Michiel in 't Zandt, Carmen Vazquez Martin and Rosa Boone for contributing to the workshop developing the LUI index. This study was funded by internal finances from the Soil Biology Group of Wageningen University, by the project "Transition to a sustainable food system" (with project number NWA.1235.18.201) which is financed by the Dutch Research Council (NWO), and by the knowledge programme KB36 biodiversity in a nature inclusive society of the ministry of LNV

(KB36-003-024).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2025.117201>.

Data availability

Data will be made available on request.

References

- Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2022. UNITE general FASTA release for Fungi. Version 16.10.2022. UNITE community. <https://doi.org/10.1093/nar/gky379>.
- Afgan, E., Baker, D., Batut, B., Van Den Beek, M., Bouvier, D., Ech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B.A., Guerler, A., Hillman-Jackson, J., Hiltmann, S., Jalili, V., Rasche, H., Soranzo, G., Goecks, J., Taylor, J., Nekrutenko, A., Blankenberg, D., 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. *Nucleic Acids Res.* 46, W537–W544. <https://doi.org/10.1093/nar/gky379>.
- Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W., Huang, S., 2018. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma* 319, 156–166. <https://doi.org/10.1016/j.geoderma.2018.01.010>.
- Allan, E., Bossdorf, O., Dormann, C.F., Prati, D., Gossner, M.M., Tschirntke, T., Blüthgen, N., Bellach, M., Birkhofer, K., Boch, S., Böhm, S., Börschig, C., Chatzinotas, A., Christ, S., Daniel, R., Diekötter, T., Fischer, C., Friedl, T., Glaser, K., Hallmann, C., Hodac, L., Hölzel, N., Jung, K., Klein, A.M., Klaus, V.H., Kleinebecker, T., Krauss, J., Lange, M., Morris, E.K., Müller, J., Nacke, H., Pašalić, E., Rillig, M.C., Rothenwöhrer, C., Schall, P., Scherber, C., Schulze, W., Socher, S.A., Steckel, J., Steffan-Dewenter, I., Türke, M., Weiner, C.N., Werner, M., Westphal, C., Wolters, V., Wubet, T., Gockel, S., Gorke, M., Hemp, A., Renner, S.C., Schöning, I., Pfeiffer, S., König-Ries, B., Buscot, F., Linsenmair, K.E., Schulze, E.D., Weisser, W.W., Fischer, M., 2014. Interannual variation in land-use intensity enhances grassland multidiversity. *Proc. Natl. Acad. Sci. USA* 111, 308–313. <https://doi.org/10.1073/pnas.1312213111>.
- Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. USA* 105, 11512–11519. <https://doi.org/10.1073/pnas.0801925105>.
- Anthony, M.A., Bender, S.F., van der Heijden, M.G.A., 2023. Enumerating soil biodiversity. *Proc. Natl. Acad. Sci. USA* 120, e2304663120. <https://doi.org/10.1073/pnas.2304663120>.
- Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806F gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. <https://doi.org/10.3354/ame01753>.
- Aslani, F., Bahram, M., Geisen, S., Pent, M., Otsing, E., Tamm, H., Jones, A., Panagos, P., Köninger, J., Orgiazzi, A., Tedersoo, L., 2024. Land use intensification homogenizes soil protist communities and alters their diversity across Europe. *Soil Biol. Biochem.* 195. <https://doi.org/10.1016/j.soilbio.2024.109459>.
- Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A.Y., Gatteringer, A., Keller, T., Charles, R., van der Heijden, M.G.A., 2019. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *ISME J.* 13, 1722–1736. <https://doi.org/10.1038/s41396-019-0383-2>.
- Barberán, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* 6, 343–351. <https://doi.org/10.1038/ismej.2011.119>.
- Barlow, J.T., Bogatyrev, S.R., Ismagilov, R.F., 2020. A quantitative sequencing framework for absolute abundance measurements of mucosal and luminal microbial communities. *Nat. Commun.* 11, 2590. <https://doi.org/10.1038/s41467-020-16224-6>.
- Bass, D., Silberman, J.D., Brown, M.W., Pearce, R.A., Tice, A.K., Jousset, A., Geisen, S., Hartikainen, H., 2016. Coprophilic amoebae and flagellates, including *Guttulinopsis*, *Rosculus* and *Helkesimastix*, characterise a divergent and diverse rhizarian radiation and contribute to a large diversity of faecal-associated protists. *Environ. Microbiol.* 18, 1604–1619. <https://doi.org/10.1111/1462-2920.13235>.
- Beentjes, K.K., Speksnijder, A.G.C.L., Schilthuizen, M., Hoogeveen, M., Pastoor, R., van der Hoorn, B.B., 2019. Increased performance of DNA metabarcoding of macroinvertebrates by taxonomic sorting. *PLoS One* 14, 1–19. <https://doi.org/10.1371/journal.pone.0226527>.
- Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Wheeler, D.L., 2005. GenBank. *Nucleic Acids Res.* 33, 34–38. <https://doi.org/10.1093/nar/gki063>.
- Betancur-Corredor, B., Lang, B., Russell, D.J., 2022. Reducing tillage intensity benefits the soil micro- and mesofauna in a global meta-analysis. *Eur. J. Soil Sci.* 73, e13321. <https://doi.org/10.1111/ejss.13321>.
- Blanchet, F.G., Cazelles, K., Gravel, D., 2020. Co-occurrence is not evidence of ecological interactions. *Ecol. Lett.* 23, 1050–1063. <https://doi.org/10.1111/ele.13525>.
- Bloor, J.M.G., Si-Moussi, S., Taberlet, P., Carrère, P., Hedde, M., 2021. Analysis of complex trophic networks reveals the signature of land-use intensification on soil communities in agroecosystems. *Sci. Rep.* 11, 18260. <https://doi.org/10.1038/s41598-021-97300-9>.
- Bowles, T.M., Jackson, L.E., Loeher, M., Cavagnaro, T.R., 2017. Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *J. Appl. Ecol.* 54, 1785–1793. <https://doi.org/10.1111/1365-2664.12815>.
- Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci.* 108, 4516–4522. <https://doi.org/10.1073/pnas.100080107>.
- Chassain, J., Joimel, S., Vieublé Gonod, L., 2024. A complex relationship between cropping systems and soil macrofauna: Influence of practice intensity, taxa and traits. *Pedobiologia (Jena)*. 105, 150974. <https://doi.org/10.1016/j.pedobi.2024.150974>.
- Chauvat, M., Wolters, V., Dauber, J., 2007. Response of collembolan communities to land-use change and grassland succession. *Ecography (Cop.)* 30, 183–192. <https://doi.org/10.1111/j.2007.0906-7590.04888.x>.
- Chen, Y.-J., Leung, P.M., Wood, J.L., Bay, S.K., Hugenholtz, P., Kessler, A.J., Shelley, G., Waite, D.W., Franks, A.E., Cook, P.L.M., Greening, C., 2021. Metabolic flexibility allows bacterial habitat generalists to become dominant in a frequently disturbed ecosystem. *ISME J.* 15, 2986–3004. <https://doi.org/10.1038/s41396-021-00988-w>.
- Chen, Y., Li, Y., Qiu, T., He, H., Liu, J., Duan, C., Cui, Y., Huang, M., Wu, C., Fang, L., 2024. High nitrogen fertilizer input enhanced the microbial network complexity in the paddy soil. *Soil Ecol. Lett.* 6, 230205. <https://doi.org/10.1007/s42832-023-0205-3>.
- Chen, Y., Yang, X., Fu, W., Chen, B., Hu, H., Feng, K., Geisen, S., 2022. Conversion of natural grassland to cropland alters microbial community assembly across northern China. *Environ. Microbiol.* 24, 5630–5642. <https://doi.org/10.1111/1462-2920.16127>.
- Clarholm, M., 1981. Protozoan grazing of bacteria in soil—impact and importance. *Microb. Ecol.* 7, 343–350. <https://doi.org/10.1007/BF02341429>.
- Clocchiatti, A., Hannula, S.E., van den Berg, M., Korthals, G., de Boer, W., 2020. The hidden potential of saprotrophic fungi in arable soil: patterns of short-term stimulation by organic amendments. *Appl. Soil Ecol.* 147, 103434. <https://doi.org/10.1016/j.apsoil.2019.103434>.
- Cozim-Melges, F., Ripoll-Bosch, R., Oggiano, P., van Zanten, H.H.E., van der Putten, W. H., Veen (Ciska), G.F., 2025. The effect of alternative agricultural practices on soil biodiversity of bacteria, fungi, nematodes and earthworms: a review. *Agric. Ecosyst. Environ.* 379, 109329. <https://doi.org/10.1016/j.agee.2024.109329>.
- Creamer, R.E., Hannula, S.E., Leeuwen, J.P.V., Stone, D., Rutgers, M., Schmelz, R.M., Rüter, P.C. d., Hendriksen, N.B., Bolger, T., Bouffaud, M.L., Buee, M., Carvalho, F., Costa, D., Dirilgen, T., Francisco, R., Griffiths, B.S., Griffiths, R., Martin, F., Silva, P. M. da, Mendes, S., Morais, P. V., Pereira, C., Philippot, L., Plassart, P., Redecker, D., Römbke, J., Sousa, J.P., Wouterse, M., Lemanceau, P., 2016. Ecological network analysis reveals the inter-connection between soil biodiversity and ecosystem function as affected by land use across Europe. *Appl. Soil Ecol.* 97, 112–124. doi: 10.1016/j.apsoil.2015.08.006.
- Cruz, A.F., Hamel, C., Hanson, K., Selles, F., Zentner, R.P., 2009. Thirty-seven years of soil nitrogen and phosphorus fertility management shapes the structure and function of the soil microbial community in a Brown Chernozem. *Plant Soil* 315, 173–184. <https://doi.org/10.1007/s11104-008-9742-x>.
- de Vries, F.T., Bardgett, R.D., 2012. Plant-microbial linkages and ecosystem nitrogen retention: Lessons for sustainable agriculture. *Front. Ecol. Environ.* 10, 425–432. <https://doi.org/10.1890/110162>.
- de Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafoulis, M.A., Bjørnlund, L., Bracht Jørgensen, H., Brady, M.V., Christensen, S., de Ruyter, P.C., D’Hertefeldt, T., Frouz, J., Hedlund, K., Hemerik, L., Hol, W.H.G., Hotes, S., Mortimer, S.R., Setälä, H., Sgardelis, S.P., Uteseny, K., van der Putten, W.H., Wolters, V., Bardgett, R. D., 2013. Soil food web properties explain ecosystem services across European land use systems. *Proc. Natl. Acad. Sci.* 110, 14296–14301. <https://doi.org/10.1073/pnas.1305198110>.
- de Vries, F.T., Wallenstein, M.D., 2017. Below-ground connections underlying above-ground food production: a framework for optimising ecological connections in the rhizosphere. *J. Ecol.* 105, 913–920. <https://doi.org/10.1111/1365-2745.12783>.
- Delgado-Baquerizo, M., Bardgett, R.D., Vitousek, P.M., Maestre, F.T., Williams, M.A., Eldridge, D.J., Lambers, H., Neuhäuser, S., Gallardo, A., García-Velázquez, L., Sala, O.E., Abades, S.R., Alfaro, F.D., Berhe, A.A., Bowker, M.A., Currier, C.M., Cutler, N.A., Hart, S.C., Hayes, P.E., Hseu, Z.-Y., Kirchmair, M., Peña-Ramírez, V.M., Pérez, C.A., Reed, S.C., Santos, F., Siebe, C., Sullivan, B.W., Weber-Grullon, L., Fierer, N., 2019. Changes in belowground biodiversity during ecosystem development. *Proc. Natl. Acad. Sci.* 116, 6891–6896. <https://doi.org/10.1073/pnas.1818400116>.
- Delgado-Baquerizo, M., Reich, P.B., Trivedi, C., Eldridge, D.J., Abades, S., Alfaro, F.D., Bastida, F., Berhe, A.A., Cutler, N.A., Gallardo, A., García-Velázquez, L., Hart, S.C., Hayes, P.E., He, J.-Z., Hseu, Z.-Y., Hu, H.-W., Kirchmair, M., Neuhäuser, S., Pérez, C.A., Reed, S.C., Santos, F., Sullivan, B.W., Trivedi, P., Wang, J.-T., Weber-Grullon, L., Williams, M.A., Singh, B.K., 2020. Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nat. Ecol. Evol.* 4, 210–220. <https://doi.org/10.1038/s41559-019-1084-y>.
- Donald, J., Murielle, J., Chave, J., Iribar, A., Louisanna, E., Manzi, S., Roy, M., Tao, S., Orivel, J., Schimann, H., Zinger, L., 2021. Multi-taxa environmental DNA inventories reveal distinct taxonomic and functional diversity in urban tropical forest fragments. *Glob. Ecol. Conserv.* 29, 2. <https://doi.org/10.1016/j.gecco.2021.e01724>.
- Edgar, R.C., 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv* 081257.
- Eldinger, A., Garland, G., Hartman, K., Banerjee, S., Degruene, F., García-Palacios, P., Hallin, S., Valzano-Held, A., Herzog, C., Jansa, J., Kost, E., Maestre, F.T., Pescador, D.S., Philippot, L., Rillig, M.C., Romdhane, S., Saghai, A., Spor, A., Frossard, E., van der Heijden, M.G.A., 2022. Agricultural management and pesticide

- use reduce the functioning of beneficial plant symbionts. *Nat. Ecol. Evol.* 6, 1145–1154. <https://doi.org/10.1038/s41559-022-01799-8>.
- Feng, Y., Delgado-Baquerizo, M., Zhu, Y., Han, X., Han, X., Xin, X., Li, W., Guo, Z., Dang, T., Li, C., Zhu, B., Cai, Z., Li, D., Zhang, J., 2022. Responses of soil bacterial diversity to fertilization are driven by local environmental context across China. *Engineering* 12, 164–170. <https://doi.org/10.1016/j.eng.2021.09.012>.
- Foissner, W., 1997. Protozoa as bioindicators in agroecosystems, with emphasis on farming practices, biocides, and biodiversity. *Agric. Ecosyst. Environ.* 62, 93–103. [https://doi.org/10.1016/S0167-8809\(96\)01142-5](https://doi.org/10.1016/S0167-8809(96)01142-5).
- French, K.E., Tkacz, A., Turnbull, L.A., 2017. Conversion of grassland to arable decreases microbial diversity and alters community composition. *Appl. Soil Ecol.* 110, 43–52. <https://doi.org/10.1016/j.apsoil.2016.10.015>.
- Frey, S., Elliott, E., Paustian, K., 1999. Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biol. Biochem.* 31, 573–585. [https://doi.org/10.1016/S0038-0717\(98\)00161-8](https://doi.org/10.1016/S0038-0717(98)00161-8).
- Gao, C., Hannula, S.E., van Bodegom, P.M., Bezemer, T.M., de Vries, F.T., Hassink, J., in 't Zandt, M.H., Moinet, G.Y.K., 2024. Land use intensity differently affects soil microbial functional communities in arable fields. *Appl. Soil Ecol.* 204, 105723. doi: 10.1016/j.apsoil.2024.105723.
- Geisen, S., Mitchell, E.A.D., Adl, S., Bonkowski, M., Dunthorn, M., Ekelund, F., Fernández, L.D., Jousset, A., Krashevska, V., Singer, D., Spiegel, F.W., Walochnik, J., Lara, E., 2018. Soil protists: a fertile frontier in soil biology research. *FEMS Microbiol. Rev.* 42, 293–323. <https://doi.org/10.1093/femsre/fuy006>.
- Geisen, S., Vulot, D., Mahé, F., Lara, E., Vargas, C. de, Bass, D., 2019a. A user guide to environmental protistology: primers, metabarcoding, sequencing, and analyses. *bioRxiv* 850610. doi: doi.org/10.1101/850610.
- Geisen, S., Wall, D.H., van der Putten, W.H., 2019b. Challenges and opportunities for soil biodiversity in the anthropocene. *Curr. Biol.* 29, R1036–R1044. <https://doi.org/10.1016/j.cub.2019.08.007>.
- Guo, J., Ling, N., Chen, Z., Xue, C., Li, L., Liu, L., Gao, L., Wang, M., Ruan, J., Guo, S., Vandenkoornhuyse, P., Shen, Q., 2020. Soil fungal assemblage complexity is dependent on soil fertility and dominated by deterministic processes. *New Phytol.* 226, 232–243. <https://doi.org/10.1111/nph.16345>.
- Guo, S., Xiong, W., Xu, H., Hang, X., Liu, H., Xun, W., Li, R., Shen, Q., 2018. Continuous application of different fertilizers induces distinct bulk and rhizosphere soil protist communities. *Eur. J. Soil Biol.* 88, 8–14. <https://doi.org/10.1016/j.ejsobi.2018.05.007>.
- Guo, Y., Liao, H.-L., Boughton, E.H., Martens-Habbena, W., Qiu, J., 2023. Effects of land-use intensity, grazing and fire disturbances on soil bacterial and fungal communities in subtropical wetlands. *Agric. Ecosyst. Environ.* 345, 108314. <https://doi.org/10.1016/j.agee.2022.108314>.
- Hartman, K., van der Heijden, M.G.A., Wittwer, R.A., Banerjee, S., Walsler, J.-C., Schlaeppi, K., 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* 6, 1–14. <https://doi.org/10.1186/s40168-017-0389-9>.
- Hartmann, M., Six, J., 2022. Soil structure and microbiome functions in agroecosystems. *Nat. Rev. Earth Environ.* 4, 4–18. <https://doi.org/10.1038/s43017-022-00366-w>.
- Heyburn, J., McKenzie, P., Crawley, M.J., Fornara, D.A., 2017. Long-term belowground effects of grassland management: The key role of liming. *The. Ecol. Appl.* 27, 2001–2012. <https://doi.org/10.1002/eap.1585>.
- Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A., Hayes, R.C., 2018. Liming impacts on soils, crops and biodiversity in the UK: a review. *Sci. Total Environ.* 610–611, 316–332. <https://doi.org/10.1016/j.scitotenv.2017.08.020>.
- Hu, X., Liu, J., Liang, A., Li, L., Yao, Q., Yu, Z., Li, Y., Jin, J., Liu, X., Wang, G., 2021. Conventional and conservation tillage practices affect soil microbial co-occurrence patterns and are associated with crop yields. *Agric. Ecosyst. Environ.* 319, 107534. <https://doi.org/10.1016/j.agee.2021.107534>.
- Hueso, S., García, C., Hernández, T., 2012. Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. *Soil Biol. Biochem.* 50, 167–173. <https://doi.org/10.1016/j.soilbio.2012.03.026>.
- Ji, L., Ni, K., Wu, Z., Zhang, J., Yi, X., Yang, X., Ling, N., You, Z., Guo, S., Ruan, J., 2020. Effect of organic substitution rates on soil quality and fungal community composition in a tea plantation with long-term fertilization. *Biol. Fertil. Soils* 56, 633–646. <https://doi.org/10.1007/s00374-020-01439-y>.
- Jia, J., Zhang, J., Li, Y., Xie, M., Wang, G., Zhang, J., 2022. Land use intensity constrains the positive relationship between soil microbial diversity and multifunctionality. *Plant Soil.* <https://doi.org/10.1007/s11104-022-05853-z>.
- Jiao, S., Lu, Y., Wei, G., 2022. Soil multitrophic network complexity enhances the link between biodiversity and multifunctionality in agricultural systems. *Glob. Chang. Biol.* 28, 140–153. <https://doi.org/10.1111/gcb.15917>.
- Jiao, S., Peng, Z., Qi, J., Gao, J., Wei, G., 2021. Linking bacterial-fungal relationships to microbial diversity and soil nutrient cycling. *mSystems* 6, 10–1128. doi: 10.1128/mSystems.01052-20.
- Jiao, S., Yang, Y., Xu, Y., Zhang, J., Lu, Y., 2020. Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. *ISME J.* 14, 202–216. <https://doi.org/10.1038/s41396-019-0522-9>.
- Kirse, A., Bourlat, S.J., Langen, K., Fonseca, V.G., 2021. Metabarcoding Malaise traps and soil eDNA reveals seasonal and local arthropod diversity shifts. *Sci. Rep.* 11, 10498. <https://doi.org/10.1038/s41598-021-89950-6>.
- Labouyrie, M., Ballabio, C., Romero, F., Panagos, P., Jones, A., Schmid, M.W., Mikryukov, V., Dulya, O., Tedersoo, L., Bahram, M., Lugato, E., van der Heijden, M. G.A., Orgiazzi, A., 2023. Patterns in soil microbial diversity across Europe. *Nat. Commun.* 14, 3311. <https://doi.org/10.1038/s41467-023-37937-4>.
- Lai, J., Zou, Y., Zhang, J., Peres-Neto, P.R., 2022. Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rda.ca.hp R package. *Methods Ecol. Evol.* 13, 782–788. <https://doi.org/10.1111/2041-210X.13800>.
- Le Provost, G., Thiele, J., Westphal, C., Penone, C., Allan, E., Neyret, M., van der Plas, F., Ayasse, M., Bardgett, R.D., Birkhofer, K., Boch, S., Bonkowski, M., Buscot, F., Feldhaar, H., Gaulton, R., Goldmann, K., Gossner, M.M., Klaus, V.H., Kleinebecker, T., Krauss, J., Renner, S., Scherreijs, P., Sikorski, J., Baulechner, D., Blüthgen, N., Bolliger, R., Börschig, C., Busch, V., Chisté, M., Fiore-Donno, A.M., Fischer, M., Arndt, H., Hoelzel, N., John, K., Jung, K., Lange, M., Marzini, C., Overmann, J., Pašalić, E., Perović, D.J., Prati, D., Schäfer, D., Schöning, I., Schrupf, M., Sonnemann, I., Steffan-Dewenter, I., Tschapka, M., Türke, M., Vogt, J., Wehner, K., Weiner, C., Weisser, W., Wells, K., Werner, M., Wolters, V., Wubet, T., Wurst, S., Zaitsev, A.S., Manning, P., 2021. Contrasting responses of above- and belowground diversity to multiple components of land-use intensity. *Nat. Commun.* 12. <https://doi.org/10.1038/s41467-021-23931-1>.
- Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129, 271–280. <https://doi.org/10.1007/s004420100716>.
- Légrand, F., Picot, A., Cobo-Díaz, J.F., Carof, M., Chen, W., Le Floch, G., 2018. Effect of tillage and static abiotic soil properties on microbial diversity. *Appl. Soil Ecol.* 132, 135–145. <https://doi.org/10.1016/j.apsoil.2018.08.016>.
- Li, H., Wang, H., Jia, B., Li, D., Fang, Q., Li, R., 2021. Irrigation has a higher impact on soil bacterial abundance, diversity and composition than nitrogen fertilization. *Sci. Rep.* 11, 16901. <https://doi.org/10.1038/s41598-021-96234-6>.
- Li, X., Liu, T., Li, H., Geisen, S., Hu, F., Liu, M., 2022. Management effects on soil nematode abundance differ among functional groups and land-use types at a global scale. *J. Anim. Ecol.* 91, 1770–1780. <https://doi.org/10.1111/1365-2656.13744>.
- Lian, J., Wang, H., Deng, Y., Xu, M., Liu, S., Zhou, B., Jangid, K., Duan, Y., 2022. Impact of long-term application of manure and inorganic fertilizers on common soil bacteria in different soil types. *Agric. Ecosyst. Environ.* 337, 108044. <https://doi.org/10.1016/j.agee.2022.108044>.
- Liang, C., Qi, J., Wu, W., Chen, X., Li, M., Liu, Y., Peng, Z., Chen, Shi, Pan, H., Chen, B., Liu, J., Wang, Y., Chen, Sanfeng, Du, S., Wei, G., Jiao, S., 2024. Smaller microorganisms outcompete larger ones in resistance and functional effects under disturbed agricultural ecosystems. *iMeta*. doi: 10.1002/imt.219.
- Liang, Y., Ning, D., Lu, Z., Zhang, N., Hale, L., Wu, L., Clark, I.M., McGrath, S.P., Storkey, J., Hirsch, P.R., Sun, B., Zhou, J., 2020. Century long fertilization reduces stochasticity controlling grassland microbial community succession. *Soil Biol. Biochem.* 151, 108023. <https://doi.org/10.1016/j.soilbio.2020.108023>.
- Magoč, T., Salzberg, S.L., 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27, 2957–2963. <https://doi.org/10.1093/bioinformatics/btr507>.
- Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet journal* 17, 10. <https://doi.org/10.14806/ej.17.1.200>.
- Morrieto, E., Hannula, S.E., Snoek, L.B., Helmsing, N.R., Zwers, H., de Hollander, M., Soto, R.L., Bouffaud, M.-L., Buée, M., Dimmers, W., Duyts, H., Geisen, S., Girlanda, M., Griffiths, R.L., Jørgensen, H.-B., Jensen, J., Plassart, P., Redecker, D., Schmelz, R.M., Schmidt, O., Thomson, B.C., Tisserant, E., Uroz, S., Winding, A., Bailey, M.J., Bonkowski, M., Faber, J.H., Martin, F., Lemanceau, P., de Boer, W., van Veen, J.A., van der Putten, W.H., 2017. Soil networks become more connected and take up more carbon as nature restoration progresses. *Nat. Commun.* 8, 14349. <https://doi.org/10.1038/ncomms14349>.
- Morugán-Coronado, A., García-Orenes, F., McMillan, M., Pereg, L., 2019. The effect of moisture on soil microbial properties and nitrogen cyclers in Mediterranean sweet orange orchards under organic and inorganic fertilization. *Sci. Total Environ.* 655, 158–167. <https://doi.org/10.1016/j.scitotenv.2018.11.174>.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., 2013. *Package Vegan*. R Packag Ver 2, 1–295.
- Oliverio, A.M., Geisen, S., Delgado-Baquerizo, M., Maestre, F.T., Turner, B.L., Fierer, N., 2020. The global-scale distributions of soil protists and their contributions to belowground systems. *Sci. Adv.* 6, 1–10. <https://doi.org/10.1126/sciadv.aax8787>.
- Orgiazzi, A., Bardgett, R.D., Barrios, E., 2016. *Global soil biodiversity atlas*. European Commission.
- Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18, 1403–1414. <https://doi.org/10.1111/1462-2920.13023>.
- Peng, Z., Qian, X., Liu, Y., Li, X., Gao, H., An, Y., Qi, J., Jiang, L., Zhang, Y., Chen, S., Pan, H., Chen, B., Liang, C., van der Heijden, M.G.A., Wei, G., Jiao, S., 2024. Land conversion to agriculture induces taxonomic homogenization of soil microbial communities globally. *Nat. Commun.* 15, 3624. <https://doi.org/10.1038/s41467-024-47348-8>.
- Pöhlme, S., Abarenkov, K., Henrik Nilsson, R., Lindahl, B.D., Clemmensen, K.E., Kausserud, H., Nguyen, N., Kjoller, R., Bates, S.T., Baldrian, P., Froslev, T.G., Adojaan, K., Vizzini, A., Suija, A., Pfister, D., Baral, H.-O., Järvi, H., Madrid, H., Nordin, J., Liu, J.-K., Pawłowska, J., Pöldmaa, K., Pärtel, K., Rønnel, K., Hansen, K., Larsson, K.-H., Hyde, K.D., Sandoval-Denis, M., Smith, M.E., Toome-Heller, M., Wijayawardene, N.N., Menolli, N., Reynolds, N.K., Drenkhan, R., Maharachchikumbura, S.S.N., Gibertoni, T.B., Læssøe, T., Davis, W., Tokarev, Y., Corrales, A., Soares, A.M., Agan, A., Machado, A.R., Argüelles-Moyao, A., Dederich, A., de Meiras-Ottoni, A., Verbeke, A., Dutta, A.K., Cui, B.-K., Pradeep, C.K., Marín, C., Stanton, D., Gohar, D., Wanasinghe, D.N., Otsing, E., Aslani, F., Griffith, G.W., Lumsch, T.H., Grossart, H.-P., Masigol, H., Timling, I., Hiesalu, I., Oja, J., Kupagme, J.Y., Geml, J., Alvarez-Manjarrez, J., Ilves, K., Loit, K., Adamson, K., Nara, K., Küngas, K., Rojas-Jimenez, K., Bitenieks, K., Irinyi, L., Nagy, L.G., Soonvald, L., Zhou, L.-W., Wagner, L., Aime, M.C., Öpik, M., Mujica, M.I., Metsoja, M., Ryberg, M., Vasar, M., Murata, M., Nelsen, M.P., Cleary, M.,

- Samarakoon, M.C., Doilom, M., Bahram, M., Hagh-Doust, N., Dulya, O., Johnston, P., Kohout, P., Chen, Q., Tian, Q., Nandi, R., Amiri, R., Perera, R.H., dos Santos Chikowski, R., Mendes-Alvarenga, R.L., Garibay-Orijel, R., Gielen, R., Phookamsak, R., Jayawardena, R.S., Rahimlou, S., Karunarathna, S.C., Tibpromma, S., Brown, S.P., Sepp, S.-K., Mundra, S., Luo, Z.-H., Bose, T., Vahter, T., Netherway, T., Yang, T., May, T., Varga, T., Li, W., Coimbra, V.R.M., de Oliveira, V. R.T., de Lima, V.X., Mikryukov, V.S., Lu, Y., Matsuda, Y., Miyamoto, Y., Kõljalg, U., Tedersoo, L., 2020. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers.* 105, 1–16. <https://doi.org/10.1007/s13225-020-00466-2>.
- Postma-Blaauw, M.B., de Goede, R.G.M., Bloem, J., Faber, J.H., Brussaard, L., 2012. Agricultural intensification and de-intensification differentially affect taxonomic diversity of predatory mites, earthworms, enchytraeids, nematodes and bacteria. *Appl. Soil Ecol.* 57, 39–49. <https://doi.org/10.1016/j.apsoil.2012.02.011>.
- Pothula, S.K., Grewal, P.S., Auge, R.M., Saxton, A.M., Bernard, E.C., 2019. Agricultural intensification and urbanization negatively impact soil nematode richness and abundance: a meta-analysis. *J. Nematol.* 51, 1–17. <https://doi.org/10.21307/jofnem-2019-011>.
- Riedo, J., Wettstein, F.E., Rosch, A., Herzog, C., Banerjee, S., Buchi, L., Charles, R., Wachter, D., Martin-Laurent, F., Bucheli, T.D., Walder, F., Van Der Heijden, M.G.A., 2021. Widespread occurrence of pesticides in organically managed agricultural soils—The ghost of a conventional agricultural past? *Environ. Sci. Technol.* 55, 2919–2928. <https://doi.org/10.1021/acs.est.0c06405>.
- Romdhane, S., Spor, A., Banerjee, S., Breuil, M.C., Bru, D., Chabbi, A., Hallin, S., van der Heijden, M.G.A., Saghai, A., Philippot, L., 2022. Land-use intensification differentially affects bacterial, fungal and protist communities and decreases microbiome network complexity. *Environ. Microbiomes* 17, 1–15. <https://doi.org/10.1186/s40793-021-00396-9>.
- Sabir, M.S., Shahzadi, F., Ali, F., Shakeela, Q., Niaz, Z., Ahmed, S., 2021. Comparative effect of fertilization practices on soil microbial diversity and activity: an overview. *Curr. Microbiol.* 78, 3644–3655. <https://doi.org/10.1007/s00284-021-02634-2>.
- Soliveres, S., Van Der Plas, F., Manning, P., Prati, D., Gossner, M.M., Renner, S.C., Alt, F., Arndt, H., Baumgartner, V., Binkenstein, J., Birkhofer, K., Blaser, S., Blüthgen, N., Boch, S., Böhm, S., Börschig, C., Buscot, F., Diekötter, T., Heinze, J., Hölzel, N., Jung, K., Klaus, V.H., Kleinebecker, T., Klemmer, S., Krauss, J., Lange, M., Morris, E. K., Müller, J., Oelmann, Y., Overmann, J., Pašalić, E., Rillig, M.C., Schaefer, H.M., Schloter, M., Schmitt, B., Schöningh, I., Schrumpf, V., Socher, S.A., Solly, E.F., Sonnemann, I., Sorkau, E., Steckel, J., Steffan-Dewenter, I., Stempfhuber, B., Tschapka, M., Türke, M., Venter, P.C., Weiner, C.N., Weisser, W.W., Werner, M., Westphal, C., Wilcke, V., Wolters, V., Wubet, T., Wurst, S., Fischer, M., Allan, E., 2016. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature* 536, 456–459. <https://doi.org/10.1038/nature19092>.
- Stefan, G., Cornelia, B., Jörg, R., Michael, B., 2014. Soil water availability strongly alters the community composition of soil protists. *Pedobiologia (Jena)*. 57, 205–213. <https://doi.org/10.1016/j.pedobi.2014.10.001>.
- Sünnemann, M., Alt, C., Kostin, J.E., Lochner, A., Reitz, T., Siebert, J., Schädlér, M., Eisenhauer, N., 2021. Low-intensity land-use enhances soil microbial activity, biomass and fungal-to-bacterial ratio in current and future climates. *J. Appl. Ecol.* 58, 2614–2625. <https://doi.org/10.1111/1365-2664.14004>.
- Szoboszlay, M., Dohrmann, A.B., Poeplau, C., Don, A., Tebbe, C.C., 2017. Impact of land-use change and soil organic carbon quality on microbial diversity in soils across Europe. *FEMS Microbiol. Ecol.* 93, 1–12. <https://doi.org/10.1093/femsec/fix146>.
- Taberlet, P., Prud'Homme, S.M., Campione, E., Roy, J., Miquel, C., Shehzad, W., Gielly, L., Rioux, D., Choler, P., Clément, J.C., Melodelima, C., Pompanon, F., Coissac, E., 2012. Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Mol. Ecol.* 21, 1816–1820. <https://doi.org/10.1111/j.1365-294X.2011.05317.x>.
- Tedersoo, L., Anslan, S., Bahram, M., Pölme, S., Riit, T., Liiv, I., Kõljalg, U., Kisand, V., Nilsson, H., Hildebrand, F., Bork, P., Abarenkov, K., 2015. Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MycKeys* 10, 1–43. <https://doi.org/10.3897/mycokeys.10.4852>.
- Tedersoo, L., Bahram, M., Pölme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., Ruiz, L.V., Vasco-Palacios, A.M., Thu, P.Q., Suija, A., Smith, M.E., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Pöldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Perts, K., Pärtel, K., Ötsing, E., Nounra, E., Njouonkou, A.L., Nilsson, R.H., Morgado, L.N., Mayor, J., May, T.W., Majuakim, L., Lodge, D.J., Lee, S.S., Larsson, K.-H., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T. W., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F.Q., Bonito, G., Anslan, S., Abell, S., Abarenkov, K., 2014. Global diversity and geography of soil fungi. *Science* (80) 346, 1052–1053. <https://doi.org/10.1126/science.1256688>.
- Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., de Ruiter, P.C., van der Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jørgensen, H.B., Christensen, S., Hertefeldt, T.D., Hotes, S., Gera Hol, W.H., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pizl, V., Stary, J., Wolters, V., Hedlund, K., 2015. Intensive agriculture reduces soil biodiversity across Europe. *Glob. Chang. Biol.* 21, 973–985. <https://doi.org/10.1111/gcb.12752>.
- Turley, N.E., Bell-Dereske, L., Evans, S.E., Brudvig, L.A., 2020. Agricultural land-use history and restoration impact soil microbial biodiversity. *J. Appl. Ecol.* 57, 852–863. <https://doi.org/10.1111/1365-2664.13591>.
- Vahter, T., Sepp, S.K., Astover, A., Helm, A., Kikas, T., Liu, S., Oja, J., Öpik, M., Penu, P., Vasar, M., Veromann, E., Zobel, M., Hiiesalu, I., 2022. Landscapes, management practices and their interactions shape soil fungal diversity in arable fields – evidence from a nationwide farmers' network. *Soil Biol. Biochem.* 168. <https://doi.org/10.1016/j.soilbio.2022.108652>.
- van Capelle, C., Schrader, S., Brunotte, J., 2012. Tillage-induced changes in the functional diversity of soil biota – a review with a focus on German data. *Eur. J. Soil Biol.* 50, 165–181. <https://doi.org/10.1016/j.ejsobi.2012.02.005>.
- van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., de Goede, R.G.M., Adams, B.J., Ahmad, W., Andriuzzi, W.S., Bardgett, R.D., Bonkowski, M., Campos-Herrera, R., Cares, J.E., Caruso, T., de Brito Caixeta, L., Chen, X., Costa, S.R., Creamer, R., da Cunha, M., Castro, J., Dam, M., Djigal, D., Escuer, M., Griffiths, B.S., Gutiérrez, C., Hohberg, K., Kalinkina, D., Kardol, P., Kergunteuil, A., Korthals, G., Krashevskaya, V., Kudrin, A.A., Li, Q., Liang, W., Magilton, M., Marais, M., Martín, J.A.R., Matveeva, E., Mayad, E.H., Mulder, C., Mullin, P., Neilson, R., Nguyen, T.A.D., Nielsen, U.N., Okada, H., Rius, J.E.P., Pan, K., Peneva, V., Pellissier, L., Pereira, C., da Silva, J., Pitteloud, C., Powers, T.O., Powers, K., Quist, C.W., Rasmann, S., Moreno, S.S., Scheu, S., Setälä, H., Sushchuk, A., Tiunov, A.V., Trap, J., van der Putten, W.S., Vestergård, M., Villenave, C., Waeyenberge, L., Wall, D.H., Wilschut, R., Wright, D.G., Yang, J., 2019. Soil nematode abundance and functional group composition at a global scale. *Nature* 572, 194–198. <https://doi.org/10.1038/s41586-019-1418-6>.
- van Eekeren, N., Bommele, L., Bloem, J., Schouten, T., Rutgers, M., de Goede, R., Reheul, D., Brussaard, L., 2008. Soil biological quality after 36 years of ley-arable cropping, permanent grassland and permanent arable cropping. *Appl. Soil Ecol.* 40, 432–446. <https://doi.org/10.1016/j.apsoil.2008.06.010>.
- Vazquez, C., de Goede, R.G.M., Korthals, G.W., Rutgers, M., Schouten, A.J., Creamer, R., 2019. The effects of increasing land use intensity on soil nematodes: a turn towards specialism. *Funct. Ecol.* 33, 2003–2016. <https://doi.org/10.1111/1365-2435.13417>.
- Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G.A.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proc. Natl. Acad. Sci.* 111, 5266–5270. <https://doi.org/10.1073/pnas.1320054111>.
- Walder, F., Schmid, M.W., Riedo, J., Valzano-Held, A.Y., Banerjee, S., Büchi, L., Bucheli, T.D., van der Heijden, M.G.A., 2022. Soil microbiome signatures are associated with pesticide residues in arable landscapes. *Soil Biol. Biochem.* 174, 108830. <https://doi.org/10.1016/j.soilbio.2022.108830>.
- Wang, D., Trimpos, K.B., Gomes, S.I.F., Jacquemyn, H., Merckx, V.S.F.T., 2024. Metabarcoding read abundances of orchid mycorrhizal fungi are correlated to copy numbers estimated using ddPCR. *New Phytol.* 242, 1825–1834. <https://doi.org/10.1111/nph.19385>.
- Wang, Z., Wang, Z., Li, T., Wang, C., Dang, N., Wang, R., Jiang, Y., Wang, H., Li, H., 2021. N and P fertilization enhanced carbon decomposition function by shifting microbes towards an r-selected community in meadow grassland soils. *Ecol. Indic.* 132, 108306. <https://doi.org/10.1016/j.ecolind.2021.108306>.
- White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols. Elsevier, San Diego, CA: Academic Press Inc., pp. 315–322. doi: 10.1016/B978-0-12-372180-8.50042-1.
- Wolejko, E., Jabłońska-Trypuć, A., Wydro, U., Butarewicz, A., Łozowicka, B., 2020. Soil biological activity as an indicator of soil pollution with pesticides – a review. *Appl. Soil Ecol.* 147, 103356. <https://doi.org/10.1016/j.apsoil.2019.09.006>.
- Xu, A., Liu, J., Guo, Z., Wang, C., Pan, K., Zhang, F., Pan, X., 2021. Soil microbial community composition but not diversity is affected by land-use types in the agro-pastoral ecotone undergoing frequent conversions between cropland and grassland. *Geoderma* 401, 115165. <https://doi.org/10.1016/j.geoderma.2021.115165>.
- Xue, L., Ren, H., Brodribb, T.J., Wang, J., Yao, X., Li, S., 2020. Long term effects of management practice intensification on soil microbial community structure and co-occurrence network in a non-timber plantation. *For. Ecol. Manage.* 459, 117805. <https://doi.org/10.1016/j.foreco.2019.117805>.
- Yin, R., Kardol, P., Eisenhauer, N., Schädlér, M., 2022. Land-use intensification reduces soil macrofauna biomass at the community but not individual level. *Agric. Ecosyst. Environ.* 337. <https://doi.org/10.1016/j.agee.2022.108079>.
- Zhang, B., Li, Y., Ren, T., Tian, Z., Wang, G., He, X., Tian, C., 2014. Short-term effect of tillage and crop rotation on microbial community structure and enzyme activities of a clay loam soil. *Biol. Fertil. Soils* 50, 1077–1085. <https://doi.org/10.1007/s00374-014-0929-4>.
- Zhao, Z.-B., He, J.-Z., Quan, Z., Wu, C.-F., Sheng, R., Zhang, L.-M., Geisen, S., 2020. Fertilization changes soil microbiome functioning, especially phagotrophic protists. *Soil Biol. Biochem.* 148, 107863. <https://doi.org/10.1016/j.soilbio.2020.107863>.
- Zhao, Z., Ma, Y., Zhang, A., Chen, Y., Zheng, Z., Zheng, W., Zhai, B., 2023. Response of apple orchard bacteria co-occurrence network pattern to long-term organic fertilizer input. *Appl. Soil Ecol.* 191, 105035. <https://doi.org/10.1016/j.apsoil.2023.105035>.
- Zhu, L., Chen, Y., Sun, R., Zhang, J., Hale, L., Dumack, K., Geisen, S., Deng, Y., Duan, Y., Zhu, B., Li, Y., Liu, W., Wang, X., Griffiths, B.S., Bonkowski, M., Zhou, J., Sun, B., 2023. Resource-dependent biodiversity and potential multi-trophic interactions determine belowground functional trait stability. *Microbiome* 11, 1–17. <https://doi.org/10.1186/s40168-023-01539-5>.
- Zinger, L., Chave, J., Coissac, E., Iribar, A., Louisanna, E., Manzi, S., Schilling, V., Schimann, H., Sommeria-Klein, G., Taberlet, P., 2016. Extracellular DNA extraction is a fast, cheap and reliable alternative for multi-taxa surveys based on soil DNA. *Soil Biol. Biochem.* 96, 16–19. <https://doi.org/10.1016/j.soilbio.2016.01.008>.