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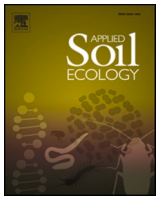

Research paper

Land use intensity differently affects soil microbial functional communities in arable fields

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ABSTRACT

Land use intensification can influence soil microbial communities and their functional potential. However, the impacts of different aspects of land use intensification on functional groups of soil microbes remain insufficiently elucidated in agroecosystems. This study investigated soil microbial groups and their functional potential in arable fields embedded in a gradient of land use intensity (LUI), integrating multiple agricultural practices in the Netherlands. The results reveal changes in soil bacterial and fungal functional groups along the LUI gradient, with a strong negative relationship between LUI and the diversity of many fungal functional groups. Changes in LUI also led to significant changes in the composition of both bacterial and fungal functional communities. Specifically, irrigation and pest control were identified as the primary practices influencing the community assembly of soil microbial functional groups. We further show that the connectivity between soil fungal functional groups decreased under higher LUI. In summary, our findings demonstrate that reducing land use intensity may have positive impacts on the functional potential of soil microbial communities, particularly for soil fungi. Sustainable management practices particularly related to irrigation and pest control may alleviate some of the observed adverse effects.

1. Introduction

The imperative of ensuring food production to sustain an increasing human population has led to intensive land management, such as high-level fertilization and tillage, and the overuse of pesticides, which can have negative impacts on soil microbes, consequently hampering multiple ecosystem functions (Thomson et al., 2015). Over the last several decades, numerous studies on soil microbial communities have shown that shifts in land use intensification can have significant effects on the ecosystem functions in agroecosystems, for example affecting plant productivity, soil carbon cycling, and nitrogen retention (Cozim-Melges et al., 2024; Romdhane et al., 2022; Tsiafouli et al., 2015).

It is increasingly recognized that individual agricultural management practices that contribute to land use intensification can have significant impacts on the diversity and community structure of soil microbial functional groups executing those functions (Hannula et al., 2021; Meyer et al., 2014; Schmidt et al., 2019; Verbruggen et al., 2010; Vermue et al., 2013). For example, intensive tillage and pesticide application resulted in a consistent reduction in the abundance, root colonization rate, and diversity of arbuscular mycorrhizal fungi (AMF) (Edlinger et al., 2022; Wetzel et al., 2014). This reduction can impede the role of mycorrhizal symbiosis in nutrient acquisition and stress mitigation, consequently hindering the goal of sustainable agriculture. There is growing evidence that intensive land use negatively impacts the biomass of soil saprotrophic fungi, which constitute 50–80 % of the overall fungal community in arable fields (Clocchiatti et al., 2020; de Vries and Bardgett, 2012; Põlme et al., 2020). These reductions in fungal biomass hamper their associated ecosystem functions, such as the cycling of carbon and nitrogen in the soil (de Vries et al., 2011; Six et al., 2006). Besides, research has shown that continuous monocultures can increase the numbers and activity of soil-borne pathogens, such as *Fusarium,* leading to increased risks of root rot e.g. in maize (Govaerts

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et al., 2008; Wang et al., 2020; Yang et al., 2021). Apart from fungi, land use intensification can also influence soil bacterial functional groups (Ikoyi et al., 2020; Lee et al., 2020). For instance, in situations where organic fertilizers are used, the activity of ammonia-oxidizing bacteria can be enhanced and this can contribute to N mineralization (Ouyang et al., 2018). In addition, the responses of different functional groups to changes in LUI may vary due to their differences in strategies of acquiring nutrients (Banerjee et al., 2019; Hannula et al., 2021). For instance, Schmidt et al. (2019) found that no-tillage management changes the symbiotroph and saprotroph ratio to favor symbiotrophs.

Most previous studies, as exemplified above, have focused on soil microbial taxonomic diversity or on individual functional groups in control experiments or fields with single or paired land use practices (Edlinger et al., 2022; Guzman et al., 2021; Romdhane et al., 2022), neglecting the fact that multiple agricultural practices are implemented simultaneously in actual agroecosystems. The impact of different aspect of agricultural management and the combination of practices (i.e. land use intensity (LUI)) on the functional and ecological diversity of whole soil microbial communities in real agroecosystems is still poorly understood (Labouyrie et al., 2023), and it remains to be clarified if these impacts differ for distinct microbial groups (Thomson et al., 2015). It is commonly known that different soil microbial functional groups are involved in a range of ecological processes (e.g., nitrogen fixation, carbon cycling, water retention) and are essential for delivering multiple ecosystem functions (Bardgett and van der Putten, 2014; Trivedi et al., 2019; Wagg et al., 2019). A better understanding of how land use intensification influences the whole suite of soil microbial functional communities is therefore key to ensure the provision of ecosystem functions and services in agroecosystems.

In addition to its well-known impacts on the diversity and structure of soil microbial communities, land use intensification may influence the interrelationships among soil microbes and change their associations. Individual agricultural practices (e.g., tillage, application of pesticde) can impact a specific taxon and initiate cascading effects throughout the entire metacommunity, facilitated by species co-variation within these multitrophic associations (Morriën et al., 2017; Banerjee et al., 2019). For instance, Chen et al. (2024) reported that excessive N fertilizer application resulted in the formation of a more complex microbial network due to intensified microbial competition in paddy soils. Han et al. (2022) observed that increasing pesticide use reduced microbial network complexity with simplified soil microbial functional diversity. Such alterations in the soil microbial network may impact the soil microbial functions owing to changes in the flow of energy and materials thorugh the entire soil communities (de Vries et al., 2018; Chen et al., 2024). Additionally, evidence is mounting that intensive agricultural practices, such as excessive use of synthetic fertilizers and pesticides, excessive tillage, and growing monocultures can disrupt the soil microbial community structure and lead to a loss of rare species in arable soils, consequently enhancing homogeneity of soil communities (Banerjee et al., 2024; Chen et al., 2024). Therefore, improved knowledge of the responses by the entire co-occurrence structure to the combined effects of land use intensification beyond those of individual agricultural practices will enable us to better predict the consequences of agricultural land management on ecosystem functions (Cozim-Melges et al., 2024).

In the study, our aim was to investigate the impacts of land use intensification on multiple functional groups of soil bacterial and fungal communities. To address this objective, we collected soil samples from 39 arable fields spanning a gradient of land use intensification in the east of the Netherlands. Land use intensification was assessed using an integrated land use intensity (LUI) index reflecting common agricultural practices applied simultaneously by farmers. We mapped ecologically relevant functions of identified bacteria and fungi based on two, annotated functional gene databases, FAPROTAX (Louca et al., 2016) and FungalTraits (Põlme et al., 2020), to examine responses of microbial functional groups. We expected that an increase in LUI would consistently reduce the diversity of soil bacterial and fungal groups. Moreover, higher LUI was expected to reduce soil microbial diversity and thereby leading to a lower complexity in the soil microbial co-occurrence network. This study provides practical insights into agricultural management practices that can sustain soil microbial biodiversity and maintain soil health.

2. Material and methods

2.1. Soil sampling

The study was conducted at 39 farms with conventional management practices in the Achterhoek region, in the east of the Netherlands, during the summer of 2022 (Table S1). At each field, a 10 m × 10 m square area was chosen for soil sampling. Each sampling area was located in the center of the field and had a buffer zone of at least 20 m from the field edges. The 10 m × 10 m square was then divided into 100 1 m × 1 m plots which were numbered from 1 to 100. Three plots were randomly selected for soil sampling in each site, where soil cores were collected (0–20 cm depth). All soil samples in each site were passed through a 2 mm sieve to homogenize the soil and to remove large roots and stones. The collected soil samples were stored at −20 °C for microbial DNA extraction.

2.2. LUI index

A land use intensity index was derived from a detailed farmer questionnaire on land management practices at each farm. Information of the questionnaire included mineral and organic fertilization of nitrogen and phosphorus, crop diversity, liming, tillage, irrigation, and pest control. For crop diversity, information was collected on diversity in space (i.e. the number of crops that are planted on the same plot per season on average between 2018 and 2022, referred to as Crop number) and time (the number of crops that are included in the crop sequence over the 2018–2022 period, referred to as Crops in rotation). We also recorded the number of years the plots was cultivated as grassland over the same 5-year period, referred to as Years in grassland. These three variables were recorded as a numerical value and were standardized relative to their maximum value to obtain intensity indices comprised between 0 (lowest intensity) and 1 (maximum observed intensity across the 39 plots). To avoid overweighing of crop diversity, we calculated a cropland diversity index (CDI) as a composite index of Crops in rotation, Crop number and Years in grassland (Eq. (1)):

$$\mathrm{CDI} = \frac{\mathit{Crops\ in\ rotation} + \mathit{Crop\ number} + \mathit{Years\ in\ grassland}}{\mathit{max}(\mathit{Crops\ in\ rotation} + \mathit{Crop\ number} + \mathit{Years\ in\ grassland})} \quad (1)$$

The other variables were recorded as categories: irrigation (0; 1–40; 41–80; >80 mm/season), tillage frequency (0; 1; 2; 3; 4; >4 times/season), mineral and organic N application (for both: 0; 1–25; 26–75; 76–125; 176–225; >225 kgN/ha), mineral and organic P fertilization (for both: 0; 1–30; 31–60; >60 kgP_2O_5/ha), pest control (yes; no) and liming (yes; no). Those variable were also transformed into normalized indices. We assigned each category a numerical value on a scale from 0 to 1, with 0 representing the lowest ordinal category and 1 representing the highest. For example, in the case of irrigation, we assigned the values 0, 0.33, 0.67 and 1 to categories 0 mm/season, 1–40 mm/season, 41–80 mm/season and > 80 mm/season, respectively. To avoid overweighing fertilization, we calculated a fertilization intensity index (FII) as the composite index of five different indices: liming, organic and mineral P fertilizer application and organic and mineral N fertilizer application (Eq. (2)).

$$\mathrm{FII} = \frac{\mathit{minN} + \mathit{orgN} + \mathit{minP} + \mathit{orgP} + \mathit{Lim}}{\mathit{max}(\mathit{minN} + \mathit{orgN} + \mathit{minP} + \mathit{orgP} + \mathit{Lim})} \quad (2)$$

where minN, orgN, minP, orgP and Lim are the normalized intensity

indices for mineral N, organic N, mineral P and organic P application, respectively, and Lim is the normalized liming intensity index.

The land use intensity was then calculated as the sum of indices of different practices normalized to the maximum of the sum, including the compiled indices FII and CDI. An assumption of this method is that all indices have a equally important contribution of the overall land use intensity index (Eq. (3)).

$$\mathrm{LUI} = \frac{FII + TII + III + PII + CDI}{max(FII + TII + III + PII + CDI)} \quad (3)$$

In addition, to visualize the different responses of microbial functional groups to LUI, the samples were divided into "low" and "high" land use intensity classes based on PCA clustering group of intensity metrics of different land use practices (Fig. S1).

2.3. Soil molecular analysis

We analyzed bacterial and fungal community composition using high-throughput amplicon sequencing of bacterial 16S ribosomal RNA genes (16S rRNA) and fungal internal transcribed spacer (ITS) regions. Genomic DNA was extracted from 0.5 g homogenized soil samples using the Power Soil DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The bacterial 16S RNA genes and fungal ITS regions were amplified with primer 515F/806R (Apprill et al., 2015; Caporaso et al., 2011; Parada et al., 2016) and ITS3/ITS4 (Tedersoo et al., 2014, 2015; White et al., 1990) respectively. PCR products were attached with IDT indexes, followed by cleanup using AMPure magnetic beads (Beckman Coulter, Brea, CA, USA) and pooling into equimolarity. The end pool was sequenced with an Illumina NovaSeq platform (BaseClear, Leiden) producing 250-bp paired-end sequences.

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 Exact Sequence Variants (ESVs) using UNOISE2 (Edgar, 2016), with $\alpha = 2$ and a minimal accepted abundance before clustering of 8 reads.

For taxonomic annotation, ESV 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 ESVs to genus level (Beentjes et al., 2019). The LCA script was performed on the top 100 hits, using the top 5 % 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), and 16S rRNA gene sequences against local copies of the respective NCBI GenBank (Benson et al., 2005) datasets (downloaded 16-03-2022). All ESVs that contributed to <0.05 % of the total reads of the sample were removed for each sample before downstream analyses. For bacteria there were nonbacterial ESVs and for fungi there were nonfungal ESVs which were removed from the abundance table.

The potential ecological functions of bacterial groups were assigned by matching the taxonomic information of the ESVs to the functional annotation of the Prokaryotic Taxa (FAPROTAX) database (Louca et al., 2016), which is a conservative algorithm currently matching 80 functions against 7600 functional annotations of 4600 prokaryotic taxa. The bacterial functional groups with a relative abundance >1 % were used for further statistical analysis. Species can belong to multiple functional groups as microbial individuals may be related to multiple functions in different life history stages or habitats. Based on the FungalTraits database (Põlme et al., 2020), fungal ESVs were assigned into functional groups: saprotrophic fungi, yeasts, potential plant pathogens (including endophytes and potential plant pathogens and saprotrophs and potential plant pathogens), molds, wood parasites and decomposers, AMF and others (functional groups with few reads, including ectomycorrhizal fungi, unclassified Chytridiomycota, mycoparasitic fungi, ericoid mycorrhizal fungi, nematophagous fungi, unclassified Mucoromycota, moss associated fungi and animal pathogenic and parasitic fungi) (Table S2). The ESVs that could not be identified into functional groups were categorized as unclassified Ascomycota, unclassified Basidiomycota, and the general category "unclassified fungi". The ESVs only occurred at one or more low-intensity sites or high-intensity sites were identified as LUI-site-specific species.

2.4. Data analysis

All statistical analyses were conducted using packages in R (v.4.2.3). We selected ESVs richness (observed ESV numbers in per sample) and Shannon index to characterize alpha diversity of soil microbial functional groups. We performed linear mixed models with crop identity (Table S1) as a random factor to analyze the relationship between land use intensity and the Shannon diversity, relative abundance of soil bacterial and fungal functional groups. We checked models for residual normality and log or square root transformation of data was used when model residuals were not normally distributed. The data on relative abundance of functional groups were Hellinger pre-transformed as the data included many zeros to avoid overemphazing the impacts of rare species (Legendre and Gallagher, 2001). Generalized linear mixed models with negative binomial residuals (glmer.nb: to account for overdispersion) were used to analyze the relationship between land use intensity and the richness of species within soil bacterial and fungal functional groups. The effect of high vs. low LUI on soil bacterial and fungal composition was estimated using PERMANOVA based on Bray-Curtis dissimilarity matrix (999 permutations) using the "vegan" package (Oksanen et al., 2013) in which we include "crop identity" as a blocking factor ("strata") to account for variation between different crops. To examine the effects of individual land use practices (e.g., irrigation intensity, fertilization intensity) on the composition of distinct functional groups, Redundancy Analysis (RDA) was carried out to reveal the relationship between individual land use practices and the composition of distinct functional groups (nine fungal functional groups and nine bacterial functional groups) with Bray-Curtis dissimilarities. The individual effect of different land use practices was examined by "rdacca.hp" package (Lai et al., 2022).

Co-occurrence network analysis has been widely used to reveal the complex interrelationships among the myriad of microbes and to elucidate the associations between different soil microbial groups (Barberán et al., 2012; Faust and Raes, 2012; Morriën 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 changes in land use intensity (LUI) on soil microbial community structure and community assembly (Banerjee et al., 2019; Hu et al., 2021). Therefore, a co-occurrence network was constructed to explore the microbial interrelationships between bacterial and fungal functional groups separately. To construct the co-occurrence network with the same number of samples, 15 samples were randomly selected from all samples under low LUI (out of 24 samples, Table S1). Only ESVs that summed to a relative abundance >0.1 % and presented in more than three samples were retained for network analysis. Co-occurrence networks of microbial functional groups were established based on Spearman's correlation matrices using the "igraph" package. We focused on ESVs that strongly co-occurred in the network with an adjusted cutoff 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 including number of nodes, average degree, average path length, and betweenness centralization were calculated in Gephi.

3. Results

3.1. Relationship between LUI and the diversity of soil microbial functional groups

There were only marginal effects of LUI on the diversity and relative abundance of soil bacterial functional groups (Table 1, Table S3), which is consistent with bacterial taxonomic results (Fig. S2). Significant responses were observed for some of bacterial functional groups, especially with nitrogen-related functions (e.g., nitrification, nitrate reduction), and these groups were non-dominant within the bacterial communities (Table S2). Increasing LUI lead to a reduction in the diversity of nitrification (Shannon diversity, $R^2 = 0.10$, $p = 0.04$), aerobic nitrite oxidation (Shannon diversity, $R^2 = 0.11$, $p = 0.02$), nitrate reduction (richness, $R^2 = 0.17$, $p < 0.01$; Shannon diversity, $R^2 = 0.14$, $p = 0.01$). For relative abundance, increasing LUI decreased the relative abundance of nitrate reduction ($R^2 = 0.11$, $p = 0.03$), while it increased the relative abundance of methylotrophy ($R^2 = 0.06$, $p = 0.09$) and anaerobic chemoheterotrophy ($R^2 = 0.11$, $p = 0.03$) (Table S3).

Significant effects of LUI were observed for several fungal functional groups (Table 1, Table S3), with ESVs richness, Shannon index and relative abundance consistently decreasing with increased LUI (Fig. 1 & Fig. S3). These findings are consistent with the fungal taxonomic diversity (Fig. S2). Among fungal functional groups, the strongest negative correlations with LUI were detected for the richness and relative abundance of wood parasites and decomposers ($R^2 = 0.17$, $p < 0.01$) and for potential plant pathogens ($R^2 = 0.15$, $p = 0.01$), for Shannon diversity of saprotrophic fungi ($R^2 = 0.24$, $p < 0.01$) and for wood parasites and decomposers ($R^2 = 0.15$, $p = 0.01$). In addition, increasing LUI lead to a significant reduction in the relative abundance of several fungal functional groups, including wood parasites and decomposers ($R^2 = 0.17$, $p = 0.08$), AMF ($R^2 = 0.07$, $p = 0.09$), mold ($R^2 = 0.12$, $p = 0.03$) and unclassified fungi ($R^2 = 0.10$, $p = 0.04$) (Table S3).

3.2. Relationship between LUI and the composition of soil microbial functional groups

We detected a significant impact of LUI on the community composition of several bacterial groups related to potential functions, including methylotrophy ($R^2 = 0.14$, $p < 0.01$), anaerobic chemoheterotrophy ($R^2 = 0.09$, $p < 0.01$), nitrification ($R^2 = 0.09$, $p < 0.01$), aerobic nitrite oxidation ($R^2 = 0.09$, $p = 0.01$), chemoheterotrophy ($R^2 = 0.06$, $p < 0.01$) and aerobic chemoheterotrophy ($R^2 = 0.06$, $p < 0.01$) (Table 2). These results align with the finding that differences in LUI influenced the community composition of both fungal and bacterial communities (Fig. S4). Similar to responses in richness, we detected that differences in LUI significantly influenced the community dissimilarity of many fungal functional groups. These groups included yeasts ($R^2 = 0.09$, $p < 0.01$), unclassified fungi ($R^2 = 0.08$, $p < 0.01$), saprotrophic fungi ($R^2 = 0.07$, $p < 0.01$), unclassified Ascomycota ($R^2 = 0.05$, $p < 0.01$), and potential plant pathogens ($R^2 = 0.04$, $p = 0.04$) (Table 2).

We further investigated how the composition of bacterial and fungal functional groups was related to specific land use practices. Irrigation and pest control were the two main practices determining the composition of dominant soil bacterial functional groups, including chemoheterotrophy, methylotrophy, anaerobic chemoheterotrophy (Fig. 2). Aerobic chemoheterotrophy and nitrogen fixation and aromatic compound degradation, were only affected by irrigation, and aromatic compound degradation was only affected by pest control. Finaly, nitrate reduction was only affected by nitrogen fertilization (Fig. 2 & Fig. S5). Fungal functional groups were also affected by diverse sets of practices. Similar to bacterial groups, saprotrophic fungi and yeasts were affected by both irrigation and pest control (Fig. 3). Potential plant pathogens and molds as well as the unclassified Ascomycota and unclassified Basidiomycota were only affected by irrigation. Finnaly, wood parasites and decomposers were only affected by nitrogen fertilization (Fig. 3 & Fig. S6).

3.3. Co-occurrence networks of soil fungal functional groups along the LUI gradient

Lastly, we explored the co-occurrence networks of soil microbes under low and high LUI. Species belonging to all fungal functional groups were presented in sites with low LUI. However, at high LUI, some functional groups such as AMF and wood decomposers were absent and the network was dominated by saprotrophic fungi and unclassified Ascomycota (Fig. 4a, b, c). Network topology parameters including the number of nodes ($p < 0.01$) and degree ($p < 0.01$) were significantly higher at sites with low LUI compared to sites with high LUI (Fig. 4d).

Table 1
The effects of land use intensity (LUI index, 0–1) on the diversity (ESVs richness and Shannon index) of soil bacterial and fungal functional groups.

Functional groups	Richness (number of ESVs)				Shannon index			
	Estimate	z value	*p*	Pseudo R^2	Estimate	t-value	*p*	Pseudo R^2
Bacterial functional groups								
Chemoheterotrophy	−0.19	−0.71	0.48	0.01	−0.25	−1.11	0.27	0.03
Aerobic chemoheterotrophy	−0.26	−0.96	0.34	0.03	−0.31	−1.37	0.17	0.05
Methylotrophy	0.85	1.25	0.21	0.04	0.65	1.11	0.27	0.03
Anaerobic chemoheterotrophy	0.63	1.10	0.27	0.03	0.65	1.17	0.24	0.03
Aromatic compound degradation	0.52	0.85	0.40	0.02	0.57	1.10	0.27	0.03
Nitrification	−1.09	−1.57	0.12	0.06	**−1.26**	**−2.04**	**0.04**	**0.10**
Aerobic nitrite oxidation	−1.07	−1.43	0.15	0.06	**−1.29**	**−2.29**	**0.02**	**0.11**
Nitrogen fixation	−0.27	−0.39	0.70	<0.01	0.02	0.05	0.96	<0.01
Nitrate reduction	**−2.37**	**−2.77**	**<0.01**	**0.17**	**−1.59**	**−2.43**	**0.01**	**0.14**
Fungal functional groups								
Saprotrophic fungi	**−0.85**	**−2.34**	**0.02**	**0.11**	**−1.22**	**−3.47**	**<0.01**	**0.24**
Yeasts	**−0.96**	**−1.73**	**0.08**	**0.09**	**−0.81**	**−1.85**	**0.06**	**0.08**
Wood parasites and decomposers	**−2.48**	**−2.79**	**<0.01**	**0.17**	**−1.75**	**−2.57**	**0.01**	**0.15**
Potential plant pathogens	**−1.61**	**−2.52**	**0.01**	**0.15**	−0.95	−1.46	0.14	0.05
AMF	**−5.42**	**−2.27**	**0.02**	**0.12**	**−1.63**	**−2.12**	**0.03**	**0.11**
Molds	**−1.97**	**−2.42**	**0.02**	**0.14**	**−1.62**	**−2.54**	**0.01**	**0.15**
Unclassified Ascomycota	−0.23	−0.47	0.64	0.01	−0.25	−0.49	0.63	0.01
Unclassified Basidiomycota	**−1.36**	**−2.32**	**0.02**	**0.11**	**−1.03**	**−1.70**	**0.09**	**0.07**
Unclassified fungi	−0.57	−1.05	0.30	0.03	**−1.03**	**−1.78**	**0.08**	**0.08**

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

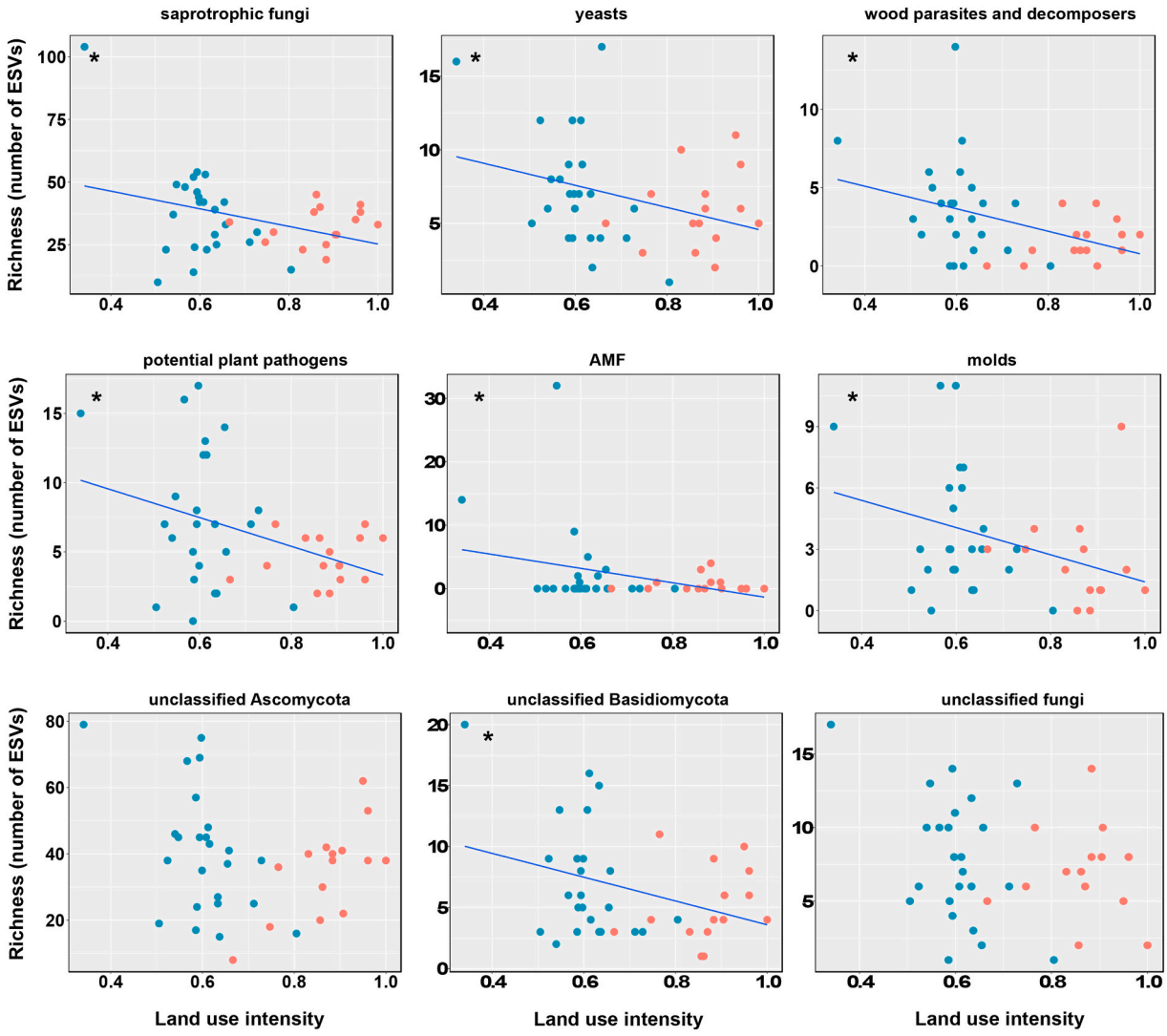

Fig. 1. Effects of LUI on the richness of fungal functional groups. Blue and red dots denote sites under low land intensity and high land use intensity, respectively. The asterisk indicates (marginally) significant impacts ($p < 0.10$) of LUI on the richness of different soil fungal functional groups. Shaded area indicate 95 % confidence intervals fitted lines.

We further investigated the fungal ESVs present at low and high LUI sites. Low-intensity sites held more LUI-site-specific ESVs than high-intensity sites, and an increase in LUI led to a drastic reduction in LUI-site-specific ESVs for all fungal functional groups (Fig. 5, Table S4). In particular, over two-thirds of LUI-site-specific AMF and mold species had disappeared in high-intensity sites.

The co-occurrence networks for bacterial communities included many ESVs were not classified into specific functional groups (Fig. S7). The nodes of bacterial co-occurrence networks were higher in sites with high LUI. Several bacterial functional groups were absent in sites with high LUI but occur in sites under low LUI. This result is consistent with the results that there were fewer LUI-site-specific bacterial ESVs, in particularly for groups including chemoheterotrophy, aerobic chemoheterotrophy and anaerobic chemoheterotrophy, under high LUI (Fig. S8).

4. Discussion

An emerging body of research suggests that land use intensification has negative effects on the taxonomic diversity of soil communities in agroecosystems which induces shifts in soil microbial composition (Banerjee et al., 2024; Cozim-Melges et al., 2024; Romdhane et al., 2022; Tsiafouli et al., 2015). However, few studies investigated the consequences on functional groups of soil microbes under real farm where multiple agricultural practices are applied simultaneously. In the present study, based on a regional sampling campaign, we investigated the effects of land use intensification integrated from multiple agricultural practices, on soil bacterial and fungal functional potentials in arable soil. Our results showed that land use intensification resulted in significant shift in diversity, community structure and co-occurrence network properties of soil bacterial and fungal functional communities. Since this study was conducted across an extensive range of arable fields at different locations with different agricultural management regimes, the reported results can be generalized and enhance our ability to maintain soil microbial functional diversity and promote sustainability in agroecosystem.

4.1. Responses of soil bacterial and fungal functional groups to LUI

Our results indicate that LUI significantly reduced the diversity within multiple soil fungal functional groups, whereas there are limited responses on the diversity of bacterial functional groups. This is in partial contrast with our first expectation, and suggests there might be higher functional redundancy in soil bacterial communities than for soil

Table 2
Summary statistics of a PERMANOVA testing the effects of LUI on the composition of soil bacterial and fungal functional groups. Presented are degrees of freedom, variance explained (R^2), F-values and *p*-values. The values in bold denote (marginally) significant correlations at $p < 0.10$.

Functional groups	df1,df2	F-value	R^2	*p*-value
Bacterial functional groups				
Chemoheterotrophy	1,37	**2.37**	**0.06**	**<0.01**
Aerobic chemoheterotrophy	1,37	**2.36**	**0.06**	**<0.01**
Methylotrophy	1,32	**5.39**	**0.14**	**<0.01**
Anaerobic chemoheterotrophy	1,34	**3.31**	**0.09**	**<0.01**
Aromatic compound degradation	1,37	1.26	0.03	0.277
Nitrification	1,34	**3.42**	**0.09**	**<0.01**
aerobic nitrite oxidation	1,34	**3.53**	**0.09**	**0.01**
Nitrogen fixation	1,37	1.04	0.03	0.34
Nitrate reduction	1,30	1.37	0.04	0.18
Fungal functional groups				
Saprotrophic fungi	1,37	**2.79**	**0.07**	**<0.01**
Yeasts	1,37	**3.74**	**0.09**	**<0.01**
Wood parasites and decomposers	1,30	**1.37**	**0.04**	**0.08**
Potential plant pathogens	1,36	**1.56**	**0.04**	**0.04**
AMF	1,11	1.09	0.09	0.29
Molds	1,33	1.52	0.04	0.13
Unclassified Ascomycota	1,37	**1.95**	**0.05**	**<0.01**
Unclassified Basidiomycota	1,37	**1.65**	**0.04**	**0.09**
Unclassified fungi	1,37	**3.07**	**0.08**	**<0.01**

fungal communities in arable soils (Li et al., 2024; Louca et al., 2018). We also found negative impacts of LUI on bacterial groups related to nitrogen cycling, but not to functional groups related to carbon cycling (Table 1). This is consistent with a recent study which showed bacterial functional redundancy for carbon metabolism in arable soils while the nitrogen fixation potential was reduced (Peng et al., 2024).

Notably, the richness and Shannon diversity of all fungal functional groups were consistently negatively affected by increased in LUI. In previous studies, the response of fungal functional diversity to LUI has been shown to vary (Clocchiatti et al., 2020; Hannula et al., 2021; Schmidt et al., 2019). For example, intensive fertilization can significantly reduce the diversity of AMF (Williams et al., 2017), whereas it can increase the diversity of saprotrophic fungi (Song et al., 2015). In addition, a field study on an abandoned arable fields observed that long-term tillage changed the ratio between saprotrophs and symbiotrophs but had no effects on the relative abundance of pathotrophs (Schmidt et al., 2019). Unlike other studies, our research was conducted under an integrated LUI context where multiple agricultural practices were implemented concurrently in operational farms in the same region. Although it is possible that different practices contributing to LUI may have distinct impacts on specific functional groups, the consistent effects on fungal functional groups highlight that increasing LUI leads to a strong reduction in the functional diversity, consequently reducing the functional redundancy of the fungal community.

4.2. Individual land use practices and composition of functional groups of soil microbes

Land use practices such as fertilization, tillage and irrigation are important factors that affect the diversity and community structure of soil microbes (Cozim-Melges et al., 2024; Navarro-Noya et al., 2013; Singh et al., 2011; Williams et al., 2017). Our results revealed relationships between the composition of soil microbial functional groups and changes in LUI. Surprisingly, we found that irrigation affected the composition of multiple bacterial and fungal groups most, including that of chemoheterotrophy, aerobic chemoheterotrophy, methylotrophy, anaerobic chemoheterotrophy and nitrogen fixation, saprotrophic fungi, yeast, molds and potential plant pathogens (Fig. S9 & Fig. S10). Additionally, we found that the effects of irrigation intensity on bacterial functional groups were inconsistent, whereas there were negative relationships between the irrigation intensity and the diversity of fungal functional groups. This suggests that an increase in the intensity of irrigation is likely to have deleterious impacts on fungal functional groups, which are assumed to be less resistant to dry-rewetting cycles than bacteria (Bapiri et al., 2010; de Vries et al., 2012). Overall, our results suggest that irrigation practice is a crucial component of LUI and that it plays an important role in determining the composition of bacterial and fungal functional groups. This is especially important in the current context of frequent summer drought events.

Additionally, we observed that pest control was one of the main practices influencing the community assembly of several microbial functional groups, such as chemoheterotrophy, methylotrophy, saprotrophic fungi and yeasts, which were dominant groups in the arable fields that we sampled (Table S2). This is in line with numerous studies that indicate that the intensive application of pesticides has negative impacts on soil communities (Riedo et al., 2021; Vahter et al., 2022; Wołejko et al., 2020), highlighting that reduction of pesticide applications can promote the soil functional diversity. In summary, these results indicated that agricultural practices for alleviating drought and controlling pathogen play a crucial role in shaping soil microbial functional communities. Adjusting these practices can be important for maintaining soil microbial functional diversity and enhancing sustainability in agroecosystems.

The composition of AMF was mainly affected by nitrogen fertilization. This finding is in agreement with recent studies showing that long-term nitrogen fertilization has significant impacts on shaping the AMF community (Williams et al., 2017; Xiang et al., 2014). Increasing fertilization inputs may change the ecological assembly processes of AMF communities towards increased competition because photosynthates from plants become an increasingly limiting resource (Liu et al., 2015). We noticed that there were more site-specific AMF taxa in fields under low LUI than under high LUI (Fig. 5), suggesting that intensive nitrogen fertilization might lead to more uniform AMF assemblages in arable fields (Verbruggen et al., 2010). This result also supports recent findings from Banerjee et al. (2024) that environmental heterogenization leads to more rare species in sites under less intensive land use. Lastly, in line with previous results which presented evidence for a relationship between wood decay fungi and nitrogen fertilization (Moore et al., 2021), we observed that nitrogen fertilization was the main driver of the composition of wood parasites and decomposers. Overall, our findings add to the growing body of evidence identifying key practices determining the abundance of soil microbes, which is important for developing microbiota management strategies for “smart farming” (Hartman et al., 2018).

4.3. LUI influences the network complexity of soil fungal functional communities

Soil functional groups do not thrive in isolation and rather form complex interconnected networks (Fierer, 2017; Morriën et al., 2017). A change in one functional group can affect other groups through interactions such as competition and antagonism among different groups (Faust and Raes, 2012; Hannula et al., 2021). Microbial co-occurrence networks can shed insight into the microbial interactions and their responses to LUI (Banerjee et al., 2019; Romdhane et al., 2022; Xue et al., 2022). In consistent with our second expectation, our study shows that the co-occurrence network of fungal functional groups was less connected across high LUI sites as compared to low LUI ones, implying the loss of associations among fungal functional groups, accompanied with a potential disruption of the interactions between functional groups. The fungal network under high LUI was also mainly constructed by dominant groups (e.g., saprotrophic fungi, yeasts and molds) and some functional groups were missing. Interestingly, the decreased network complexity follows the response observed for fungal diversity, which suggests that changes in soil fungal diversity were mirrored in fungal networks. The reduction in the fungal network complexity could be

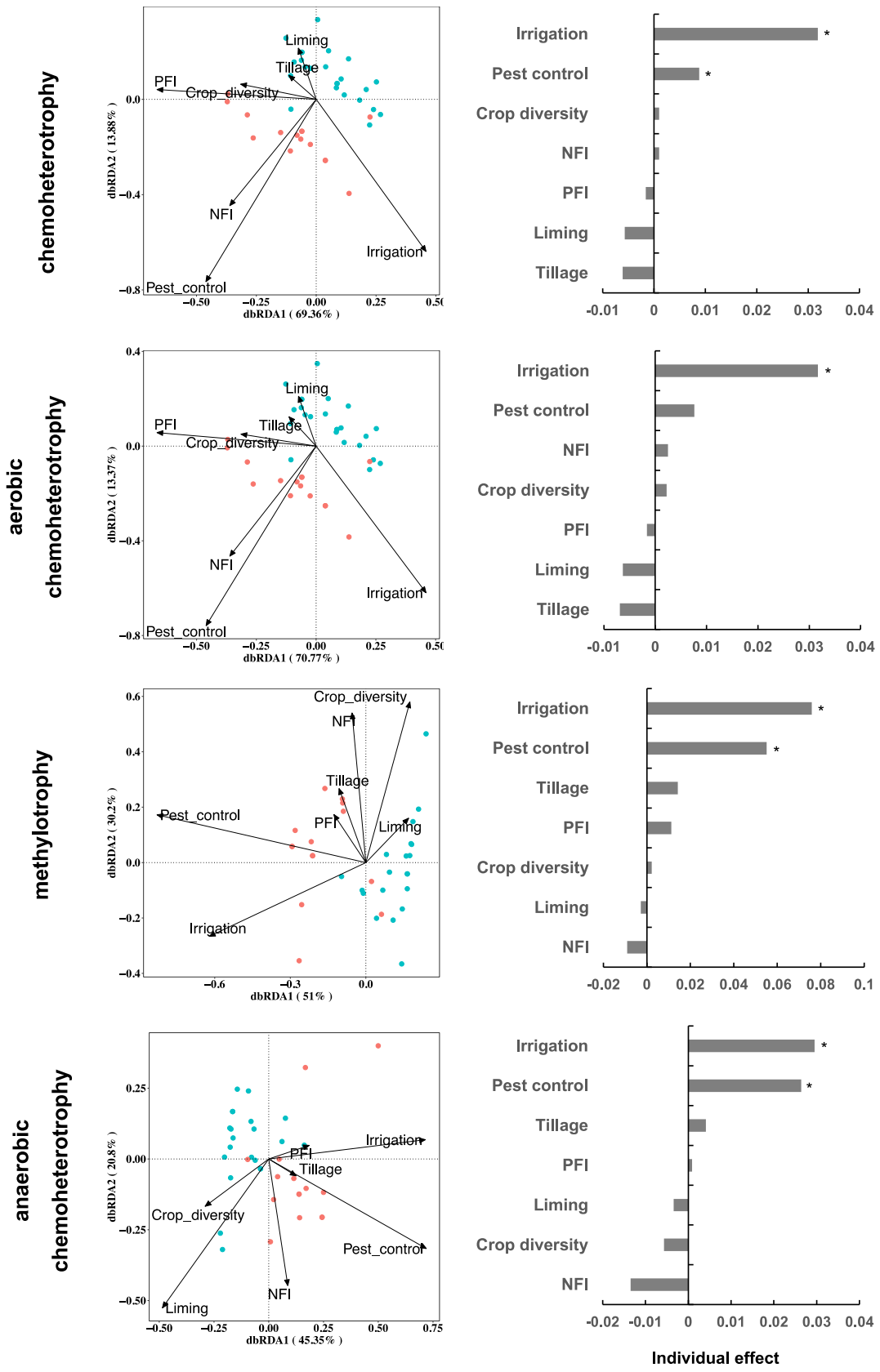

Fig. 2. Redundancy analysis of main soil bacterial functional groups, chemoheterotroph (n = 39), aerobic chemoheterotrophy (n = 39), methylotrophy (*n* = 34), anaerobic chemoheterotrophy (*n* = 36), as affected by different agricultural practices. Blue and red dots denote sites under low land intensity and high land use intensity, respectively. Bar plots show the relative importance (calculated based on the rdacca.Hp package) of individual agricultural practices on the composition of soil fungal functional groups. * indicates significant impacts of individual practices on the community composition of different soil fungal functional groups. Note: PFI, phosphorus fertilization intensity; NFI, nitrogen fertilization intensity.

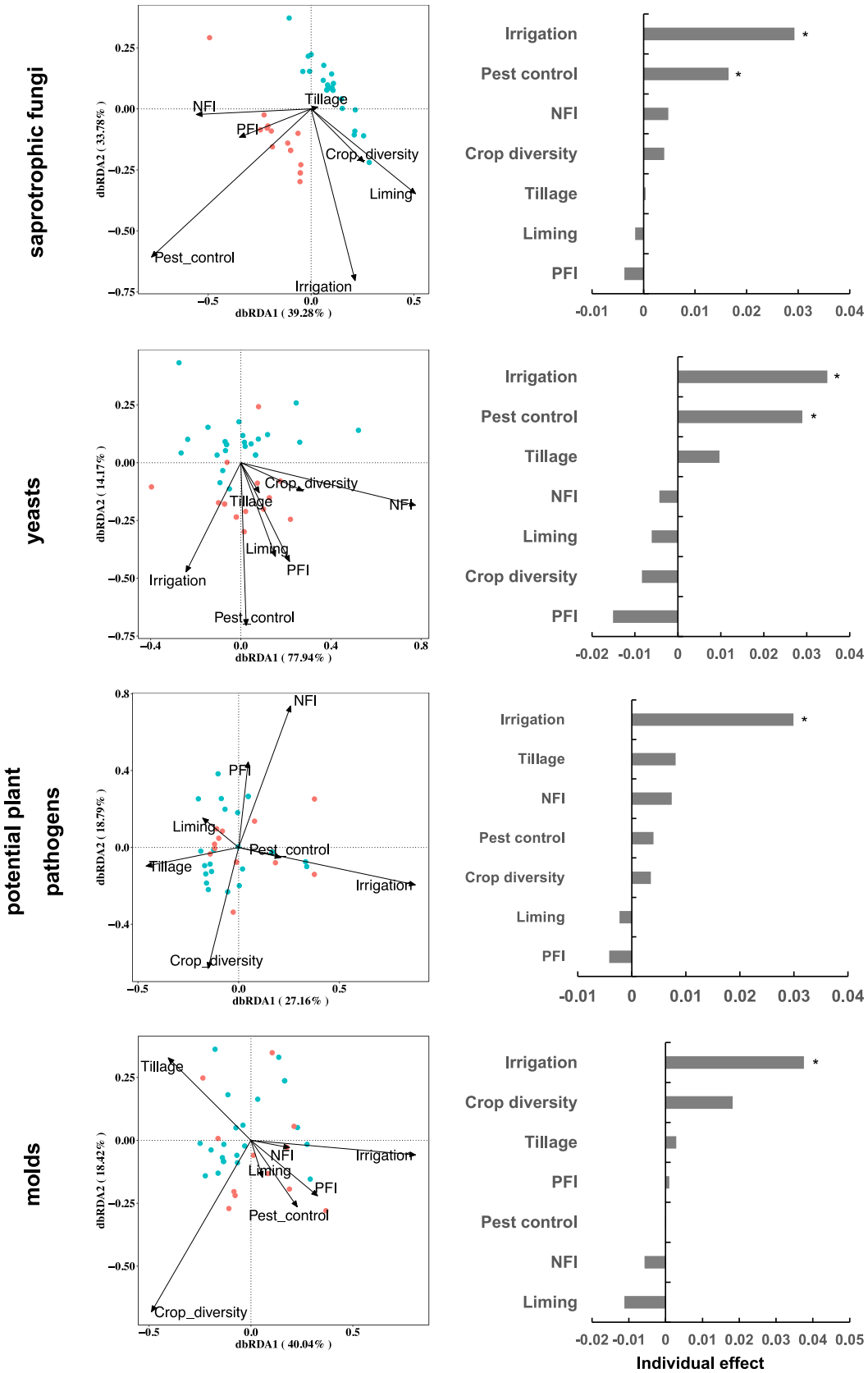

Fig. 3. Redundancy analysis of main soil fungal functional groups, saprotrophic fungi (n = 39), yeasts (n = 39), potential plant pathogens (n = 38), molds (n = 35), as affected by different agricultural practices. Blue and red dots denote sites under low land intensity and high land use intensity, respectively. Bar plots show the relative importance (calculated based on the rdacca.Hp package) of individual agricultural practices on the composition of soil fungal functional groups. * indicates significant impacts of individual practices on the community composition of different soil fungal functional groups. Note: PFI, phosphorus fertilization intensity; NFI, nitrogen fertilization intensity.

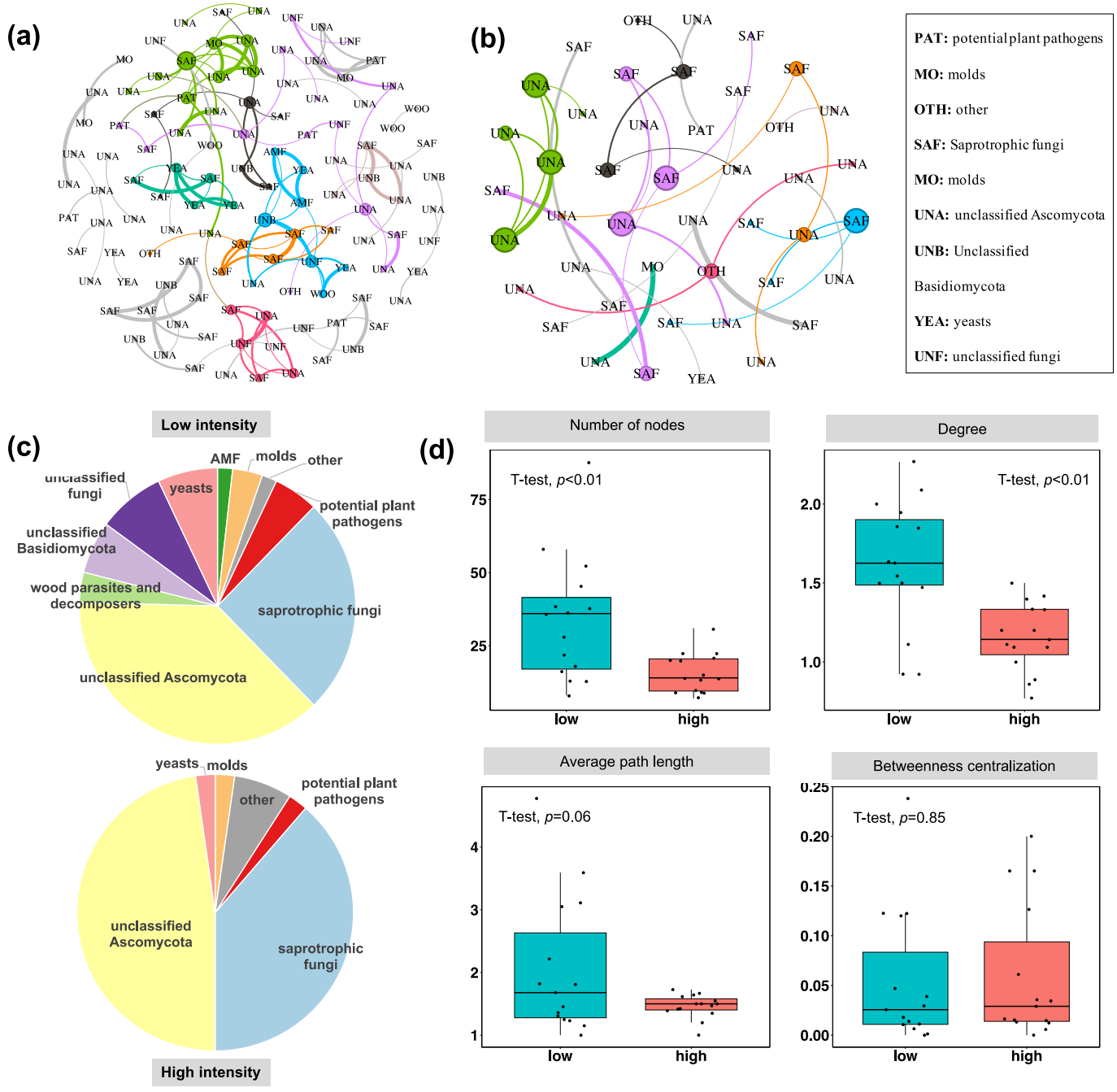

Fig. 4. Meta-community co-occurrence network of soil fungi with nodes colored according to network modularity for low land use intensity (a), and high land use intensity (b). Number of involved functional groups in co-occurrence networks (c). Node-level topological features under low and high land use intensities (d).

attributed to the drastic loss of LUI-site-specific ESVs of individual functional groups with increasing LUI. Most fungal groups lost >50 % of the site-specific species under high LUI. Especially AMF lost many LUI-site-specific species (75 %) (Table S4), suggesting a biotic homogenization among fungal functional groups with increasing LUI (Gossner et al., 2016).

The co-occurrence network of soil bacteria showed different patterns, with increased nodes under high LUI. However, we also observed that fewer species were involved in bacterial co-occurrence networks in high LUI sites, and that LUI-site-specific species within bacterial functional groups were missing with increased LUI (Fig. S8). Taken together, these findings are in accordance with recent studies that reports that land use intensification can exert a strong homogenizing effect on soil microbial communities (Aslani et al., 2024; Peng et al., 2024), resulting in functional homogenization with a reduced occurrence of rare species (Banerjee et al., 2024). Since the soil microbial functional groups are a fundamental component of ecosystem functions (Bardgett and van der Putten, 2014; Trivedi et al., 2019; Wagg et al., 2019), functional homogenization arising from land use intensification can lead to the potential loss of ecosystem functions (Hautier et al., 2017; van der Plas et al., 2016).

5. Conclusions

Using an analysis of bacteria and fungi in real farms across a gradient of LUI, we showed that land use intensification has consistent negative effects on the diversity of fungal functional groups and only marginal effects on the diversity of soil bacterial functional groups. We observed a

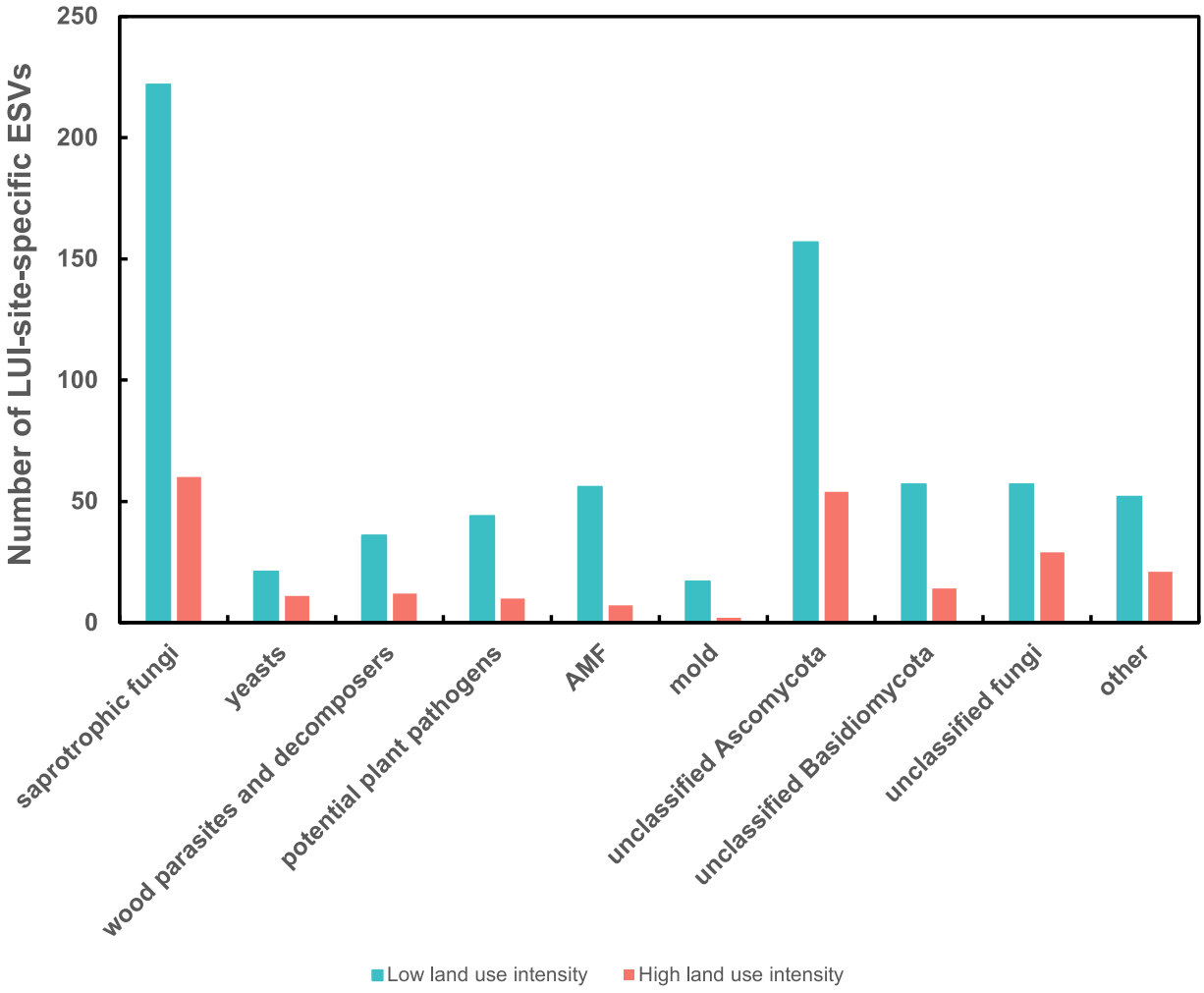

Fig. 5. Richness of the LUI-site-specific fungal ESVs in sites under low land use intensity and high land use intensity.

clear reduction in the network complexity of soil fungal communities, and species composition was homogenized in both bacterial and fungal functional communities with increasing LUI. These findings show that reduced LUI can foster more diverse soil microbial functional communities, potentially improving soil functional redundancy which is crucial for the resilience of agroecosystems. Notably, our study identifies irrigation and pest control as the most important land use practices shaping the composition of most bacterial and fungal functional groups along the LUI gradient in arable fields. More real-world surveys such as ours are needed to explore optimal combinations of agricultural practices for conserving soil biodiversity and supporting sustainable agricultural systems. This study highlights the importance to comprehensively assess the impacts of land use intensification on soil functional diversity in agroecosystems, rather than using a single or few taxonomic-based indicators under specific agricultural practices, as different bacterial and fungal functional groups exhibit differential responses.

CRediT authorship contribution statement

Chenguang Gao: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Emilia Hannula:** Writing – review & editing, Visualization, Methodology, Formal analysis, Conceptualization. **Peter M. van Bodegom:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **T. Martijn Bezemer:** Writing – review & editing, Methodology, Formal analysis. **Franciska T. de Vries:** Writing – review & editing, Methodology, Formal analysis. **Jan Hassink:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation. **Michiel H. in 't Zandt:** Writing – review & editing, Methodology, Investigation, Data curation. **Gabriel Y. K. Moinet:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2024.105723.

Data availability

Data will be made available on request.

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