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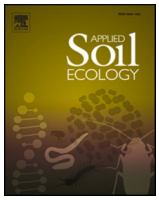

Research paper

Soil depth shapes the microbial response to land use and climate change in agroecosystems

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ABSTRACT

Soil microbial communities are vital for ecosystem functions and are strongly affected by land use and climate change, yet the specific impacts in deeper topsoil layers remain unclear. This study investigates these effects across three topsoil layers after eight years of experimental treatments at the Global Change Experimental Facility (GCEF) in order to unravel the role of different topsoil layers in the response of microbial communities to land use and climate change. Distinct effects of land use and climate change on microbial biomass, community structure, and functions in agroecosystems were observed, with the upper 15 cm of soil exhibiting the strongest responses, and more pronounced land use impacts than those of climate change. Although spring climate treatment including higher precipitation and higher temperature provided favorable conditions for microbes, negative effects, possibly a legacy from previous summer droughts, persisted. Despite a decrease in microbial abundance and activity with depth, a diverse microbial community persisted throughout the topsoil due to organic material input. Grasslands exhibited greater changes in microbial community structure and reduced biomass and functionality with depth, whereas tilled croplands showed less pronounced depth effects. Thus, deeper topsoil layers were more critical for soil functionality in croplands. Surprisingly, responses to experimental treatments were partly reversed in deeper soil layers compared to the uppermost layer, suggesting a buffering role of deeper layers against disturbances. These findings emphasize the importance of considering soil depth and land management practices in global change studies to fully understand impacts on soil health and ecosystem functioning. However, croplands' reliance on deeper soil layers suggests vulnerability to additional stressors, underscoring the need of balanced land management practices to ensure long-term ecosystem resilience.

1. Introduction

Terrestrial ecosystems have undergone drastic changes due to industrialization and globalization (IPBES, 2019; Isbell et al., 2023). Agroecosystems now cover more than 40 % of the terrestrial surface, ensuring high crop production in order to meet the growing demand for food. However, agroecosystems provide additional vital functions, including nutrient and water cycling, carbon fixation, habitat provisioning and maintenance of air quality, among others (FAO, 2020). Global change poses a significant threat to these ecosystem functions by reducing soil biodiversity, as highlighted by recent reports of IPBES (2019) and IPCC (2023). The negative impacts on soil biota, particularly on the soil microbial community, strongly impair ecosystem functioning, as soil microorganisms play a pivotal role in organic matter decomposition, nutrient cycling, plant growth promotion, and pathogen prevention (van der Heijden et al., 2008; Zhang et al., 2023a). For this reason, an increasing number of studies have focused on the influence of global change on the diversity, structure and functions of soil microbial

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communities (Jansson and Hofmockel, 2020). The majority of these studies have primarily focused on the uppermost soil layer - average depth of 18 cm for soil biology studies (Yost and Hartemink, 2020) - which contains the highest microbial biomass, diversity, and activity due to the direct access to organic matter input (Blume et al., 2002), but also exhibits the strongest responses to environmental changes (Han et al., 2017). However, knowledge about global change effects in deeper soil is limited. Deeper soil horizons exhibit distinct physicochemical properties, such as, reduced organic matter and oxygen availability, which shape microbial diversity, community structure and functions in unique ways. However, microbial communities in these layers can be activated and may contribute notably to C transformations (Stone et al., 2014; Jones et al., 2018; Min et al., 2021). Hence, deeper topsoil layers may serve as important buffer reservoir of microbial activity in the context of global change (Huang et al., 2024). However, the extent to which microbial communities in subsoil layers respond to environmental changes remains understudied. Addressing this gap is essential for a more comprehensive understanding of microbial processes across soil profiles, particularly in the context of climate change and land use type.

Both land-use intensification and climate change are considered to be the most relevant and increasingly important drivers of decreasing biodiversity and ecosystem functioning (Sala et al., 2000; Phillips et al., 2023). These global change factors strongly influence the characteristics of agroecosystems, thereby altering soil habitat conditions. Common agricultural management practices, such as tillage, not only enhance SOC loss (Baker et al., 2007; Jiao et al., 2012), but also contribute to soil erosion and nutrient leaching (Lal, 2007; Abbas et al., 2020; Bhattacharyya et al., 2022). Moreover, high use of synthetic fertilizers leads to nutrient imbalances, acidification, and salinization of soils (Guo et al., 2010; Qu et al., 2014). On the other hand, climate change, including increased mean temperatures as well as more frequent and severe droughts, reduces water availability leading to decreased nutrient availability in soils (Borken and Matzner, 2009; Nielsen and Ball, 2015). This, in turn, leads to reduced plant productivity, and consequently to a decline in the input of fresh organic matter (litter, root exudates) into the soil (Berdugo et al., 2020). In order to develop strategies to mitigate the effects of global change and maintain essential agroecosystem functions, it is crucial to understand the effects of global change factors on microbial communities and their functioning. While there is a large body of research in this area, the effect of different global change factors as well as the implementation of realistic land-use and climate scenarios have been largely neglected in the majority of ecosystem studies. Many approaches fail to capture the interactions between drivers, even though they frequently occur together and interact in complex, additive (Rillig et al., 2019), or antagonistic ways (Côté et al., 2016). In addition, most climate change experiments tend to use manipulations that are disproportionate compared to model projections, such as extreme temperatures or severe drought (Korell et al., 2020), likely leading to unrealistically strong responses.

To avoid these drawbacks, we studied the impact of land use and climate on soil microbial communities using a unique experimental field platform in Central Germany, the Global Change Experimental Facility (GCEF). The GCEF comprises five types of land use (conventional and organic farming, intensive meadow, extensive meadow, and sheep pasture) that are managed according to common regional practices and are exerted to ambient as well as to future climatic conditions. The future climate scenario has been adapted from regional climate models, and includes a moderate increase in average temperature, increased precipitation in spring and autumn, and increased summer drought (Schädler et al., 2019). Previous studies at the GCEF focusing on the soil microbial community found that land use strongly influences soil microbial biomass, diversity and activity, with a higher microbial biomass, functional diversity and respiration in grassland compared to croplands (Kostin et al., 2021; Sünnemann et al., 2021a). These land-use effects were dependent on management intensity. Intensively managed land-use systems showed lower fungal biomass and fungi-to-bacteria ratios, but on the other hand higher invertebrate feeding activity (Siebert et al., 2019) as well as higher microbial activity in the intensively managed cropland (Breitkreuz et al., 2021; Sünnemann et al., 2021b). Climate effects were found to be less pronounced, but negative impacts of the future climate were observed for soil microbial activity (Siebert et al., 2019), microbial biomass in extensive grasslands (Kostin et al., 2021), soil biodiversity (Scherzinger et al., 2024), and soil multifunctionality (Sünnemann et al., 2023). The extent of the responses to land use and climate showed intra- and interannual variability. Based on this existing knowledge and complementing previous studies, we focused on the spring season, which is a crucial phase within the vegetation period in Central Europe. Due to the already warm weather with good water supply, the middle and end of the spring season correspond to the peak in biomass production in agroecosystems, where plant-soil microbe interactions play a critical role for plant nutrient supply, health, stress resistance, and ultimately productivity (Zak et al., 2003). This might be related to the particularly pronounced land-use effects on soil characteristics and biological activity that have been observed in the spring season (Sünnemann et al., 2021b). According to climate prediction models, the spring months in the study region will become warmer and slightly wetter (Schädler et al., 2019), likely improving the growth and activity of soil microbes that in turn may lead to an improved agroecosystem performance. Therefore, one of the objectives of this study was to quantify the impact of the improved environmental conditions during the main growing season in agroecosystems of Central Europe on the structure and functions of the soil microbiome. The second main objective of this study was to elucidate the responses of the soil microbial community to land use and climate change in deeper topsoil layers. In accordance with the vast majority of ecosystem studies, previous GCEF-related studies have concentrated on the uppermost soil layer (0–15 cm), which demonstrates the most pronounced responses to environmental or management alterations, while deeper soil layers may be less or not affected (Dove et al., 2021). Nevertheless, the entire topsoil, at the study site reaching a depth of 50–60 cm, is typically a hotspot of microbial abundance and activity, and valuable insights into ecosystem processes may be lost by neglecting deeper topsoil layers. These soil layers are of particular importance for sustainable agriculture, given the challenges posed by climate change (Gross and Harrison, 2019), as they can act as a buffer against climate variability and extremes (Huang et al., 2024). By accessing water and nutrients in deeper soil layers, plants are better able to withstand periods of drought, heat stress, or heavy rainfall. To elucidate the importance of these deeper topsoil layers on the microbial response on the combined influence of land use and climate, our study was extended beyond the typical 0–15 cm depth, encompassing a second layer (15–30 cm), that is ploughed in the two arable fields but not in the grasslands, as well as a third layer (30–50 cm), that remains undisturbed across all land-use types.

The main hypotheses to be tested were as follows: we expected that (i) deeper soil layers are less affected than the uppermost soil layer by land use and climate, acting as a buffer against environmental changes. We further hypothesized (ii) that grasslands show a more diverse and functional microbial community than croplands due to a more diverse range of rhizodeposits, but expected different depth gradients for microbial community indices between tilled croplands and non-tilled grassland. Moreover, we postulated that (iii) low-intensity management promotes higher microbial biomass, diversity and activity. As low intensity systems have been shown to exhibit higher sustainability and enhanced resilience to climate change, we expected that deeper soil layers under low-intensity management will be less affected by land use and climate compared to those under high-intensity management. We further anticipated that (iv) the experimental future climate will have a positive impact on soil microbial biomass, diversity and activity in the uppermost soil layer, given that the climate manipulation in spring comprises increased rainfall and temperature. However, due to the small deviation from the ambient climate, we expected no effects of the future

climate treatment in deeper soil layers. Finally, we hypothesized that (v) the responses of the microbial community to land use and climate are less pronounced when the entire topsoil layer (0-50 cm) was considered, as opposed to the common approach of examining only the uppermost 0–15 cm.

2. Methods

2.1. Study site

The Global Change Experimental Facility (GCEF) is located at the field research station of the Helmholtz Centre for Environmental Research - UFZ in Bad Lauchstädt, Central Germany (51°23′35″N, 11°52′55″E, 118 m a.s.l.). The region has a temperate climate with a mean annual temperature of 9.0 °C and a mean annual precipitation of 483 mm between 1896 and 2021. The soil at the site is highly fertile and loamy, classified as Haplic Chernozem with an A horizon extending 50 to 60 cm depth (Altermann et al., 2005). The GCEF experimental platform was established in 2013 and comprises various land-use and climate treatments, as described in detail by Schädler et al. (2019). It includes two croplands, conventional farming (CF) and organic farming (OF), as well as three grasslands, intensive meadow (IM), extensive meadow (EM) and extensive pasture (EP). For CF, a three-year crop rotation (winter rape seed, winter wheat, winter barley) was established. While in years with winter wheat and winter barley, OF shares the same crop with CF, a nitrogen-fixing legume is planted instead of rapeseed on OF plots: (2020: Persian clover). The crop in the year of sampling was winter barley for both farming systems. In CF, mineral NPK fertilizer, growth regulators, and pesticides are used, while in OF pesticides are omitted and a more moderate fertilization is applied, including the incorporation of N-fixing legumes in the crop rotation, as well as applying less-processed rock phosphate (P-Ca-Mg) and patent kali (K-Mg-S) every three years with the legumes. In IM, a combination of five forage grasses was initially sown in 2014. Due to the summer drought in previous years, IM was reestablished in 2020 with the same species composition. This treatment involves the application of mineral NPK fertilizer, and up to four mowing events annually. In both the EM and EP treatments, a total of 56 plant species, including legumes, herbs, and grasses, were sown representing the typical species pool found in the region. No fertilizer is applied. The EM treatment is mowed twice per year, while the EP treatment is grazed by sheep three times per year, with each grazing event lasting 24 h per plot.

Half of the plots of the GCEF experience the current ambient climate (A), while the other half is subjected to future climate conditions (F). The future climate conditions include a warming treatment (average increase of mean daily temperature of 0.55 °C), accomplished by passive night warming of the plots through closed roof and side panels. The panels, together with rain sensors and an irrigation system, are also used to alter the precipitation patterns (−20 % in summer, +10 % in spring and fall) according to future climate scenarios. For further details, see Schädler et al. (2019).

2.2. Soil sampling

Bulk soil samples were taken in May 2022 from all plots, from three depths (D1: 0–15 cm, D2: 15–30 cm and D3: 30–50 cm). To account for plot heterogeneity two independent subsamples per plot were collected, each comprising a composite of three soil cores (⌀ 1.5 cm), (5 land-use types x 2 climates × 5 replicates × 3 depths × 2 subsamples = 300 samples). Samples were immediately processed through sieving (< 2 mm) and litter and roots were removed. Portions of each sample were frozen at −80 °C for amplicon sequencing, at −20 °C for quantification of N_{min}, as well as of available P and K, stored at 4 °C (for microbial biomass, basal respiration and enzymatic activity potential analysis) or air-dried for determination of pH, TC, and TN.

2.3. Abiotic soil parameters

Plant available phosphorus and potassium were extracted from fresh soil using the double lactate method (1:50 *w/v*, pH 3.6, 1.5 h). Phosphorus concentration was determined calorimetrically with the molybdenum blue method (Murphy and Riley, 1962). Plant available K was quantified from the same extracts with an ion-selective electrode (Mettler Toledo SevenExcellence pH/Ion meter, Gießen, Germany). For pH analysis, air-dried soil was suspended in 0.01 M $CaCl_2$ solution (1:2.5 (*w/v*)) and equilibrated at room temperature for 1 h. The pH was determined using a pH electrode (Mettler SevenEasyse pH meter, Gießen, Germany). TN and TC were determined in duplicates in a CHN elemental analyzer (Vario EL III, Elementar Analysensysteme GmbH, Langenselbold, Germany). Mineral nitrogen (NO_3^- and NH_4^+) was extracted from 5 g of fresh soil with 1 M KCl (1:4 w/v). The suspension was shaken horizontally (1.5 h) and filtered (Whatman Schleicher and Schuell 595 1/5 Ø 270 mm filter). The concentrations of NH_4^+-N and NO_3^--N in the clear extracts were determined using a flow injection analyzer (FIAstar 5000, Foss GmbH, Rellingen, Germany). Soil moisture content was measured with a halogen moisture analyzer (Kern DBS60–3, Kern & Sohn GmbH, Germany) and used to calculate nutrient concentrations per g of dry soil.

2.4. Microbial biomass

Microbial C and N content was determined with the chloroform-fumigation-extraction method (Vance et al., 1987). Briefly, fresh soil was fumigated with chloroform gas over 24 h to lyse microbial cells and release microbial C and N. Soluble C and N were extracted from both fumigated and non-fumigated soil with 0.05 M K_2SO_4 (1:4 w/v). The mixture was then horizontally shaken for 30 min. Clear extracts were obtained through centrifugation and C and N in the extracts were determined with a flow injection analyzer (FIAstar 5000, Foss GmbH, Rellingen, Germany) (Multi N/C, Analytik Jena, Germany). Correction factors ($k_{EC} = 0.45$ for C and $k_{EN} = 0.54$ for N) were used to account for the non-extractable part of N and C in the microbial biomass (Joergensen, 1996; Joergensen and Mueller, 1996).

2.5. Illumina MiSeq amplicon sequencing of 16S rDNA and ITS2 region

DNA was extracted from soil according to manufacturer instruction using the Qiagen DNeasy PowerSoil Pro Kit. DNA concentration in the extracts was measured with a Nanodrop ND-8000 spectrophotometer (Thermo Fischer Scientific, Dreieich, Germany). 16S rDNA fragment (for bacterial and archaeal community) and ITS2 region (for fungal community) were PCR-amplified using the KAPA HiFi DNA Polymerase and primers (515f: GTGYCAGCMGCCGCGGTAA, 805r: GGACTACHVGGGTWTCTAAT for 16S rDNA and ITS4: TCCTCCGCTTATTGATATGC and fITS7: GTGARTCATCGAATCTTTG for ITS2 region). PCR-amplification was conducted in triplicates for each sample and target region (40 ng DNA template used per PCR reaction). Success of PCR was checked with agarose gel electrophoresis (1.5 %). Amplicons from the PCR-triplicates were pooled and purified with AmpPure XP Beads, indexed in an additional PCR (Illumina Nextera XT index primers) and purified again with AmpPure XP Beads. Concentration of indexed and purified PCR-products was determined with Nanodrop ND-8000 spectrophotometer. DNA of fungal and prokaryotic amplicons were equimolarly pooled. The prokaryotic and fungal amplicon pools were combined for Illumina MiSeq paired-end sequencing. Sample libraries and the PhiX control library were diluted and denatured following the MiSeq Illumina v2 reagent kit instructions and injected into an Illumina MiSeq flow-cell (Illumina, San Diego, CA, USA) for paired-end sequencing.

2.6. Bioinformatics

MiSeq raw data was processed with the dadasnake pipeline (version 10) for primer removal, read quality control, rarefaction, ASV assignment and taxonomy assignment (Weißbecker et al., 2020). We used the SILVA 138 SSU database for 16S rDNA taxonomy assignment and UNITEv9 for ITS2 region. A total of 66,893 prokaryotic and 6581 fungal ASV entered the analysis of microbial community structure with the phyloseq (McMurdie and Holmes, 2013) and vegan package in R studio (Oksanen et al., 2012). The data set was normalized with DESeq2 (Love et al., 2014). Alpha diversity indices were calculated with the estimate_richness function in phyloseq using the unfiltered non-normalized dataset and subsequently analyzed with linear mixed model and ANOVA (Hellinger transformed data for phylum relative abundances). For beta diversity analysis, ASVs with a prevalence of less than once in 10 % of the samples were filtered from the data set. PERMANOVA (number of permutations = 999, $\alpha = 0.05$) using the same linear mixed model as in ANOVA analysis was performed for statistical analyses of Bray-Curtis dissimilarity between treatments. To analyze land-use specific depth effects, pairwise PERMANOVA was conducted for each land-use type and depth (pairwise.adonis package). Since the field site exhibits a significant pH gradient, a conditioning was introduced using the capscale function in vegan (bray-curtis_dis ~ 1 + Condition (pH)). The influence of abiotic soil parameters on the community structure was determined with the envfit function of the vegan package on both, the unconstrained PCoA ordination (using all soil parameters) and the pH-conditioned ordination (excluding pH). In both cases, fitting was performed using only one of the two field plot replicates to include the full set of soil parameters, since mineral N was not determined for the second field plot replicate.

2.7. Functional parameters of microorganisms

Hydrolytic soil enzyme activity potentials (hereafter referred to as 'enzyme activity') for six marker enzymes (cellulase, xylosidase, *N*-acetyl-glucosaminidase, sulfatase, acid phosphatase and β -glucosidase) were determined under standardized and substrate-saturating conditions using a fluorometric assay (4-methylumbelliferone (MUF)-fluorescence). Fresh soil samples were solubilized in sodium acetate buffer (50 mM, pH 5) and homogenized in an ultrasonic bath for 5 min at 30 °C. Soil suspension was incubated with 300 μM MUF-linked substrates at 25 °C (assay volume 250 μl). Standards of MUF (1.25 and 2.5 μM) incubated with and without soil suspension were measured in parallel to account for quenching effects for each soil sample specifically. Enzymatic reaction was stopped upon addition of 30 μl NaOH (1 M) after 1 h. Fluorescence of MUF was analyzed with an Infinite 200 PRO instrument (Tecan Group Ltd., Männedorf, Switzerland (ex/em: 360 nm/465 nm). Enzymatic activity was defined as turnover rate of substrate in nmol h^{-1} g^{-1} dry soil. Basal respiration was determined using an O_2-microcompensation system to measure the respiratory response of soil microorganisms using approximately 6 g of fresh soil (Scheu, 1992). Soils were acclimatized for 3 days in an airtight container at 20 °C before determining soil microbial activity (measured in μl O_2 h^{-1} g^{-1} soil dry weight and converted to μg-C CO_2 h^{-1} g^{-1}) in an 24 h interval.

2.8. Statistical analyses

All statistical analyses were conducted in R studio (R version 4.2.2). The data set was divided in the three depth levels. Climate, land use, and their interaction were fixed factors in a linear mixed model (lme4 package) (Bates et al., 2015). The split-plot design of the GCEF (Schädler et al., 2019) was analyzed with climate as the mainplot factor and land use as the subplot factor. Additionally, a linear mixed model including all depths (i.e., split-split-plot design with depth, climate, and land use as fixed factors, and depth as the sub-subplot factor) was analyzed. Abiotic soil parameters, microbial biomass, and enzyme activity differences between treatments were analyzed with ANOVA ($\alpha = 0.05$) after confirming normality and homogeneity of variances with Q-Q-plots and Levene's Test using the car package (Fox and Weisberg, 2019). In case the criteria for ANOVA was not met, log transformed data were used. The data was further analyzed with the lmerTest package (Kuznetsova et al., 2017) using a Tukey HSD post hoc test. To account for the average across the sampled depth profile (0–50 cm), weighted average values were calculated:

$$weighted\ average = \frac{D1(0-15\ cm)*15 + D2\ (15-30\ cm)*15 + D3(30-50\ cm)*20}{50}$$

Rcorr function of the Hmisc package (Harrell Jr., 2024) was used to calculate Pearson's R correlation coefficient, and respective *p*-values were approximated through the t- distribution.

3. Results

3.1. Effects on microbial community structure

Soil prokaryotic and fungal community structure were strongly dependent on the depth layer (Table S1, full model). PERMANOVA analysis based on Bray-Curtis dissimilarity revealed highly significant and land-use specific depth effects for both fungal and prokaryotic community structures (Fig. S1, Table S1). Remarkably, prokaryotic community structure of grasslands differed between all depths (Fig. S1a). In contrast, prokaryotic community structure in the croplands and fungal community across all land-use types exhibited higher structural similarity between D1 and D2, with highly significant distinction from those in D3 (Fig. S1 a, b).

In the uppermost soil layer, fungal communities showed distinct, land-use specific patterns, i.e. land-use types exhibited clear differences between their fungal community structures, except for similar community structures in extensively managed meadows (EM) and pastures (EP) (Fig. 1d and Fig. S2d). Besides the impact of soil pH and associated phosphorus availability (Fig. S2d-f), soil ammonium content strongly explained the variation in fungal community structure in D1. In deeper soil layers, fungal community structure differed mainly between croplands and grasslands (Fig. 1e, f and Fig. S2e, f).

Land-use effects on the prokaryotic community structure in D1 were less pronounced (Fig. 1a and Fig. S2a). However, a clear separation between croplands and grasslands were observed in all depth layers. Prokaryotic community structure in D1 was strongly affected by pH, P availability and TC content (Table S2, Fig. S2a), but also driven by K, P, and nitrate availability in D1, D2 and D3 respectively (Table S2, Fig. 1a-c).

3.2. Microbial community alpha diversity measures

In line with the community structure, prokaryotic and fungal alpha diversity were strongly affected by soil depth (Table S3, full model). In D1, prokaryotic alpha diversity indices were significantly influenced by land use and climate, with lowest and highest Shannon indices in the

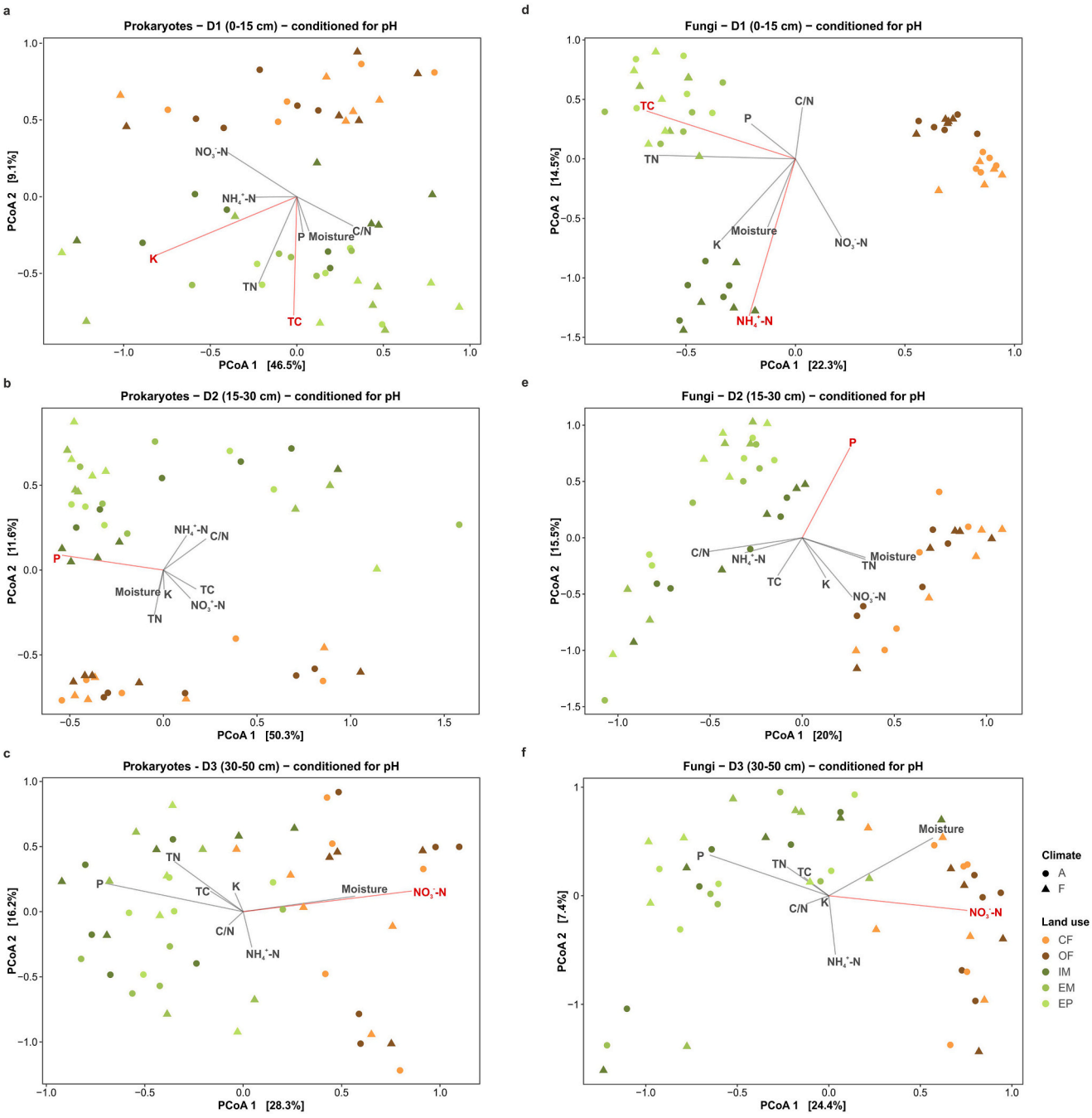

Fig. 1. Ordination of prokaryotic (left) and fungal (right) community structures separated by depth. Samples are plotted on the first two PCoA axes, PCoA1 and PCoA2, using the capscale function (vegan) with a null model but a conditioning variable (pH). The ordination plots are based on Bray-Curtis dissimilarity matrices and are grouped by land use (CF: conventional farming, OF: organic farming, IM: intensive meadow, EM: extensive meadow, EP: extensive pasture) and climate (A – ambient climate, F- future climate). Group differences were tested with PERMANOVA *(adonis2* function) and are summarized in Table S14. Influence of environmental variables on community structure was determined using the *envfit* function (vegan package), environmental variables most strongly correlated with community structure ($p < 0.1$) are marked in red color.

extensive pasture and intensive meadow, respectively. In organic farming (OF), the richness was significantly higher under future climate conditions compared to the ambient climate (Fig. S3a, Table S3 – land use x climate interaction). In contrast, richness and Shannon index of fungal ASVs were not significantly affected by climate, but only by land-use type (Table S3, Fig. S3g, j). The richness and Shannon index of fungal species in D1 were lowest in CF, followed by OF, while highest in the three grasslands.

Land-use effects on prokaryote diversity in D2 and D3 were similar to D1, while climate effects on prokaryotes were not observed (Fig. S3b, c, e, f). In contrast, a significant climate effect on fungal richness in D2 was found for the extensive meadow, with a significantly lower fungal richness in future climate versus ambient climate (Fig. S3h, Table S3, S4). Additionally, land-use effects on fungal diversity were slightly different compared to D1, with the highest and lowest richness in IM and CF, respectively. In D3, neither land-use nor climate effects were found.

Considering the complete topsoil layer (Fig. 2a-d), both prokaryotic richness and diversity were significantly higher in OF compared to those in the grasslands, which is in line with the pattern observed across all individual layers (Fig. S3). Since an opposing pattern emerged in deeper

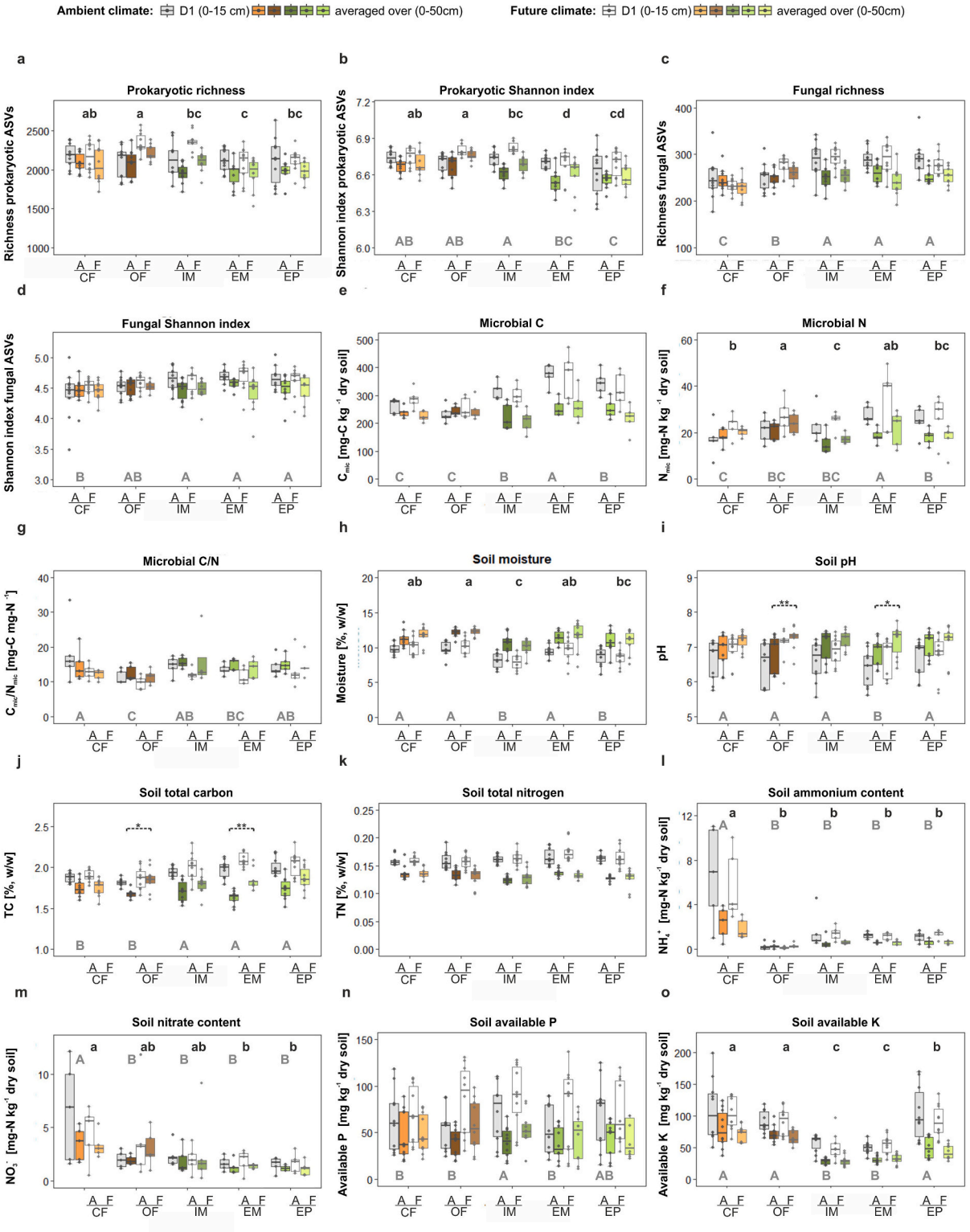

Fig. 2. Abiotic soil parameters, microbial biomass and prokaryotic alpha diversity indices across all sampling depths (0–50 cm) separated by land use and climate treatment. For each parameter the respective values of D1 (0–15 m) are plotted (grey boxplots) left from the corresponding averaged values for 0–50 cm (colored boxplots) for comparison. CF = conventional farming, OF = organic farming, IM = intensive meadow, EM = extensive meadow, EP = extensive pasture, A = ambient climate, F = future climate. Between group differences were determined for each depth layer using Tukey's HSD post hoc test. Land use types that differed significantly from each other within D1 ($p < 0.05$) are marked with a different uppercase letter, significant climate effects within one land use type are marked with: $0.1 < p < 0.05$ (.), $0.05 < p < 0.01$ (*), $0.01 < p < 0.001$ (**), $p < 0.001$ (***). Land use types that differed significantly from each other on the basis of averaged values ($p < 0.05$) are indicated by a different lowercase letter, and climate effects within a land use type are marked by: $0.1 < p < 0.05$ (.), $0.05 < p < 0.01$ (*), $0.01 < p < 0.001$ (**), $p < 0.001$ (***).

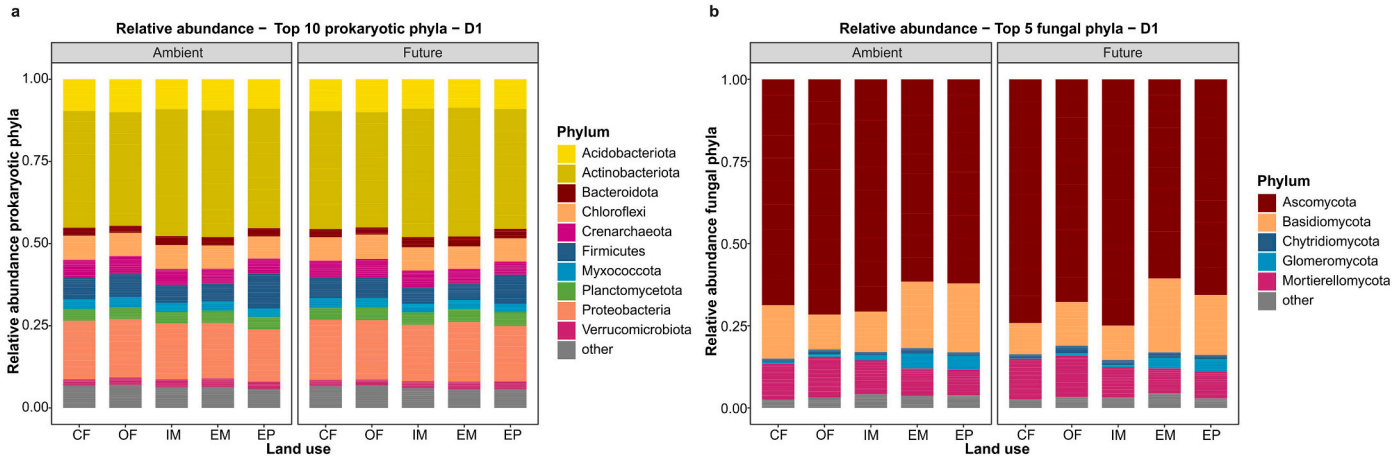

Fig. 3. Relative abundance of prokaryotic and fungal phyla separated by land use type and climate treatment in D1 (0–15 cm). (a) The ten most abundant prokaryotic phyla and (b) the five most abundant fungal phyla, as well as the remaining non-assigned ASVs indicated by "other". Ambient = ambient climate, Future = future climate, CF = conventional farming, OF = organic farming, IM = intensive meadow, EM = extensive meadow, EP = extensive pasture. Between group differences, which were determined with Tukey's HSD post hoc test, are summarized in Tables S10 and S12.

soil layers for fungal alpha diversity compared to the upper soil (Fig. S3), land-use differences on fungal richness and diversity vanished when considering the whole topsoil layer (Fig. 2c, d).

3.3. Relative abundance of dominant microbial phyla

In total, we identified 49 prokaryotic phyla, with over 90 % of the total abundance covered by ten phyla. Actinobacteriota was the dominant phylum in all land-use types, followed by Proteobacteria and Acidobacteriota. Within D1, the relative abundances of the dominant prokaryotic phyla were not influenced by climate but significantly influenced by land use, except of Myxococcota, Planctomycetota and Chloroflexi (Fig. 3a, Tables S5 and S6). Actinobacteriota exhibited significantly higher relative abundances in intensive and extensive meadows compared to the other land-use types. Interestingly, the relative abundance of Firmicutes was elevated in the pasture compared to the remaining land-use types. Relative abundance of Crenarchaeota was significantly lower in extensive meadow and pasture than in IM, OF, and CF (Table S6).

In total 15 fungal phyla were found, with over 95 % of the total abundance attributed to five phyla (Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Mortierellomycota). In D1, these five fungal phyla were significantly affected by land use, while climate effects were only significant for Chytridiomycota (Tables S7, S8). The fungal community in D1 was dominated by Ascomycota across all land-use types. Their relative abundance as well as the one of Mortierellomycota was higher in CF, OF and IM than in the two extensive grasslands. In contrast, the relative abundances of Basidiomycota and Glomeromycota were significantly higher in less intensively managed grasslands, EM and EP (Table S8).

Between D1 and D2 relative abundance of dominant phyla changed significantly in grasslands, but not in croplands (Table S18). Thus, treatment effects on the relative abundances of prokaryotic phyla were less pronounced in D2 compared to D1 (Fig. S4, Tables S5 and S6). Land-use effects were found for Bacteroidota, Myxococcota and Proteobacteria, while no climate effects were determined. Proteobacteria and Myxococcota showed a significantly higher relative abundance in croplands compared to grasslands, while the relative abundance of Bacteroidota was significantly higher in organic farming compared to grasslands. The relative abundance of prokaryotic phyla changed significantly between D2 and D3 in all land-use types (Table S18), with more pronounced land-use effects in D3 than in D2. In contrast, treatment effects on fungal community in D2 were highly comparable to those of D1 (Fig. S4, Tables S7 and S8), except lacking climate effects on Chytridiomycota but an additional climate effect on Glomeromycota, where the relative abundance was higher under ambient, than under future climate. There were no climate effects on fungal phyla in D3, and also less pronounced land-use effects, which were observed only for Ascomycota and Glomeromycota.

3.4. Microbial carbon and nitrogen

Microbial biomass C (C_{mic}) as well as microbial N (N_{mic}) in D1 were significantly lower in croplands compared to grasslands, with the highest C_{mic} and N_{mic} in EM (Fig. S5a, S5d). The microbial C/N ratio was lowest in organic farming, but highest in conventional farming (Fig. S5g, Table S10). In D2, land-use effects remained significant, but the land-use patterns were partly reversed. Microbial C and N contents increased in both croplands, while it decreased in all grasslands compared to D1. Consequently, C_{mic} and N_{mic} in croplands were significantly higher than in grasslands in D2. This pattern also remained in the deepest topsoil layer (D3) but with strongly reduced contents of microbial C and N compared to D2. Due to comparable effects on microbial C and N, the land-use pattern of the microbial C to N ratio was similar in all three depth layers, with higher C to N ratios in grasslands than in croplands. Climate effects on microbial carbon and nitrogen were not observed within the individual depth layers. However, climate effects emerged differently over different the depth layers for microbial N (Tables S9, S17) with a significant reduction of in N_{mic} in future climate between D1 and D2, but not in the ambient climate scenario.

Considering the complete topsoil layer, no significant climate effects were found for C_{mic}, N_{mic} and the microbial C to N ratio (Fig. 2e-g). While for N_{mic} land-use effects were pronounced with the highest and lowest microbial N in organic farming and intensive meadow, respectively (Fig. 2f), land-use effects for C_{mic} and C_{mic}/N_{mic} across the complete topsoil layer vanished (Fig. 2e, g).

3.5. Soil community functional parameters

3.5.1. Soil basal respiration

Soil depth strongly affected soil basal respiration (Table S9, full model). In D1, basal soil respiration was significantly affected by land use, with higher respiration rates in grasslands compared to croplands (Fig. 4a, Table S10). Respiration strongly decreased in grasslands but not in croplands between D1 and D2, resulting in similar respiration rates across all land-use types (Fig. S6). Equal respiration rates across all land-

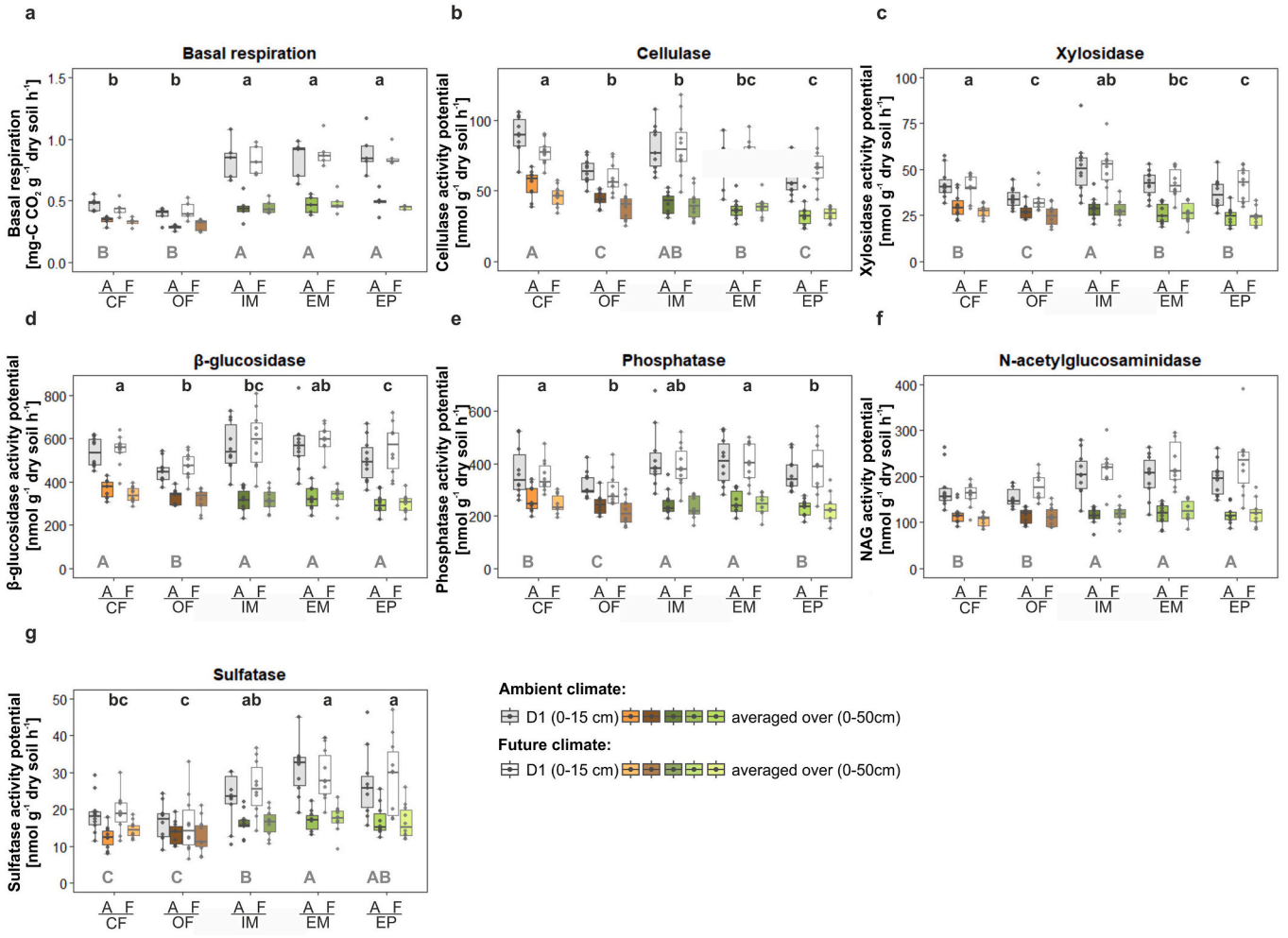

Fig. 4. Microbial functional parameters across all sampling depths (0–50 cm) separated by land use and climate treatment. For each parameter the respective values of D1 (0–15 cm) are plotted (grey boxes) left from the corresponding averaged values (colored boxes) for comparison. CF = conventional farming, OF = organic farming, IM = intensive meadow, EM = extensive meadow, EP = extensive pasture, A = ambient climate, F = future climate. Between group differences were determined for each depth layer using Tukey's HSD post hoc test. Land use types that differed significantly from each other ($p < 0.05$) are marked with a different letter, significant climate effects within one land use type are marked with: $0.1 < p < 0.05$ (.), $0.05 < p < 0.01$ (*).

use types were also observed for D3, but at lower rates than in D2. Consequently, the pattern observed for the complete topsoil layer followed the pattern observed for the uppermost soil layer (Fig. 4a). Climate effects were observed neither for individual nor the complete topsoil layer (Table S9).

3.5.2. *Soil enzymatic activity*

Enzymatic activities decreased significantly with increasing soil depth (Table S11, Fig. 4b-g, Fig. S7). The most pronounced differences between land-use types were observed for the uppermost soil layer D1. In contrast, in the deepest topsoil layer (D3), significant differences were only found between grasslands and croplands, with enzymatic activities being higher in croplands compared to grasslands.

Within D1, land use significantly affected all enzyme activities, but the observed patterns were enzyme-specific (Tables S11, S12). The activities of enzymes involved in decomposition of primary plant-originated substrates (cellulase, xylosidase, β -glucosidase) and that of phosphatase were significantly lower in organic farming compared to all other land-use types (Fig. 4). *N*-acetyl-glucosaminidase (NAG) and sulfatase activities were significantly lower in both croplands compared to those in grassland soils. Climate did not significantly affect enzymatic activities. However, when analyzing croplands separately, a marginally significant negative effect of future climate on cellulase activity was found (Table S11).

In D2, enzyme activities decreased strongly in the grasslands compared to D1, while only decreasing moderately and slightly in conventional and organic farming soil, respectively (Fig. S7). Land-use effects were highly significant for enzymes involved in carbon decomposition and for phosphatases, whereas there were no significant differences on NAG and sulfatase activities. In both croplands, cellulase, xylosidase, β -glucosidase and phosphatase activity were significantly higher than in the grasslands in D2. In D3, all enzymatic activities were further reduced in all land-use types compared to the upper soil layers, while the overall pattern remained similar to D2. Additionally, sulfatase showed a significant interaction between land use and climate with higher activity in future climate in extensive meadow. Within the individual soil layers, climate was not a significant factor.

For the complete topsoil layer, the activities of cellulase, xylosidase, β -glucosidase, and phosphatase were significantly lower in organic farming than in conventional farming (Fig. 4). For the same enzymes, the extensive pasture showed the lowest activity among all land-use types. Interestingly, we observed significantly lower activities for β -glucosidase and phosphatase in the extensive pasture compared to the extensive meadow when considering the entire topsoil layer. Across all depths, no differences were observed for NAG activity between the land-use treatments, and sulfatase activity showed similar patterns to those of

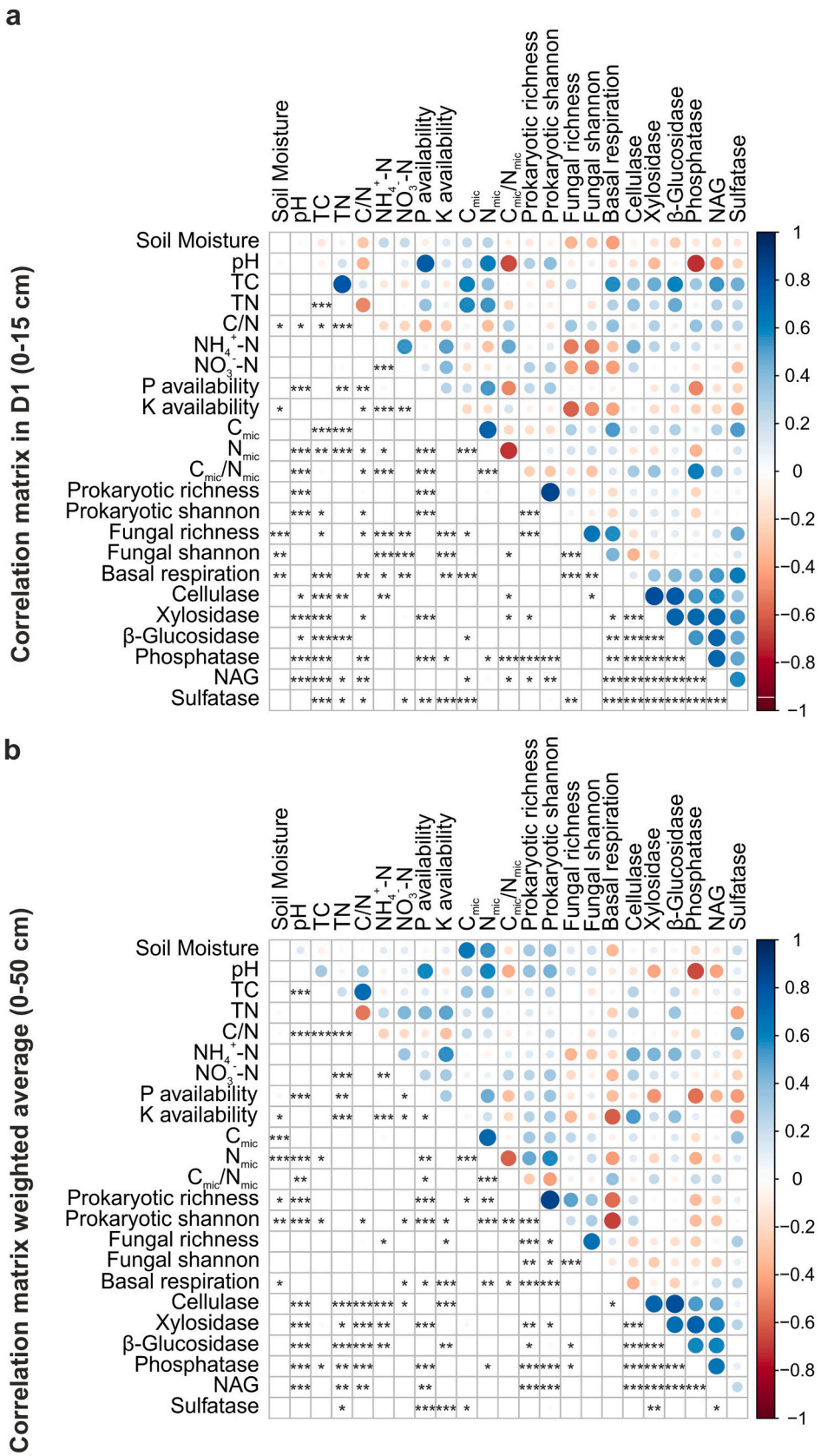

Fig. 5. Correlation analysis between soil abiotic parameters and soil functional parameters across D1 (0–15 cm) and averaged across all sampling depths (0–50 cm). Correlation analysis was conducted using Pearson's Correlation coefficient. Positive and negative correlation between variables is indicated by color, significant correlations are indicated by $0.05 < p < 0.01$ (*), $0.01 < p < 0.001$ (**), $p < 0.001$ (***). All R-values and *p*-values are summarized in Tables S22-S25.

the individual depth layers. In line with the separate data for the three depth layers, cellulase activity was lower under future climate than under ambient climate in croplands.

3.6. Correlation analysis

Within D1, prokaryotic alpha diversity was positively correlated with pH (Fig. 5a). In contrast, fungal alpha diversity was negatively correlated with soil moisture, ammonium and nitrate availability. Microbial C and N were positively correlated with TC and TN. Additionally, TC correlated positively with all soil functional parameters (basal respiration and enzymatic activities). Basal respiration and sulfatase activity were positively correlated with fungal richness and microbial C. Moreover, nearly all functional parameters positively correlated with each other (Tables S15).

Different patterns were found when averaged values across the sampling depths are considered (Fig. 5b). Prokaryotic richness and diversity showed a weak positive relationship with soil moisture, pH, P. In contrast, fungal richness was only weakly negatively correlated with ammonium and K availability. Soil moisture, TC and TN correlated positively with microbial C and N (Fig. 5b). In contrast to D1, ammonium availability positively correlated with activity of all C-cycling enzymes activities. K availability positively correlated with cellulase, while strongly negatively correlating with basal respiration. There was a weak positive relationship between TN and Cellulase and β -glucosidase, but a negative relationship of TN and sulfatase. While the correlation of basal respiration and sulfatase activity with microbial C and basal respiration disappeared, an additional positive correlation between prokaryotic diversity with microbial N was found. Soil functional parameters were less strongly correlated among each other compared to D1. Strong correlation of basal respiration and sulfatase with the other functional parameters disappeared when considering the average values (Fig. 5b, Table S16).

4. Discussion

After eight years of experimental treatments at the GCEF platform, we observed distinct effects of land use and climate on microbial biomass and microbial community structure and functions, with more pronounced responses to land use than to climate change. Further, the effects were differentially expressed at different soil depths. The pattern of abiotic and microbial parameters across different topsoil layers varied depending on the land-use type. While grasslands showed a clear biomass and functionality decrease with increasing soil depth, the tilled croplands showed a much stronger dependence on deeper topsoil layers for soil functionality. Surprisingly, the direction of treatment effects was partially reversed in deeper soil layers compared to the uppermost soil layer. This suggests an important buffering role of deeper topsoil layers against disturbances, as those layers contribute to resilience and productivity in arid climate (Zhang et al., 2023b).

4.1. Importance of soil depth in soil research

For both, land use and climate change, the strongest responses were observed in the upper 15 cm, which is well in the range of soil depths usually investigated in ecological studies. This finding confirms our first hypothesis that the uppermost soil layer is more susceptible to the effects from land use and climate change than deeper soil layers, which are not directly exposed to the altered conditions. However, not only this layer, but the complete topsoil (A horizon) plays a pivotal role in supporting ecosystem functioning as it is the hotspot of nutrient cycling and decomposition processes (Blume et al., 2002). And even though microbial abundance and activity along with carbon and nutrient availability decrease with increasing soil depth (Allison et al., 2007), a high microbial biomass with an active, species-rich and diverse microbial community is found in the complete topsoil, due to dense plant rooting and regular input of fresh organic material via litter and root exudates (Blume et al., 2002; Hsiao et al., 2018). At our specific study site, the soil type is a nutrient-rich and highly fertile Haplic Chernozem, with an A horizon ranging until a depth of 50–60 cm (Altermann et al., 2005). By covering the complete topsoil layer at the site, the results of this study revealed that the land-use and climate change induced patterns of the uppermost soil layer, are not only less pronounced in deeper topsoil layers, but partly reversed, as particularly evident for microbial biomass and enzymatic activities.

4.2. Land-use effects unfold differently over soil depth in croplands and grasslands

Microbial community diversity was strongly shaped by land-use type. In line with our second hypothesis, lower fungal alpha-diversity in the croplands compared to grasslands was observed in the uppermost soil layer. Fungal communities are more sensitive towards tillage practices in croplands, as frequent soil structure disruptions destroy fungal hyphae networks (Young and Ritz, 2000). Additionally, due to the monoculture in both croplands, the heterogeneity of rhizodeposits and litter was low. Consequently, niche formation is limited and diversity of taxa that are relevant for the initial steps of organic matter decomposition is negatively affected (Heijboer et al., 2018). Land use was also the main driver of fungal beta-diversity, which might be related to the strong link between plant and fungal communities (Cassman et al., 2016). However, fungal community structure was clearly different between the two croplands although the same crop was grown. These differences may be attributed to variations in the crop rotation, particularly the inclusion of a legume in organic farming, which was grown two years prior and may have influenced community composition (Paungfoo-Lonhienne et al., 2017; Yu et al., 2021). This suggests that management practices, such as mineral N fertilization, affected fungal community structure, as indicated by the significant correlation between the fungal community structure and the soil ammonium content. In contrast to fungi, prokaryotic community structure was less affected by land use, with pH being the main driver, which has been reported in many studies before (Rousk et al., 2010; Kaiser et al., 2016). This indicates that neither the prokaryotic to fungal nor the prokaryotic to plant community link is very strong (Cassman et al., 2016). Management intensity did not affect prokaryotic alpha-diversity in croplands. However, the higher prokaryotic Shannon index in the intensive meadow compared to extensively managed grasslands contrasts our third hypothesis supported by a recent meta-analysis showing, that bacterial community diversity is increased in non-tilled soils with moderate N fertilization, which corresponds to the management of the intensive meadow in our experiment (Li et al., 2020).

The relative abundances of the most abundant prokaryotic and fungal phyla were very similar across all land-use types. Higher relative abundance of Actinobacteriota in grasslands than in croplands, may possibly be related to their ability to decompose chitin (Hjort et al., 2010), which originates from fungi and insects that are more abundant in grasslands than in croplands (Andersson et al., 2004). Higher relative abundance of Firmicutes in the pasture may be related to the high abundance of these taxa in the sheep gut microbiome and stool (Cholewińska et al., 2023). Fungal communities were dominated by Ascomycota, which typically prevail in soils (Egidi et al., 2019). However, higher relative abundance of Basidiomycota in grasslands, than in croplands, was likely due to the more diverse litter in grasslands, which includes more lignin-rich substrates (Barel et al., 2019), predominantly decomposed by Basidiomycota (Blackwood et al., 2007).

Depth effects on the microbial community structure were more pronounced in the prokaryotic than in the fungal community. As hypothesized, different depth-related patterns were observed for grasslands and croplands, with gradual community shifts in each layer for grasslands, but only a strong shift below 30 cm in croplands. The latter pattern was most likely caused by tillage-induced only small differences

in soil properties between the two upper layers. Interestingly, for the fungal community structure this pattern was observed across all land-use types. We conclude that this pattern is caused by the strong link between the fungal community structure and the plant community that shapes the habitat conditions in the upper 30 cm via root traits and rhizodeposition (Francioli et al., 2021). Alpha-diversity of prokaryotes and fungi decreased with increasing soil depth in grasslands but not in croplands, which can be explained by the positive relationship between Shannon diversity and TC, TN and C/N (Will et al., 2010).

Microbial biomass, diversity, and functionality serve as indicators of soil health (Stewart et al., 2018), and it is crucial to identify the abiotic parameters that drive these factors. Within the uppermost soil layer, the positive correlations between TC, microbial C and soil functional parameters suggest that higher TC levels enhance microbial biomass and microbial activity in the soil, which has been shown at the site before (Sünnemann et al., 2021a). Consequently, higher microbial carbon in grasslands compared to croplands can be related to a higher allocation of assimilated carbon from shoot to roots and also higher net rhizodeposition of grassland plants compared to crops (Pausch and Kuzyakov, 2018). Moreover, higher plant diversity enhances primary productivity and root biomass, which promote microbial biomass and activity (Prommer et al., 2020). Interestingly, the application of N fertilizer was not reflected by higher microbial N in the intensively managed cropland (CF) and grassland (IM), indicating that applied mineral N was mainly taken up by plants rather than transferred to the microbial pool. In the studied season, a particular high proportion of available N is taken up by plants due to an increase of rooting density (Jaeger et al., 1999; Kuzyakov and Xu, 2013).

The differentially pronounced depth effect between grasslands and croplands induced a completely different land-use pattern in the 15–30 cm depth layer, with generally higher C_{mic} and N_{mic} in croplands than in grasslands. Further, the particularly low C_{mic}/N_{mic} ratio in croplands points to a low proportion of fungi within microbial biomass (Cleveland and Liptzin, 2007).

Lower basal respiration within the uppermost soil layer in croplands compared to grasslands is likely due to the lower amount of microbial biomass in croplands (Sünnemann et al., 2021a). Interestingly, land-use effects diminished in deeper soil, because basal respiration decreased between D1 and D2 only in grasslands, following the pattern of microbial biomass (Fang and Moncrieff, 2005).

In croplands, intensive management generally increased enzymatic activities, while in grasslands, only xylosidase and cellulase activities increased with intensive management, mainly opposing our third hypothesis. In the uppermost soil layer, enzymatic activity in grasslands was for the majority of enzymes significantly higher compared to those in the croplands, which is linked to the significantly higher microbial biomass in grasslands compared to croplands. Although winter barley was grown in both farming systems, we observed significantly lower enzymatic activities in organic farming than in conventional farming. This is in concert with (Breitkreuz et al., 2021) who observed consistently higher enzyme activities in wheat rhizosphere in CF than OF, suggesting that differences in edaphic parameters were the main drivers of enzyme activities. We assume that the main reason for this difference is the lower N availability in OF, as nitrogen is the most prevalent limiting nutrient in terrestrial ecosystems (LeBauer and Treseder, 2008) and is an essential element for the biosynthesis of enzymes (Allison and Vitousek, 2005). N input in organic farming is solely accomplished through nitrogen fixation by legumes that were cultivated two years prior to our sampling. We assume that due to a strong N limitation, enzyme production in the organic farming system was restrained (Allison and Vitousek, 2005). Further, for croplands with low TN or receiving an input of crop residues with high C:N ratios, a strong N immobilization by microorganisms was revealed (Rathke et al., 2006). Our results support this assumption by a high microbial N content, but a low N availability in organic farming. In contrast, mineral N fertilization in CF, but also in IM, improved N availability and increased enzymatic activities as shown in previous studies (Carreiro et al., 2000; Geisseler and Scow, 2014). Thus, contrary to our third hypothesis, mineral N fertilization in intensively managed systems may promote microbial activity. Additionally, enzymatic activities may be related to land-use dependent differences in microbial community structure. Fungi produce extracellular enzymes that are able to degrade more complex organic compounds like lignocellulose polymers (Sánchez, 2009). Hence, distinctly structured and more diverse fungal communities in grasslands may contribute to a higher activity of xylosidase as observed especially in the intensive meadow. NAG activity was significantly higher in grasslands than in croplands. Since NAG activity is related to fungal biomass turnover (Latgé, 2007; Qu et al., 2022), the higher microbial biomass in grasslands determined in this study along with the previously reported higher proportion of saprophytic fungi in grasslands (Sünnemann et al., 2021a), suggest higher fungal biomass and thus more available substrate for NAG in grasslands. While N fertilization had beneficial effects on C-cycling enzymes, the opposite effect was observed on NAG activity. In presence of ammonium the decomposition of organic N compounds may be reduced due to easier available nitrogen from ammonium (Wild et al., 2019), explaining the particularly low NAG activity in conventional farming. Interestingly, there were significantly lower enzymatic activities for cellulases and phosphatases in EP compared to EM, although the same plant species pool was sown in both land-use types during treatment implementation. The differences may be attributed to soil compaction and trampling damage caused through grazing (Proffitt et al., 1995), as well as changes in plant community composition due to selective grazing (Lyseng et al., 2018). Thus, a shift in plant community structure may be related to a less diverse litter composition, explaining the lower microbial biomass and the observed reduction in enzymatic activity in EP, although microbial community structures in EM and EP remained highly similar. The distinct patterns for sulfatase, which correlated more positively with basal respiration and significantly with microbial C in the uppermost soil layer than other enzymes, indicate that sulfatase activity might be particularly sensitive to microbial biomass and respiration processes. Although sulfatase is often not considered in soil enzyme studies (Jian et al., 2016), it may be a valuable indicator of microbial functionality, since it cleaves sulfur bridges in proteins and therefore, indicates turnover of microbial residues.

While a general reduction in enzyme activity with increasing depth was expected and demonstrated before (Aon and Colaneri, 2001; Liu et al., 2018), we observed a partial pattern reversal between land-use types in deeper topsoil layers. For example, carbon and phosphorous cycling enzymatic activities in croplands were significantly higher than in grasslands within D2. The same pattern was found for the C-cycling enzymes in D3. The differential depth effect on prokaryotic community structure, as well as on microbial biomass, were most likely directly linked to the reduction of enzymatic activities in grasslands from D1 and D2, while remaining similar in croplands in D1 and D2. Moreover, we conclude that the N fertilization effect in conventional farming levels off in deeper soil, resulting in comparable activities in OF and CF, confirming our third hypothesis that intensive management will exhibit stronger depth effects. Additionally, tillage practices in croplands incorporate litter and SOM into deeper soil layers, inducing higher enzymatic activities compared to grasslands. These findings and the similar patterns of microbial biomass and enzymatic activity point to a strong link between microbial community and SOM decomposition across all topsoil layers.

4.3. Climate effects

The responses of soil parameters to the experimental treatment were way more pronounced for land use than for climate as assumed in our fourth hypothesis. This finding may be explained by the particular GCEF climate treatment which is relatively mild, but represents a realistic climate scenario (Korell et al., 2020). In addition, soils exhibit a high

buffering capacity against environmental changes (Fridley et al., 2011), which might be particularly relevant for soils with a high capacity to store nutrients and water like in this study. However, individual parameters showed land-use dependent responses to the future climate.

The higher alpha-diversity in organic farming under future versus ambient climate likely is a response to the warmer and wetter future spring conditions which might enhance the range of accessible habitats and facilitate new habitat formation, allowing more diverse taxa to occupy the respective niches (Metze et al., 2024). Surprisingly, and contrary to our third hypothesis we found climate effects also in deeper soil layers, e.g. a decreased fungal richness in the extensive meadow in D2 under future, compared to ambient climate. Generally, drought is assumed to increase fungal dominance and richness (Zhou et al., 2020), supported by the negative correlation of soil moisture with fungal alpha diversity in our dataset. Community structure was not affected by climate, which may be explained by long time scales for community structure adaptation upon climatic changes (Rinnan et al., 2007).

Additionally, microbial nitrogen responded significantly depth dependent to the experimental climate treatment. While microbial nitrogen under the future climate decreased significantly between D1 and D2 microbial carbon did not follow this pattern, suggesting that N_{mic} is more sensitive to climate than C_{mic}. A lower microbial C to N ratio is an indicator of a bacterial dominated microbial biomass (Strickland and Rousk, 2010). This further supports the conclusion that fungi were particularly negatively affected by future climatic conditions, i.e. changes in litter composition (Bontti et al., 2009), leaving fungi more vulnerable to climate change impacts (Treseder et al., 2016). However, other studies found opposite or inconclusive results. Even at our study site, the fungi-to-bacteria ratio was reported to be unaffected by climate change (Kostin et al., 2021). This effect may have accumulated over time, or may be seasonally differentially expressed, as future climate models predict an increase in spring and autumn precipitation in Central Europe (Christensen and Christensen, 2007), but a reduction of summer precipitation in Germany (Chan et al., 2020). These intra-annual differences at one site need to be considered as well (Siebert et al., 2019).

At the specific time of sampling in May 2022, the future climate treatment comprised 10 % more precipitation for more than two months and increased average temperatures in comparison to the ambient control, which should increase respiration rates (Walker et al., 2018). Thus, we expected beneficial effects on functional indices such as higher basal respiration and enzymatic activity of the microbial community under the future climate. However, in contrast to our third hypothesis, no such positive effects on functional indices were observed. Positive effects are likely masked by negative legacy effects induced by previous summer droughts on the soil biota, which have been described for i.e. decomposition activity of invertebrates, and basal respiration (Siebert et al., 2019; Kostin et al., 2021; Sünnemann et al., 2021b) as well as on the plant community (Korell et al., 2024) at the experimental site. Thus, substrate availability for soil microbial decomposition is reduced (Domeignoz-Horta et al., 2023). Interestingly, we even found a negative impact of future climate on cellulase activity in croplands, which is in contrast to a global meta-analysis (Xiao et al., 2018). Due to the precipitation increase by 10 % in fall and spring as well as no climate treatment in winter because of technical limitations, legacy effects resulting from the previous summer drought are remarkable, especially considering the rapid responses of soil enzymes to changed environmental conditions (Sinsabaugh et al., 2008; Sinsabaugh et al., 2009). Legacy effects are complex and studies on duration of legacy effects especially regarding repeated drought events are rare (Müller and Bahn, 2022). We assume that the legacy effect is connected to the drought induced lower crop productivity in the year before, resulting in lower availability of organic matter for enzymatic decomposition. No climate effects on the enzyme activity were observed in the grasslands consistent with a previous study showing that climate change influenced microbial functional profiles in croplands, but not grasslands, at the GCEF (Bei et al., 2023), underlining that croplands are less resilient to climate change than grasslands and that legacy effects persist through the next growing season.

4.4. Impact of climate and land use on soil functionality

Soil functionality is a product of the cumulative contributions from different soil layers, particularly within the topsoil, where most root activity, nutrient uptake, and microbial interactions occur. Thus, even if the uppermost soil layer undergoes changes, the layers beneath can buffer these effects, maintaining overall soil functionality.

We therefore analyzed all recorded parameters, except microbial community structure, using weighted averages over the entire topsoil layer (0–50 cm). For this dataset, and in line with our fifth hypothesis, climate effects, which have been detected in individual soil layers, disappeared. Similarly, some land-use effects disappeared, while other effects remained. If present, they were, however, much less pronounced than revealed by focusing on the uppermost soil layer only, demonstrating the buffering capacity of deeper soil layers. While strong land-use-related responses were evident in the uppermost soil layer, TC and microbial C showed no between croplands and grasslands differences when considering the complete topsoil layer. This can be attributed to the incorporation of litter into deeper soil layers in tilled croplands, which creates substrate reservoirs and thus habitable soil (Angers et al., 1997). A recent study reported a strikingly high contribution of enzymes located in depths below 20 cm to overall soil enzymatic activity (Dove et al., 2020). In line with these findings we determined considerable enzyme activities in deeper topsoil layers, although the activity was land-use type dependent. Consistent with our data, Dove et al. (2021) showed how the effect of soil chemical properties becomes a more dominant driver of microbial community composition than climate as soil depth increases. The weighted average enzyme activities were generally lowest in the pasture, which is contrasting to the results observed for the uppermost soil layer. The results clearly reveal that disregarding deeper topsoil layers leads to an underestimation of enzymatic activity in organic farming, while overestimating the activity in grasslands. The lower enzymatic activities observed for the complete topsoil layer in the extensive pasture compared to the extensive meadow, while having similar microbial communities, underlines the decoupling of community structure and function between mowing and grazing management in extensive grasslands. Interestingly, no significant differences were found for the weighted average activity of NAG between the land-use types and the effects detected between grasslands and croplands in the uppermost soil layer vanished. This suggests that microbial communities in deeper soil layers are C limited due to the lower availability of readily decomposable organic C sources (Jones et al., 2018) as well as less microbial necromass, which provides NAG substrates, in deeper topsoil layers of grasslands compared to those in croplands.

In sum, our results highlight the depth dependence of global change effects on soil microbial activity parameters, and indicates that the buffering capacity of deeper layers of topsoil in croplands reduces land-use and climate impacts, maintaining soil functionality. As a result, aboveground responses to global change, such as plant productivity may be less pronounced. However, since this study reports only data from a single year, it is important to note that the observed effects may vary in other years. Interannual variability in environmental conditions, such as a wetter than average spring, could alter microbial responses and soil nutrient dynamics. Long-term studies are necessary to assess the stability of these patterns and whether the observed depth effects persist under annually varying conditions. Nonetheless, the fact that croplands already extensively exploit this buffering zone suggests that croplands may be operating at the edge of their resilience. This reliance on deeper layers of topsoil could potentially limit the ability to cope with additional stressors, making arable land more vulnerable to these stressors than grassland, and ultimately pushing croplands to tipping points where important ecosystem functions may be irreversibly lost. Once the

buffering capacity is exceeded, more severe responses to global change can be expected (Steffen et al., 2015; Davis et al., 2019). It underscores the trade-off between short-term stability and long-term sustainability and highlights the intricate balance that land management practices must strike between maximizing current productivity and ensuring the long-term health and resilience of an ecosystem.

5. Conclusion

This study highlights the need for more focus on deeper soil layers, as these are largely understudied in soil biology but are important contributors to ecosystem functioning. Global change factors that mainly affect the uppermost soil layer, may cause immediate observable changes, but the deeper layers play a crucial role in buffering these effects and sustain soil health and functionality. Our data showed not only that the composition of prokaryotic and fungal communities and enzyme activities changed with depth, but also that the impact of land use and climate decreased with depth. In contrast to strongly pronounced land-use effects, the future climate treatment had only minor impacts on the soil of the five agroecosystems after eight years of experimental treatments, what can be attributed to the mild but realistic climate treatment and possibly also to adaptation of microbial taxa, allowing to maintain soil functions and process rates under climate change conditions. However, microbial community structure and traits partly showed distinct, land-use specific responses, which will have a greater impact on soil functions and properties in the long term. Microbial community diversity, structure, and traits were less impacted in grasslands than in cropland, indicating a higher resilience of grassland systems. Future research on global change effects on soil functionality should be extended from the traditional 10–15 cm to at least 30 cm depth or, even consider the complete topsoil layer. Additionally, identifying key stone microbial taxa and functional groups across the complete topsoil layer and over multiple years, would provide valuable insights into specific community functions and dynamics over time under global change. This would help to understand the interconnected processes within the entire topsoil profile in order to develop sustainable soil management practices that enhance overall soil functionality without compromising its resilience.

CRediT authorship contribution statement

Lena Philipp: Writing – original draft, Investigation, Formal analysis. **Marie Sünnemann:** Writing – review & editing, Methodology, Investigation. **Martin Schädler:** Writing – review & editing, Project administration, Methodology. **Evgenia Blagodatskaya:** Writing – review & editing, Methodology. **Mika Tarkka:** Writing – review & editing, Conceptualization. **Nico Eisenhauer:** Writing – review & editing, Conceptualization. **Thomas Reitz:** Writing – review & editing, Methodology, 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.

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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.2025.106025.

Data availability

Sequences are available in the Sequence Read Archive (BioProject ID: PRJNA1234075).

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