Science of the Total Environment 885 (2023) 163683

Contents lists available at ScienceDirect

Science of the Total Environment

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

Soil microbial biodiversity promotes crop productivity and agro-ecosystem functioning in experimental microcosms

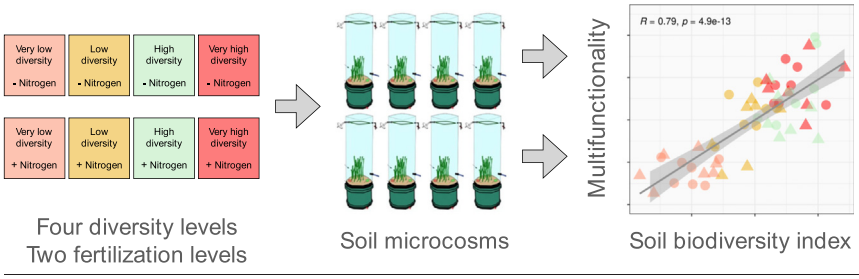
Ferran Romero [a,*], Sarah Hilfiker [a], Anna Edlinger [a], Alain Held [a], Kyle Hartman [a], Maëva Labouyrie [a,b,c], Marcel G.A. van der Heijden [a,b,**]

[a] Plant-Soil Interactions, Research Division Agroecology and Environment, Agroscope, 8046 Zurich, Switzerland
[b] Department of Plant and Microbial Biology, University of Zurich, 8008 Zurich, Switzerland
[c] European Commission, Joint Research Centre Ispra (JRC Ispra), Via Enrico Fermi 2749, 21027 Ispra, Italy

HIGHLIGHTS

- Soil biodiversity in agro-ecosystems faces stressors including nitrogen fertilizers.
- Little is known about the impact of fertilizers on soil biodiversity-functioning relationships.
- A factorial design revealed strong soil biodiversity-multifunctionality correlations.
- Mineral nitrogen application reduced nitrogen uptake from decomposing litter.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Charlotte Poschenrieder

Keywords:
Soil biodiversity
Multifunctionality
Fertilizer
Microcosms
Soil microorganisms

ABSTRACT

Soil biota contribute substantially to multiple ecosystem functions that are key for geochemical cycles and plant performance. However, soil biodiversity is currently threatened by land-use intensification, and a mechanistic understanding of how soil biodiversity loss interacts with the myriad of intensification elements (e.g., the application of chemical fertilizers) is still unresolved. Here we experimentally simplified soil biological communities in microcosms to test whether changes in the soil microbiome influenced soil multifunctionality including crop productivity (leek, *Allium porrum*). Additionally, half of microcosms were fertilized to further explore how different levels of soil biodiversity interact with nutrient additions. Our experimental manipulation achieved a significant reduction of soil alpha-diversity (45.9 % reduction in bacterial richness, 82.9 % reduction in eukaryote richness) and resulted in the complete removal of key taxa (i.e., arbuscular mycorrhizal fungi). Soil community simplification led to an overall decrease in ecosystem multifunctionality; particularly, plant productivity and soil nutrient retention capacity were reduced with reduced levels of soil biodiversity. Ecosystem multifunctionality was positively correlated with soil biodiversity (R = 0.79). Mineral fertilizer application had little effect on multifunctionality compared to soil biodiversity reduction, but it reduced leek nitrogen uptake from decomposing litter by 38.8 %. This suggests that natural processes and organic nitrogen acquisition are impaired by fertilization. Random forest analyses revealed a few members of protists (i.e., *Paraflabellula*), Actinobacteria (i.e., *Micolunatus*), and Firmicutes (i.e., *Bacillus*) as indicators of ecosystem multifunctionality. Our results suggest that preserving the diversity of soil bacterial and eukaryotic communities within agroecosystems is crucial to ensure the provisioning of multiple ecosystem functions, particularly those directly related to essential ecosystem services such as food provision.

* Corresponding author.
** Correspondence to: M.G.A. van der Heijden, Plant-Soil Interactions, Research Division Agroecology and Environment, Agroscope, 8046 Zurich, Switzerland.
E-mail addresses: ferran.romeroblanch@agroscope.admin.ch (F. Romero), marcel.vanderheijden@agroscope.admin.ch (M.G.A. van der Heijden).

http://dx.doi.org/10.1016/j.scitotenv.2023.163683
Received 30 January 2023; Received in revised form 18 April 2023; Accepted 19 April 2023
Available online 3 May 2023

1. Introduction

Soil harbours the most diverse biological community on earth, as it is habitat to a wide range of macro- and microorganisms (Crowther et al., 2019; Fierer, 2017). Soil biodiversity plays a pivotal role in numerous ecological functions (i.e., ecosystem multifunctionality) that, in turn, sustain ecosystem services such as the production of food or climate regulation (Banerjee and van der Heijden, 2023; Byrnes et al., 2014). Despite their ecological importance, soils currently face the negative impacts of many anthropogenic activities, including land-use change, and the intensification of agricultural practices (Winkler et al., 2021), which is leading to decreased soil biodiversity globally (Carmona et al., 2020; Tsiafouli et al., 2015; Zhou et al., 2020). Until now, most research efforts focused on understanding the role of aboveground biodiversity (e.g., plants and animals) on ecosystem functions, especially those related to natural ecosystems (Isbell et al., 2011), but the role of belowground biodiversity in shaping soil functions and ecosystem performance remains unclear. Recent studies however suggest that microbial diversity could be used as a proxy to predict functioning in natural biomes including grasslands, arid ecosystems, and tropical forests (Delgado-Baquerizo et al., 2016, 2020).

Ecological theory suggests that a reduction in soil biodiversity (e.g., taxa richness) does not necessarily imply a loss of soil functions, as compensation may occur according to the degree of functional niche overlap among species, particularly at high levels of diversity (Louca et al., 2018; Pierre-Alain et al., 2018). However, quantitatively assessing the relationship between soil biodiversity and soil functions has, until now, been obstructed by a lack of experimental approaches allowing for precise manipulation of taxa abundance and composition. The dilution-to-extinction method, for example, involves sequentially diluting a microbial inoculum (Garland and Lehman, 1999), and has been used in soil microbial ecology as an approach capable of non-specifically reducing soil biodiversity (Chen et al., 2020; Delgado-Baquerizo et al., 2020; Wertz et al., 2007). On the other hand, it is also possible to specifically select for certain taxa by fractionating soil organisms according to size, e.g., using filters of decreasing mesh size (Wagg et al., 2019). This experimental approach has effectively revealed that soil microbial diversity is directly linked to multiple soil ecosystem functions (i.e., soil multifunctionality) in grasslands (Wagg et al., 2014, 2019). Whether such relationships can also be found in anthropogenic environments (e.g., agroecosystems) still needs experimental confirmation. Consequently, there is a lack of mechanistic understanding on the importance of soil belowground biodiversity for plant productivity and ecosystem multifunctionality in agroecosystems.

If soil biodiversity determines soil multifunctionality in anthropogenic environments, the biodiversity-multifunctionality relationship is likely to be affected by the numerous environmental disturbances that are typical of these environments. To date, various studies have observed changes in soil microbial diversity in response to disturbances, such as those related to intensive agricultural practices (e.g., tillage, fertilization, pesticide application) (Banerjee et al., 2019; de Graaff et al., 2019; Wittwer et al., 2021). Among these disturbances, the release of reactive nitrogen into the environment as part of agricultural fertilization processes generally increases plant productivity but may have major impacts on soil communities (Wang et al., 2021; Wittwer et al., 2021). Recent studies employing metabarcoding techniques demonstrated that soil microbial diversity changes following nitrogen fertilization; these include shifts towards soil bacterial communities dominated by Proteobacteria, Actinobacteria and Firmicutes, while the relative abundance of Acidobacteria and Verrucomicrobia tends to decrease after nitrogen application (Dai et al., 2018; Wang et al., 2021; Zeng et al., 2016). Similarly, nitrogen fertilization tends to increase the relative abundance of certain fungal taxa (e.g., Ascomycota), while reducing others (e.g., Basidiomycota) (Paungfoo-Lonhienne et al., 2015), although this seems to be context-dependent (Qi et al., 2021). Other studies showed that intensive tillage, fertilizer application, and pesticide use negatively impact important groups of soil biota including earthworms, arbuscular mycorrhizal fungi, and nitrogen-fixing bacteria (Datta et al., 2016; Reinprecht et al., 2020; Wittwer et al., 2021). Importantly, nitrogen fertilization has also been demonstrated to consistently favor pathogenic over mutualistic fungi in 25 grassland soils distributed across four continents (Lekberg et al., 2021). Although variability exists, these and other studies are in line with the copiotrophic hypothesis, in which taxa adapted to nutrient-rich environments are more likely to increase under nutrient-rich conditions (i.e., after nitrogen fertilization), while oligotrophic taxa tend to decrease (Fierer et al., 2007). Finally, the response of other microbial eukaryotes (e.g., protozoa) to nitrogen deposition is still unclear, although experimental evidence suggests that they might be more sensitive to nitrogen fertilization than other soil microorganisms (Zhao et al., 2019).

Various studies assessed the impacts of fertilization on ecosystem functioning (Allison et al., 2009; Jian et al., 2016; Ward et al., 2017). For example, a meta-analysis covering 65 published nitrogen fertilization experiments concluded that nitrogen fertilization stimulated carbon and phosphorus-associated hydrolase activity, while reducing oxidase activity (Jian et al., 2016). Experimental evidence also points towards a negative effect of nitrogen fertilization on soil respiration (Ward et al., 2017). Other soil functions have showed contrasting responses to nitrogen fertilization; for example, litter decomposition responses to nitrogen deposition ranges from positive or neutral (Allison et al., 2009; Hobbie et al., 2012; Ren et al., 2018) to negative (Zhou et al., 2017). These and other studies show that the effects of nitrogen fertilization on soil biodiversity and ecosystem functioning have been effectively addressed separately (although not fully resolved), but it is still unclear how nitrogen fertilization alters the relationship between soil biodiversity and ecosystem multifunctionality.

In this study, we evaluated the impact of soil community simplification on ecosystem multifunctionality and crop productivity. Moreover, we also investigated whether the relationship between soil biodiversity and ecosystem multifunctionality depends on soil fertility. To test this, we established soil microcosms under sterile conditions and experimentally manipulated (i.e., reduced) soil biodiversity by adding soil inoculum that was sieved through different meshes following earlier work (Wagg et al., 2014, 2021). The size-based reduction of soil diversity employed in this study aligns with the impact of intensive land management practices, such as soil tillage, that physically damages soil organisms depending on their size (Jansa et al., 2003; Wagg et al., 2018). In addition, half of the microcosms were fertilized to test whether biodiversity-ecosystem multifunctionality relationships are altered when mineral nitrogen fertilizer is applied. Microcosms were harvested after eleven weeks, and we recorded soil parameters, a set of ecosystem functions, and analyzed bacterial and eukaryotic communities. We hypothesized that (i) soil community simplification would reduce overall ecosystem multifunctionality, (ii) mineral nitrogen fertilizer would increase plant productivity but reduce soil community diversity and (iii) the interaction between soil diversity loss and nitrogen deposition would be additive, leading to an even more simplified soil community than under single-factor conditions, with negative impacts on ecosystem multifunctionality.

2. Materials and methods

2.1. Maintenance of microcosms and experimental conditions

We established microcosms within closed systems of 6.5 L soil volume: 23.5 cm in diameter and 20.4 L ventilated lids (Fig. S1). The 65 cm-high lids received air and water through 0.22 μm sterile filters to avoid contamination of the microcosms by water and air-borne microorganisms. As a substrate for community establishment and plant growth, we used a 7.2 Kg 1:1 mixture of field soil from a nearby field (47°25′40.3″N 8°31′04.7″E, 444 asl) and quartz sand (water-holding capacity [WHC] of the mixture was 0.348 L Kg^{-1}). The substrate mixture was sterilized by autoclaving at 121 °C for 90 min. Four different inocula were created by sequentially sieving soil through decreasing mesh sizes as described in Wagg et al. (2014) and Wertz et al. (2007). The most diverse community was established by inoculating with soil sieved through a 5-mm mesh, followed by 100 μm, 11 μm, and sterile soil. Mesh sizes were selected based on our previous findings regarding the capacity of different meshes to discard specific

soil organisms: for example, the 100 μm mesh was expected to reduce mycorrhizal abundance, while the 11 μm mesh was expected to completely remove mycorrhizal fungi and nematodes, while also reducing total fungal and bacterial biomass (Wagg et al., 2014). The sieving procedure was performed by adding 800 mL of sterilized deionized water. The fraction of soil not passing the sieves was collected and sterilized and added to the microcosms to ensure that the same amount of soil inoculum was added to each microcosm. Each microcosm was filled with the sterilized 1:1 soil-sand mixture and 400 g of soil community inoculum. After inoculation, microcosms were incubated for six weeks under the experimental conditions described in van der Heijden et al. (2016) and Wagg et al. (2014) in the greenhouse before planting 15 seedlings of *Allium porrum* (i.e., leek) variety *Nipper* per microcosm. To improve overall plant survival, leek seeds were surface-sterilized and pre-germinated on water-agar under sterile conditions for six days before planting. Two weeks after planting the seedlings, leek seedlings were selectively thinned to nine plants per microcosm. *A. porrum* was selected as model plant because previous research suggests that it greatly depends on soil microbial diversity (Milleret et al., 2009).

A total of four soil biodiversity treatments were established (i.e., receiving inoculum sieved through a 5 mm, 100 μm, and a 11 μm sieve, plus a treatment receiving only sterilized soil), and each soil biodiversity treatment was set up with or without a nitrogen fertilization treatment (2 levels; high nitrogen vs. control) in a random-block design consisting of 7 blocks. High nitrogen microcosms received an equivalent of 145 kg N ha^{-1} applied as 3 sequential applications of ammonium nitrate 0 (directly after inoculation), 2 and 7 weeks after planting. Each soil treatment was replicated 7 times; for a total of 56 experimental microcosms (Fig. S1).

2.2. Characterization of soil properties and ecosystem functions

At the end of the experimental phase (21 weeks after setting up the microcosms and 17 weeks after planting leek seedlings), the microcosms were harvested, and the soil was collected and mixed. Soil samples (200 g) were collected and dried at 40 °C and sieved (2 mm Ø); the resulting soil fraction was used for analysis of Olsen phosphorus (P-Olsen) and pH (Watanabe and Olsen, 1965) (Table S1). We also quantified nine functions known to be linked to the functioning of soil ecosystems: plant primary production (i.e., above- and belowground biomass), soil respiration (i.e., CO_2 emissions), nitrous oxide (N_2O) emissions, leaching of inorganic forms of nitrogen (i.e., ammonium and nitrate) and phosphorus (i.e., phosphate), decomposition of leaf litter, and nitrogen turnover between leaf litter and leek plants. To assess overall ecosystem functioning, we then combined these ecosystem functions into an ecosystem multifunctionality index (EMF, see below Section 2.3.3).

2.2.1. Gas accumulation measurements

Gas fluxes (i.e., CO_2 and N_2O) were measured in a closed loop during 4 min by directly plugging a Picarro G2508 Greenhouse Gas Analyzer (Picarro Inc) into the gas outlet of every microcosm. To account for potential variation, gas fluxes were repeatedly measured for one week: every eight hours for the three first days, then every 12 h during the two following days, and every 24 h during the last two days. Cumulative gas fluxes were then calculated as described in Bender et al. (2014) and expressed as N_2O-N or CO_2-C g m^2 h^{-1}.

2.2.2. Plant biomass measurements

Leek plants were harvested from microcosms by cutting the stalk just above the soil surface. Leek plants were then collected in paper bags, dried in an oven at 60 °C and weighed (aboveground dry biomass). Roots were collected from the soil by hand and sieving and the same procedure was subsequently followed to estimate root (i.e., belowground) dry biomass.

2.2.3. Nutrient concentrations in leachate

After plants were harvested and soil respiration was measured, each microcosm was watered to bring the soil to saturation (10 % above the WHC) and to induce leaching. Leachate percolating through the soil column was collected from an outlet at the bottom of the microcosms and filtered through 0.45 μm pore size filters. Ammonium, nitrate, and phosphate concentrations were then determined in filtered leachate samples by means of standard protocols (Murphy and Riley, 1962; van der Heijden, 2010).

2.2.4. Leaf litter decomposition and nitrogen turnover

Litter decomposition was assessed with 0.5 mm propyltex mesh litterbags (6 × 6 cm) containing ^{15}N-labeled sterilized *Lolium multiflorum* shoots that were added to microcosms at the start of the experiment (2 bags of 1 g per microcosm). The amount of the initial 1 g of litter lost was calculated as decomposition. The ability for the plants to acquire N through litter mineralization was estimated using the $\delta^{15}N$ signal in the *L. multiflorum* shoots at the end of the experiment. Briefly, dried shoots were milled with a Tissue Lyser (Qiagen) and loaded into sample boats (1.5 mg). Isotopic composition was then analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) following standard procedures (You et al., 2021).

2.3. Characterization of soil microbial community

We analyzed the colonization of leek roots by arbuscular mycorrhizal fungi and extracted DNA from soil samples to characterize the bacterial and eukaryotic microbial community using next-generation sequencing (NGS) of the 16S rRNA and the 18S rRNA genes, respectively. To assess overall soil community, we then combined these structural parameters into a soil biodiversity index (see next Section 2.3.2).

2.3.1. Arbuscular mycorrhizal fungi

To analyze the colonization of roots by arbuscular mycorrhizal fungi (AMF), we cut freshly harvested roots into 1.5 cm pieces and stored them in 70 % ethanol. Later, roots were washed in deionized water and decolorized in 10 % KOH at 80 °C for 17 min. Afterwards, roots were washed again and incubated in an ink-acidic mix at 80 °C for 20 min to colorize fungi (Vierheilig et al., 1998). AMF colonization was then assessed under a light microscope (× 200 magnification) following the standard gridline intersect method (McGonigle et al., 1990).

2.3.2. Soil DNA extraction and Illumina sequencing

At each harvest, six soil cores (1.7 mm diameter) were taken to the depth of the microcosm (~ 20 g of fresh soil). Soil cores were homogenized, and a 0.25 g subsample was frozen at − 20 °C for DNA extraction using the Nucleospin 96 Soil DNA extraction kit (Macherey-Nagel, Düren, Germany). Microbial community composition was determined on an Illumina MiSeq platform using the Nextera XT DNA Library Preparation kit (Illumina Inc., CA, USA). Primers 515F and 806R targeting the V3-V4 region of the 16S rRNA gene were used to characterize the bacterial community, and primers 1391F/EukBR were selected to target the eukaryotic community (Cui et al., 2022; Delgado-Baquerizo et al., 2021). Metabarcoding of the 18S rRNA gene failed for two samples (replicate number one of the sterile non-fertilized treatment, and replicate number four of the 11-μm fertilized treatment). Thus, all results related to eukaryotic diversity measures were calculated on $n = 54$ (instead of 56). All sequences are available at the Sequence Read Archive (SRA) database under BioProject accession numbers PRJNA956349 (bacteria) and PRJNA956556 (eukaryotes).

Demultiplexed fastq files were processed using the DADA2 (v1.16) package (Callahan et al., 2016) in R software (3.6). Filter and trimming (function *filterandtrim*) parameters were *max*N = 0, *max*EE = c (2,2). *trunc*Q = 2. *Max*EE corresponds to the maximum expected errors calculated from the quality score as sum(10^(− Q/10). *Trunc*Q = 2 parameter truncate reads at the first instance of a quality score less than or equal to two. *Max*N is maximum number of accepted 'N' bases. The error rates were estimated using the function *learnErrors*. Exact amplicon sequence variants (ASV) were resolved using *dada* function, and chimeric sequences were removed using the function *removeBimeraDenovo*. Taxonomic assignment was performed using the *assignTaxonomy* function on the sequence

table (seqtab.nochim) against the Silva reference database (v132) for 16S rRNA gene dataset and the PR2 database (v4.14.0) for the 18S rRNA gene dataset (Guillou et al., 2012; Quast et al., 2012). After removing sequences classified as *Chloroplast* or *Mitochondria*, all reads from individual taxa were expressed as percentages of the total number of counts in a sample.

2.3.3. Ecosystem multifunctionality (EMF) and Soil biodiversity (SBI) indices

We calculated an ecosystem multifunctionality index to estimate the ability of soil microbial communities to sustain multiple functions simultaneously (Byrnes et al., 2014). For this, we standardized all ecosystem function data by z-transformation (overall mean of 0 and standard deviation of 1) and expressed multifunctionality as the mean of all the z-transformed variables (Maestre et al., 2012). Leaching data (NH_4^+, NO_3^-, and PO_4^{3+}) and N_2O loss were multiplied by -1 because we consider higher values of these functions to reflect an undesirable ecosystem state. As such, increasingly negative multifunctionality values indicate a decline in overall ecosystem functioning (Wagg et al., 2014). Similarly, as a general indicator of soil biodiversity, we calculated a biodiversity index for each microcosm comprised of the mean of z-transformed values of 16S and 18S Shannon diversity and percentage of AMF colonization.

2.4. Statistical analyses

We examined the response of the nine ecosystem functions and the community-level metrics (alpha diversity) in each microcosm ($n = 56$) using R Software v3.6.0. For each ecosystem function and each community-level metric, we first plotted the residuals (qqplot) and then ran Levene's and Shapiro-Wilk tests to check for homogeneity of variances and normality of error distributions (functions *leveneTest* and *shapiro.test*). Data not meeting either one or both assumptions (i.e., *p-value* < 0.05 on Levene's and/or Shapiro-Wilk test) was transformed using the *bestNormalize* function (Table S2). We then ran a 2-way ANOVA using the *aov* function (v3.6.2) with the factors *Diversity* (four levels: 5 mm, 100 μm, 11 μm, sterile) and *Nitrogen* (two levels: control, high) as fixed factors, as well as the interaction between both factors (*Diversity*Nitrogen*). We also ran 2-way ANOVA on the relative abundance of the 10 most abundant bacterial and eukaryotic groups (phyla for bacteria, supergroup for eukaryotes). We used Tukey's honest significant differences (HSD) for post hoc pairwise comparisons. The effects of the two factors (*Diversity* and *Nutrient*) and their interaction on microbial community composition were evaluated by means of permutational multivariate ANOVA (PERMANOVA) ran using the *adonis* function (Package vegan v2.4-2). We constructed a dissimilarity matrix using Bray-Curtis distances to reveal differences in bacterial (i.e., 16S rRNA gene) and eukaryotic (i.e., 18S rRNA gene) community composition, and visualized the results from the similarity matrix in non-metric multidimensional scaling (nMDS) plots. Relationships among variables (e.g., ecosystem functions and indices) were explored by means of Pearson's correlation using the *stat_cor* function within the ggpubr package (v4.0). To further explore which microbial taxa could be good predictors of ecosystem multifunctionality, we ran random forest analyses on the different functions ($n = 9$) and the ecosystem multifunctionality index (EMF) using the most abundant amplicon sequence variants (ASVs >0.1 % of relative abundance) as predictors. We also included alpha-diversity parameters as predictors (i.e., richness and Shannon's diversity of bacterial and eukaryotic ASVs). Random forest analyses were performed with 100 permutations and 5000 trees using the *rfPermute* package. The ASVs significant at $p < 0.01$ were selected as predictors of ecosystem multifunctionality.

3. Results

3.1. Soil biodiversity measures

3.1.1. Arbuscular mycorrhizal fungi (AMF)

The different soil biodiversity treatments had a significant effect on AMF root colonization (Table 1). Plant roots in microcosms inoculated with sterilized or 11-μm sieved inocula were hardly colonized by AMF (≤ 1 % of total root surface showing AMF colonization), demonstrating that soil sterilization removed AMF and that AMF propagules cannot pass the 11-μm sieve. In microcosms inoculated with 100-μm and 5-mm sieved inocula, AMF colonization rates reached 48 ± 12 % and 47 ± 16 % of total root surface, respectively (Fig. S2). Nitrogen addition did not significantly alter AMF colonization, and no interaction between diversity and nitrogen addition was observed (Table 1).

Table 1
Analysis of variance output (F-values) demonstrating the effect of soil biodiversity manipulation, nitrogen fertilization and the interaction of both factors on a range of ecosystem functions and biodiversity parameters. Results are also shown for the calculated indices (EMF and SBI). Df; degrees of freedom. Significance: ***; p-value < 0.001, ***; p-value < 0.01, ***; p-value < 0.05. Significant results are highlighted in bold.

	Soil biodiversity (df = 3)	Nitrogen (df = 1)	Interaction (df = 3)
Ecosystem functions			
Belowground biomass	**17.55** ***	1.06	0.41
Aboveground biomass	**38.31** ***	0.78	1.94
CO_2 emissions	**5.98** **	< 0.01	2.02
N_2O emissions	0.33	2.98	1.39
Nitrogen turnover (δ15N)	**8.04** ***	**17.04** ***	0.04
Litter decomposition	**3.22** *	0.65	1.23
NH_4 leaching	**16.88** ***	0.26	1.37
NO_3 leaching	**3.07** *	**4.06** *	0.29
PO_4 leaching	**24.88** ***	0.02	**3.13** *
Ecosys. Multif. Index (EMF)	**27.79** ***	1.82	2.03
Biodiversity			
ASV richness (16S rRNA)	**8.81** ***	0.09	1.04
ASV Shannon (16S rRNA)	**8.93** ***	0.74	0.93
ASV richness (18S rRNA)	**18.16** ***	0.71	0.10
ASV Shannon (18S rRNA)	**17.49** ***	1.05	1.95
AMF colonization	**46.83** ***	< 0.01	0.15
Soil Biodiversity Index (SBI)	**66.11** ***	0.10	0.34

3.1.2. Bacterial and eukaryote community composition based on DNA metabarcoding

High-throughput sequencing of the 16S rRNA and the 18S rRNA genes was employed to assess the impact of the different soil biodiversity treatments on alpha-diversity metrics and the community composition of the established microbial communities. After removing short and low-quality sequences, the 16S rRNA gene data set consisted of 6′442′862 (average read number per sample: 115′051 ± 21′078). A total of 9046 amplicon sequence variants (ASVs) were observed. Bacterial richness (i.e., observed ASVs in the 16S rRNA gene data set) ranged from 258.86 ± 61.95 (microcosms inoculated

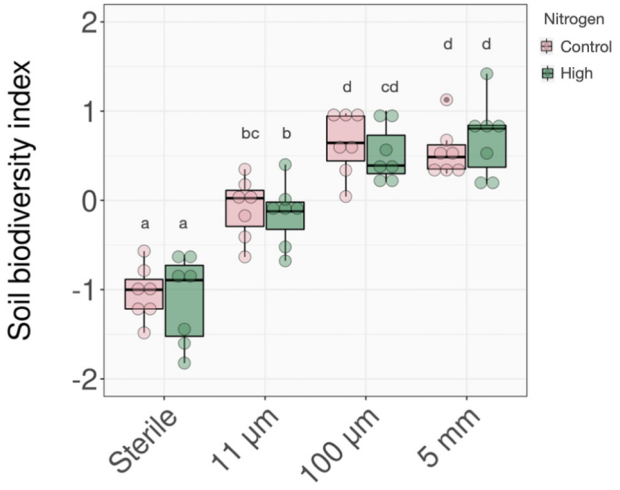


Fig. 1. Soil biodiversity index (SBI) for the four soil biodiversity (sterile, 11 μm, 100 μm, and 5 mm) and nitrogen (control, high) treatments. Different letters indicate significant differences between groups (Tukey post-hoc test, p < 0.05).

with sterilized soil enriched with N) to 477.43 ± 125.76 (microcosms inoculated with soil sieved through 5 mm and enriched with N) (Fig. S3). Similarly, Shannon-Wiener Index (H′) ranged from 4.71 ± 0.43 (sterile treatment enriched with N) to 5.47 ± 0.30 (5-mm treatment enriched with N) (Fig. S3). The Eukaryote (18S) rRNA gene dataset consisted of 1'729'181 reads (average read number per sample: 32'020 ± 12'370), that clustered into 1824 ASVs. Eukaryote richness ranged from 25.83 ± 14.05 (sterile treatment non-enriched with N) to 146.43 ± 30.45 (5 mm-N treatment) (Fig. S3). Eukaryote Shannon's diversity ranged from 1.53 ± 0.79 (sterile treatment non-enriched with N) to 3.84 ± 0.24 (10 μm-N treatment) (Fig. S3). Overall, soil sieving through decreasing mesh sizes had a strong impact on all alpha-diversity parameters (Table 1).

Non-metric multidimensional scaling (NMDS) showed that the overall bacterial and eukaryotic community composition (beta-diversity of 16S and 18S rRNA genes ASV) in sterile soil was clearly separated from the 5-mm, 100-μm and 11-μm treatments (Fig. S4). The effect of sieving on microbial community composition was further supported by PERMANOVA on both bacterial and eukaryote communities. Nitrogen addition effects on microbial community composition were low, and no significant interaction between diversity and nitrogen addition was found (Table 1). To further explore the effect of sequential sieving on the composition of bacterial and eukaryotic communities, we repeated this procedure (Bray-Curtis distances calculation, NMDS, and PERMANOVA) without the samples from the sterile treatment. This allowed us to show a separation of the 11-μm treatment community from the 100-μm and 5-mm treatments, especially for the eukaryotic community.

Overall, soil bacterial community composition was dominated by Actinobacteria, Proteobacteria, and Firmicutes, while the supergroups Archaeplastida, Opisthokonta, and Amoebozoa dominated the eukaryote community (Fig. S5). Out of the top-10 most abundant bacterial classes, six were significantly impacted by the diversity treatment, while three were impacted by the addition of mineral nitrogen (Fig. S6, Table S3). The interaction

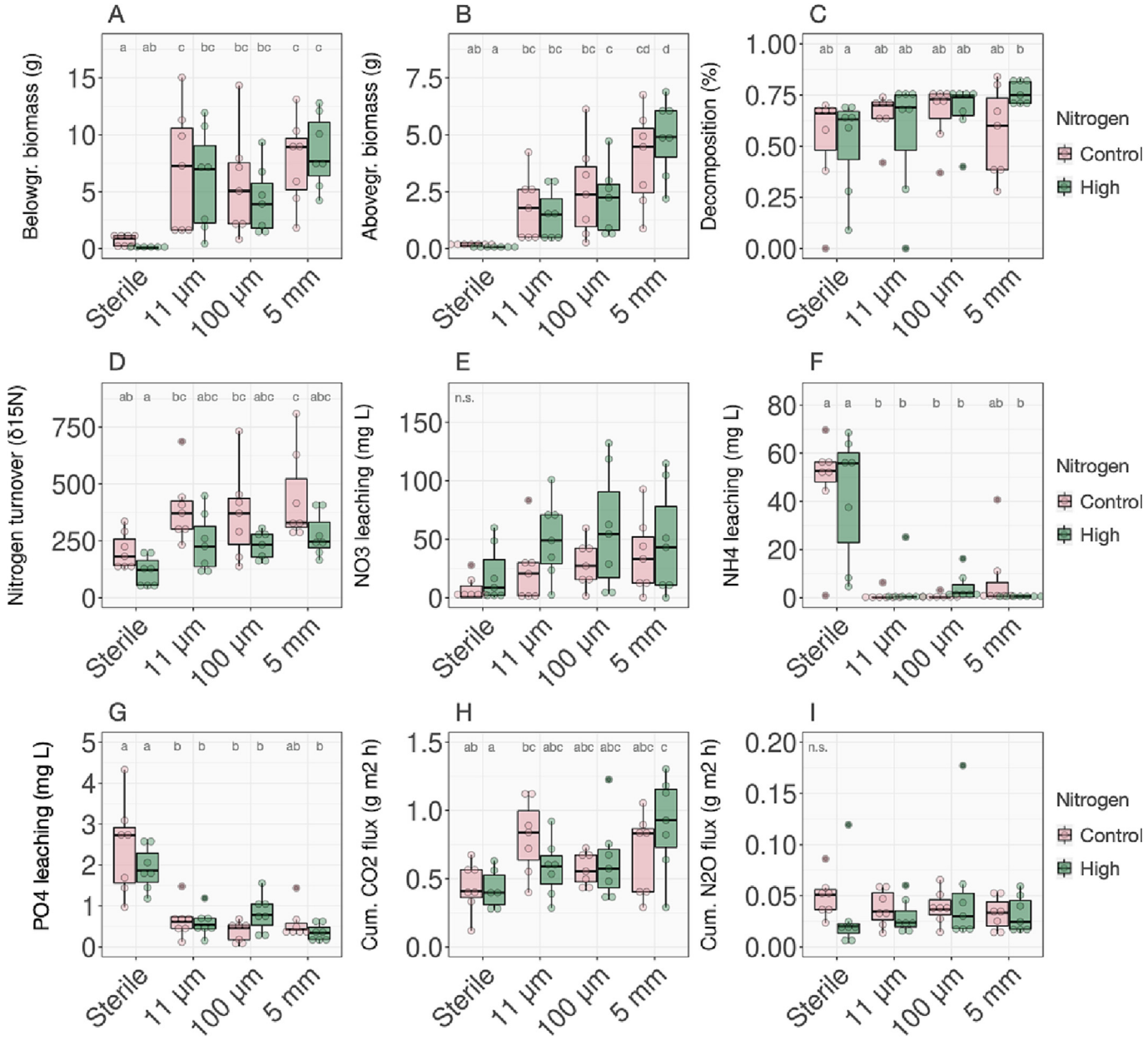


Fig. 2. Changes in below- and aboveground biomass (A, B), Lolium decomposition rate (C), nitrogen turnover (D), nutrient leaching (E-G), and gases emissions (H, I) for each treatment along the diversity gradient (Sterile - 11 μm - 100 μm - 5 mm). Boxplot color indicates whether microcosms were supplemented with mineral nitrogen (green) or not (pink). Different letters within each ecosystem function indicate statistically significant differences at a significant level of $p < 0.05$ (Tukey's HSD test).

between diversity and nitrogen addition was only significant for one bacterial class (i.e., Gemmatimonadetes). Regarding the eukaryotic community, seven out of the ten most abundant classes were affected by the diversity treatment, and none of them was significantly affected by nutrient addition, nor by the interaction between both factors (Fig. S7, Table S3).

3.1.3. Soil biodiversity index (SBI)

The calculated SBI values ranged from −1.82 to 1.42 (Fig. 1) and were the highest in microcosms inoculated with 5-mm sieved soil inocula and supplemented with nitrogen (0.69 ± 0.43). The lowest values of the SBI were recorded in microcosms inoculated with sterile soil (−1.12 ± 0.49). In line with the observations made for alpha-diversity parameters and AMF colonization, only the factor diversity significantly influenced the calculated soil biodiversity index (Table 1).

3.2. Links between soil biodiversity and ecosystem functioning

3.2.1. Plant biomass and gas (CO_2 and N_2O) emissions

We observed that sterilization of the inoculum lead to very low above- and belowground biomass production (Fig. 2). Aboveground biomass in microcosms containing the sterilized inoculum averaged 0.12 ± 0.06 g, and belowground biomass averaged 0.40 ± 0.49 g. The above- and belowground biomass produced significantly increased in microcosms receiving a live soil inoculum (Table 1) with the maximum biomass production observed in microcosms inoculated with 5-mm sieved inoculum (total biomass: 12.50 ± 5.32 g). Nitrogen addition only had a minor impact on biomass production, and no significant effect was reported (Table 1). Inoculation of microcosms with live soil communities also tended to increase CO_2 emissions from soil (Fig. 2), while no effect on N_2O was observed (Fig. 2, Table 1).

3.2.2. Nutrient leaching

Ammonium and phosphate leaching values were highest in microcosms receiving the sterile inoculum, averaging 44.78 ± 23.38 mg NH_4^+ L^{-1} and 2.17 ± 0.90 mg P L^{-1} (Fig. 2). However, sterile treatments showed the lowest nitrate concentrations in their leachates (13.43 ± 19.24 mg NO_3^- L^{-1}). Microcosms supplemented with nitrogen had significantly higher nitrate concentrations present in their leachates (43.96 ± 40.69 mg NO_3^- L^{-1}) compared to the control microcosms without nitrogen fertilization (24.15 ± 25.65 mg NO_3^- L^{-1}) (Fig. 2, Table 1).

3.2.3. Litter decomposition and ^{15}N turnover

Across all microcosms, we found that an average of 61.37 ± 20.59 % of the sterilized *Lolium multiflorum* shoots added to assess litter decomposition were decomposed by the end of the experiment. We found a trend towards increased decomposition rates in treatments with soil sieved through increasing mesh sizes (Fig. 2), although with some noticeable intra-treatment variation, particularly in the 5-mm treatment. The ability of leek plants to acquire N through litter mineralization was estimated using the δ15N signal in the shoots at the end of the experiment. The δ15N signal ranged from 160.54 ± 85.80 (sterile treatment microcosms) to 358.86 ± 173.93 (5-mm treatment microcosms). Microcosms with nitrogen added showed significantly lower δ15N signals (215.49 ± 106.48) than control microcosms without nitrogen fertilization (352.18 ± 179.58) (Table 1), indicating that N fertilization suppresses the importance of biological N supply sources.

3.2.4. Ecosystem multifunctionality index (EMF)

The calculated EMF values ranged from −0.87 to 0.98 and were highest in microcosms receiving the 5-mm sieved soil inoculum (Fig. 3). Microcosms inoculated with soil sieved through 100 μm showed an average EMF of 0.21 ± 0.38 (11 μm; −0.02 ± 0.32). The lowest values of the EMF were recorded in microcosms inoculated with sterile soil (−0.59 ± 0.16). We ran correlations between the EMF values and each ecosystem function (Fig. S8), and observed that aboveground biomass ($R = 0.89$, $p < 0.001$), followed by belowground biomass ($R = 0.86$, $p < 0.001$) best correlated with EMF.

3.3. Biodiversity-ecosystem functioning relationships

Soil biodiversity correlated significantly with ecosystem multifunctionality (Fig. 3). Ecosystem multifunctionality and soil biodiversity did not vary strongly between microcosms inoculated with 5-mm and 100-μm sieved soil, but these indices decreased in microcosms inoculated with 11-μm sieved soil and, particularly, in microcosms inoculated with sterilized soil had the lowest EMF. The correlation between soil biodiversity and ecosystem multifunctionality was strongly driven by the sterile treatment, but it was still significant when microcosms inoculated with sterile soil were removed from the ordinary least-squares regression model ($R = 0.50$, $p < 0.001$; Fig. S9). Among the ecosystem functions included in the EMF, plant dry biomass and mineral nutrient leaching showed the highest dependency on soil biodiversity (Fig. S10). All soil biodiversity measures included in this study (i.e., bacterial and eukaryotic alpha-diversity, and AMF colonization) significantly correlated with ecosystem multifunctionality (Fig. S11), the strongest correlation being with Shannon's diversity of eukaryotic amplicon sequence variants ($R = 0.61$, $p < 0.001$). However, the correlation between multifunctionality and AMF colonization was strongly driven by the microcosms with no AMF colonization (i.e., sterile and 11 μm treatments); this

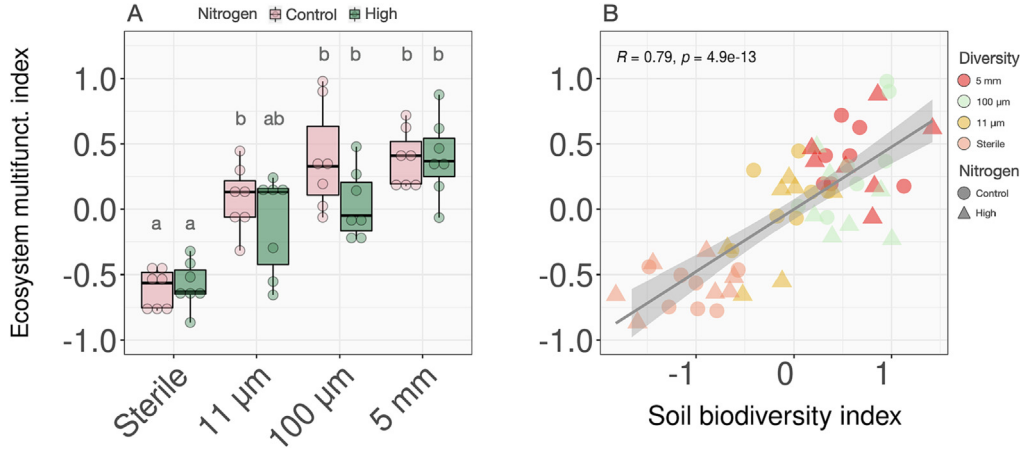


Fig. 3. Ecosystem multifunctionality index (A) along diversity (sterile, 11 μm, 100 μm, and 5 mm) and nitrogen (control, high) treatments. Different letters indicate significant differences between groups (Tukey post-hoc test, $p < 0.05$). Relationship between soil biodiversity and ecosystem multifunctionality in microcosms receiving different soil inoculum (B). Fit statistics (Pearson's R and P-value) are also provided. Points are individual microcosms. Color indicates soil biodiversity treatment, and shape indicates whether microcosms were supplemented with nitrogen (triangles) or not (circles).

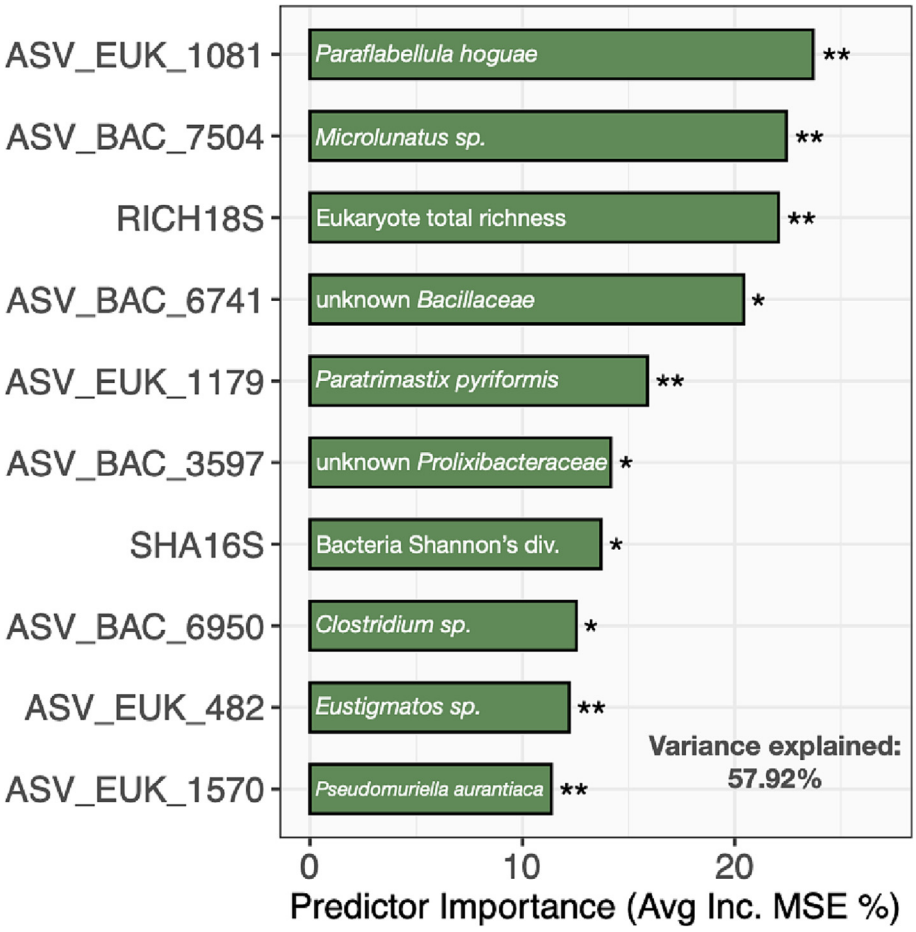


Fig. 4. Top 10 predictors of ecosystem multifunctionality according to random forest regression model. Amplicon sequence variants (ASV) are assigned to the lowest taxonomic group, and taxonomy is indicated in each bar plot. Predictor importance is indicated as % increase in mean square error (MSE). Significance: **; p-value < 0.01, *; p-value < 0.05. Correlations between these predictors and multifunctionality are shown in Fig. S12.

correlation became non-significant (p-value > 0.05) when the microcosms receiving sterilized inoculum were removed from the correlation. Random forest analysis further determined that 28 eukaryotic amplicon sequence variants (ASVs) and 36 bacterial ASVs were significantly ($p < 0.01$) associated to changes in one or several ecosystem functions (Table S4). Random forest analysis also determined that alpha-diversity parameters (i.e., richness and Shannon's diversity) of eukaryotic sequences were strongly associated to changes in ecosystem multifunctionality Fig. 4, Table S4). Bacterial ASVs associated to ecosystem functioning were mainly related to members of the Firmicutes phylum (i.e., *Clostridium* and *Domibacillus*), while eukaryotic ASVs spanned many supergroups including Stramenopiles, Rhizaria, Opisthokonta, Archaeaplastida, and Alveolata (Table S4).

4. Discussion

Here we manipulated soil biodiversity in experimental microcosms by sequentially sieving soil inoculum through decreasing mesh sizes. This allowed us to assess whether reductions in soil biodiversity decrease ecosystem multifunctionality. Among the ecosystem functions assessed, plant dry biomass was strongly affected by soil community simplification, suggesting that soil biodiversity is of crucial importance for crop yield in agroecosystems. We used leek as crop in this study. It is well known that the growth of leek is promoted by soil biota such as arbuscular mycorrhizal fungi (Salomon et al., 2022), and further studies need to mechanistically assess whether other crops are also affected so much when soil biodiversity is changed.

Our study found a sequential decrease in soil biodiversity across the diversity treatments, but this reduction was not fully mirrored at the multifunctionality level. Although there was a noticeable trend, we did not observe any significant differences in multifunctionality among the microcosms that received inoculum sieved through 11 μm, 100 μm, and 5 mm. This indicates that there is a certain level of functional redundancy. Our results indicate that reducing biodiversity in soil can have a negative impact on ecosystem multifunctionality.

Fertilization showed only small effects on soil biodiversity and ecosystem multifunctionality, and these effects were limited to nitrogen turnover and leaching of mineral nitrate. Interestingly, the fraction of plant nitrogen derived from decomposing litter declined strongly upon fertilization; the δ15N signal on leek shoots from fertilized microcosms was on average 38.8 % lower than the signal on control (non-fertilized) microcosms, indicating that fertilization impairs the natural fertilizer role of soil biota at multiple diversity levels. The small effect of nitrogen fertilization on soil biodiversity could be due to the relative short duration of this experiments (21 weeks) or because the primers used did not detect important groups of microbes that are sensitive to nitrogen fertilization (e.g., taxa involved in the nitrogen cycle including nitrifying and denitrifying bacteria).

The correlation between overall ecosystem multifunctionality and soil biodiversity was strong and remained significant even after microcosms receiving sterile inoculum were removed from the analysis. Random forest determined that alpha diversity of 18S rRNA gene amplicons were better predictors of ecosystem multifunctionality than 16S rRNA. In line with this, previous studies have shown that microbial eukaryotes (e.g., fungi and protists) might be better predictors of soil multifunctionality than bacteria (Guo et al., 2021; Hu et al., 2021; Li et al., 2019). Further studies are necessary and a mechanistic understanding on the relative importance of different soil taxa is lacking, as other studies show the opposite trend

(Wagg et al., 2019). Random forest analysis pointed in this study towards protists belonging to Amoebozoa (i.e., *Paraflabellula*) and Excavata (*Paratrimastix*) as good predictors of ecosystem multifunctionality. The genera *Paraflabellula* and *Paratrimastix* are known to primarily feed on bacteria, and we therefore suggest that future studies should address their potential top-down control of bacterial community composition and how this drives soil multifunctionality (Hampl, 2017; Roger Anderson, 2017). Random Forest also indicated towards members of Actinobacteria (i.e., *Microlunatus*), Bacteroidetes (i.e., *Prolixibacteraceae*), and Firmicutes (i.e., *Bacillus*) as good predictors of ecosystem multifunctionality.

The method employed here to experimentally reduce diversity has successfully been employed before to manipulate both composition and alpha diversity in soil communities (Wagg et al., 2014, 2021). Compared to the dilution-to-extinction method, where the filtering of species is nonspecific, our approach has the advantage of increased predictability over the community composition present in the inoculum. However, reductions in overall soil diversity with the method employed here are equivalent to those observed following the dilution-to-extinction method (Chen et al., 2020). By sequentially sieving a soil inoculum, we achieved a 45.9 % reduction in bacterial richness and a 13.9 % reduction in bacterial diversity (Shannon's Index). The effect on eukaryotic community was larger: richness was on average 82.9 % lower in microcosms inoculated with sterilized soil compared to microcosms inoculated with 5-mm sieved inoculum (Shannon's diversity index was 60.2 % lower). Whereas the dilution-to-extinction method should produce similar effects on bacterial and eukaryotic communities, we here show that our approach was more efficient in reducing eukaryotic rather than bacterial diversity. In line with this, we fully removed arbuscular mycorrhizal fungi (AMF) from the sterile and 11 μm treatments. Importantly, AMF colonization rates observed in 100 μm and 5 mm treatments (ca. 50 %) were like those found on conventional and no-tillage farming systems (Banerjee et al., 2019). Consistent with our expectations, the microcosms inoculated with sterilized soil exhibited the greatest within-treatment variation in soil diversity. We attribute this finding to reduced interspecific competition and increased stochastic reassembly, which likely allowed the soil community to evolve rapidly in various directions and reach distinct equilibria. This suggests that the initial conditions of the soil community strongly influence its subsequent diversity and composition. As previous research has demonstrated, sterilization may have reset the community to a state where stochastic processes played a more prominent role in shaping its assembly, resulting in greater heterogeneity among replicate microcosms (Kim et al., 2013; Santillan et al., 2019).

Our first hypothesis predicted that soil community simplification would reduce overall ecosystem multifunctionality, and this prediction was supported by our results. We here provide solid experimental evidence that soil microbial diversity is strongly associated to ecosystem multifunctionality. Importantly, moderate-to-small reductions in microbial richness led to considerable decreases of ecosystem multifunctionality in this study, especially in the case of plant primary production and soil capacity to retain inorganic nutrients. In line with previous studies, we found that microcosms receiving sterilized inoculum showed the largest ammonium concentrations in their leachates (Bonkowski and Roy, 2005). Similarly, other studies have found that soil biota enhance agricultural sustainability by reducing nitrogen (Bender and van der Heijden, 2015; Thiele-Bruhn et al., 2012) and phosphorus (Wagg et al., 2014) losses. Our results are therefore in line with a growing body of literature pointing at the role of soil microbial diversity on supporting multiple ecosystem functions (Delgado-Baquerizo et al., 2016; Shi et al., 2020; Wagg et al., 2014; Wang et al., 2022). We add to previous studies pointing at losses of microbial richness over 50 % as a tipping point over which ecosystem multifunctionality is seriously compromised (Wagg et al., 2021). It is important to note that it is not possible to directly compare the results of this experiment with agricultural fields and further research needs to establish tipping point values for agricultural fields. Also, the levels of microbial diversity here are lower compared to values observed in some field studies (Cui et al., 2022; Delgado-Baquerizo et al., 2021). It is possible that there is a strong link between microbial diversity and ecosystem performance below a specific basic level of microbial diversity. Above such a level (e.g., a tipping point) microbial diversity might be less important, as there is functional redundancy. Future studies need to assess this hypothesis. Finally, we highlight here the need to include additional ecosystem functions in future studies, such as those related to carbon utilization potential, to further enhance our understanding of how soil biodiversity drives multifunctionality (Garland et al., 2021).

Importantly, the correlation between soil biodiversity and ecosystem multifunctionality was maintained even when the treatment receiving sterilized inoculum was removed from the analysis, indicating a reliable biodiversity-ecosystem function (BEF) relationship in our microcosms. Furthermore, we show that integrating several biodiversity components into a soil biodiversity index, together with the integration of nine ecosystem functions into an ecosystem multifunctionality index, allows evaluation of BEF relationships in an integrative manner. In line with this, the strong correlation between ecosystem multifunctionality and soil biodiversity indicated that the soil community characteristics included in the SBI were adequate indicators of soil diversity in our microcosms. The use of biodiversity/functioning indices has been previously proposed to efficiently represent the interactive nature of soil biodiversity networks (Wagg et al., 2014). We observed that the function showing the largest dependency on soil biodiversity was aboveground biomass, which is of great interest from the food production perspective.

Our second hypothesis predicted that mineral nitrogen fertilization would increase plant productivity, while decreasing diversity in soil. We could not observe a clear increase in plant biomass in microcosms supplemented with ammonium nitrate, suggesting that leek plants in our experiment were not nitrogen limited. However, we systematically recorded higher relative abundances of Alpha- and Gammaproteobacteria in microcosms supplemented with nitrogen, while the relative abundance of Clostridia decreased. Previous studies have also shown shifts towards Proteobacteria-dominated communities after nitrogen application (Dai et al., 2018; Stefan et al., 2021). In our study, the change in bacterial community composition following nitrogen addition was accompanied by lower nitrogen turnover rates between *Lolium* litter and leek shoots, indicating that changes in soil bacterial community structure might impair nitrogen acquisition by plants. We here show that decreased nitrogen acquisition by leek plants occurs independently of the diversity treatment, with an average decrease of 38.8 %. Previous research suggests that mineral nitrogen might partially decimate nitrifying communities (e.g., ammonia-oxidizing bacteria), therefore leading to reduced uptake of oxidized forms of nitrogen (i.e., nitrate) by plants (Fan et al., 2011; Kong et al., 2019). With the random forest analysis, we further demonstrated that changes in nitrogen turnover rates could have been driven by shifts in the relative abundance of bacterial taxa such as *Domibacillus* and Bacteroidales. The order Bacteroidales has recently been significantly correlated to enzymatic activities in mangrove soils (Craig et al., 2021), therefore it is expected that members of this group actively make nitrogen available for plant growth in natural and anthropogenic environments. Similarly, the *Bacillaceae* family (including *Domibacillus*) and other members within the phylum Firmicutes are efficient decomposers of plant litter and are known to play an important role in the nitrogen cycle (Ines et al., 2015). On the other hand, addition of mineral fertilizer significantly increased nitrate concentrations in leachate. Overall, this indicates that excess nitrogen in microcosms supplemented with ammonium nitrate was kept in the soil rather than used by plants. Despite we did not measure initial nitrogen concentrations in our microcosms, a previous experiment using the same soil recorded initial nitrogen concentrations around 10 mg of inorganic N per kg of soil (Wagg et al., 2014). In line with this, we argue that nitrogen was not a limiting factor in our experiment, therefore only minor differences in ecosystem multifunctionality were observed following application of mineral nitrogen.

Our third hypothesis predicted that the interaction between soil diversity loss and nitrogen deposition would be additive, leading to a soil community even more simplified than under single-factor conditions, with negative impacts on ecosystem multifunctionality. This hypothesis was not confirmed, as we did not observe additive effects between diversity direct manipulation and nitrogen addition. We argue that the lack of interaction might have been because nitrogen was not a limiting factor in our

experiment. We argue that future studies should include quantitative measures of nitrogen-cycle genes (e.g., qPCR of denitrification genes) to fully understand how soil diversity and mineral fertilization interact to alter ecosystem multifunctionality. Moreover, only one sampling was performed in this experiment, limiting our capacity to predict single and interactive effects at different time points (Wagg et al., 2021) and over a longer period. Finally, future studies should use other primer pairs to capture sequences that might have been overlooked in this experiment, and that belong to potentially important taxa for ecosystem multifunctionality, like arbuscular mycorrhizal fungi (Higo et al., 2020).

Overall, this study suggests that preserving the diversity of soil bacterial and eukaryotic communities within agroecosystems is crucial to ensure the provisioning of multiple ecosystem functions, particularly those directly related to essential ecosystem services such as food provision.

CRediT authorship contribution statement

F.R.: Formal analysis, Visualization, Writing - Original Draft, Writing - Review & Editing.

S.H.: Conceptualization, Methodology, Investigation, Formal analysis.

A.E.: Conceptualization, Methodology, Supervision, Writing - Review & Editing.

A.H.: Resources, Methodology, Writing - Review & Editing.

K.H.: Formal analysis, Writing - Review & Editing.

M.L.: Formal analysis, Writing - Review & Editing.

M.vd.H.: Initiation, Conceptualization, Project administration, Funding acquisition, Writing - Review & Editing.

Data availability

Data will be made available through Figshare and sequence read archive (SRA) upon acceptance

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Data produced and analyzed in this paper were generated in collaboration with the Genetic Diversity Centre (GDC), ETH Zurich (Zurich, Switzerland). Authors sincerely thank to the Swiss National Science Foundation for funding through grant number 310030-188799.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.163683.

References

Allison, S.D., LeBauer, D.S., Ofrecio, M.R., Reyes, R., Ta, A.-M., Tran, T.M., 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biol. Biochem. 41, 293–302.

Banerjee, S., van der Heijden, M.G.A., 2023. Soil microbiomes and one health. Nat Rev Microbiol 21, 6–20. https://doi.org/10.1038/s41579-022-00779-w.

Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A.Y., Gattinger, A., Keller, T., Charles, R., van der Heijden, M.G.A., 2019. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. ISME J. 13, 1722–1736.

Bender, S.F., van der Heijden, M.G.A., 2015. Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. J. Appl. Ecol. 52, 228–239.

Bender, S.F., Plantenga, F., Neftel, A., Jocher, M., Oberholzer, H.-R., Köhl, L., Giles, M., Daniell, T.J., van der Heijden, M.G., 2014. Symbiotic relationships between soil fungi and plants reduce N2O emissions from soil. ISME J. 8, 1336–1345.

Bonkowski, M., Roy, J., 2005. Soil microbial diversity and soil functioning affect competition among grasses in experimental microcosms. Oecologia 143, 232–240.

Byrnes, J.E.K., Gamfeldt, L., Isbell, F., Lefcheck, J.S., Griffin, J.N., Hector, A., Cardinale, B.J., Hooper, D.U., Dee, L.E., Emmett Duffy, J., 2014. Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. Methods Ecol. Evol. 5, 111–124. https://doi.org/10.1111/2041-210X.12143.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from illumina amplicon data. Nat. Methods 13, 581–583.

Carmona, C.P., Guerrero, I., Peco, B., et al., 2020. Agriculture intensification reduces plant taxonomic and functional diversity across European arable systems. Funct Ecol. 34, 1448–1454. https://doi.org/10.1111/1365-2435.13608.

Chen, Q.-L., Ding, J., Zhu, Y.-G., He, J.-Z., Hu, H.-W., 2020. Soil bacterial taxonomic diversity is critical to maintaining the plant productivity. Environ. Int. 140, 105766. https://doi.org/10.1016/j.envint.2020.105766.

Craig, H., Antwis, R.E., Cordero, I., Ashworth, D., Robinson, C.H., Osborne, T.Z., Bardgett, R.D., Rowntree, J.K., Simpson, L.T., 2021. Nitrogen addition alters composition, diversity, and functioning of microbial communities in mangrove soils: an incubation experiment. Soil Biol. Biochem. 153, 108076.

Crowther, T.W., van den Hoogen, J., Wan, J., Mayes, M.A., Keiser, A.D., Mo, L., Averill, C., Maynard, D.S., 2019. The global soil community and its influence on biogeochemistry. Science 365 (1979), eaav0550. https://doi.org/10.1126/science.aav0550.

Cui, H., Vitousek, P.M., Reed, S.C., Sun, W., Sokoya, B., Bamigboye, A.R., Verma, J.P., Mukherjee, A., Peñaloza-Bojacá, G.F., Teixido, A.L., 2022. Environmental filtering controls soil biodiversity in wet tropical ecosystems. Soil Biol. Biochem. 166, 108571.

Dai, Z., Su, W., Chen, H., Barberán, A., Zhao, H., Yu, M., Yu, L., Brookes, P.C., Schadt, C.W., Chang, S.X., 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of actinobacteria and proteobacteria in agro-ecosystems across the globe. Glob. Chang. Biol. 24, 3452–3461.

Datta, S., Singh, Joginder, Singh, S., Singh, Jaswinder, 2016. Earthworms, pesticides and sustainable agriculture: a review. Environ. Sci. Pollut. Res. 23, 8227–8243.

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat. Commun. 7, 10541. https://doi.org/10.1038/ncomms10541.

Delgado-Baquerizo, M., Reich, P.B., Trivedi, C., Eldridge, D.J., Abades, S., Alfaro, F.D., Bastida, F., Berhe, A.A., Cutler, N.A., Gallardo, A., García-Velázquez, L., Hart, S.C., Hayes, P.E., He, J.-Z., Hseu, Z.-Y., Hu, H.-W., Kirchmair, M., Neuhauser, S., Pérez, C.A., Reed, S.C., Santos, F., Sullivan, B.W., Trivedi, P., Wang, J.-T., Weber-Grullon, L., Williams, M.A., Singh, B.K., 2020. Multiple elements of soil biodiversity drive ecosystem functions across biomes. Nat. Ecol. Evol. 4, 210–220. https://doi.org/10.1038/s41559-019-1084-y.

Delgado-Baquerizo, M., Eldridge, D.J., Liu, Y.-R., Sokoya, B., Wang, J.-T., Hu, H.-W., He, J.-Z., Bastida, F., Moreno, J.L., Bamigboye, A.R., 2021. Global homogenization of the structure and function in the soil microbiome of urban greenspaces. Sci. Adv. 7, eabg5809.

Fan, F., Yang, Q., Li, Z., Wei, D., Cui, X., Liang, Y., 2011. Impacts of organic and inorganic fertilizers on nitrification in a cold climate soil are linked to the bacterial ammonia oxidizer community. Microb. Ecol. 62, 982–990. https://doi.org/10.1007/s00248-011-9897-5.

Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15, 579–590. https://doi.org/10.1038/nrmicro.2017.87.

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.

Garland, J.L., Lehman, R.M., 1999. Dilution/extinction of community phenotypic characters to estimate relative structural diversity in mixed communities. FEMS Microbiol. Ecol. 30, 333–343. https://doi.org/10.1111/j.1574-6941.1999.tb00661.x.

Garland, G., Banerjee, S., Edlinger, A., Miranda Oliveira, E., Herzog, C., Wittwer, R., Philippot, L., Maestre, F.T., van der Heijden, M.G.A., 2021. A closer look at the functions behind ecosystem multifunctionality: a review. J. Ecol. 109, 600–613. https://doi.org/10.1111/1365-2745.13511.

de Graaff, M.-A., Hornslein, N., Throop, H.L., Kardol, P., van Diepen, L.T.A., 2019. Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: a meta-analysis. Adv. Agron. 155, 1–44.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., 2012. The protist ribosomal reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41, D597–D604.

Guo, S., Xiong, W., Hang, X., Gao, Z., Jiao, Z., Liu, H., Mo, Y., Zhang, N., Kowalchuk, G.A., Li, R., Shen, Q., Geisen, S., 2021. Protists as main indicators and determinants of plant performance. Microbiome 9, 64. https://doi.org/10.1186/s40168-021-01025-w.

Hampl, V., 2017. Preaxostyla. In: Archibald, J.M., Simpson, A.G.B., Slamovits, C.H. (Eds.), Handbook of the Protists. Springer International Publishing, Cham, pp. 1139–1174 https://doi.org/10.1007/978-3-319-28149-0_8.

van der Heijden, M.G.A., 2010. Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91, 1163–1171.

van der Heijden, M.G.A., de Bruin, S., Luckerhoff, L., van Logtestijn, R.S.P., Schlaeppi, K., 2016. A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. ISME J. 10, 389–399. https://doi.org/10.1038/ismej.2015.120.

Higo, M., Tatewaki, Y., Iida, K., Yokota, K., Isobe, K., 2020. Amplicon sequencing analysis of arbuscular mycorrhizal fungal communities colonizing maize roots in different cover cropping and tillage systems. Sci. Rep. 10, 1–13.

Hobbie, S.E., Eddy, W.C., Buyarski, C.R., Adair, E.C., Ogdahl, M.L., Weisenhorn, P., 2012. Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. Ecol. Monogr. 82, 389–405.

Hu, W., Ran, J., Dong, L., Du, Q., Ji, M., Yao, S., Sun, Yuan, Gong, C., Hou, Q., Gong, H., Chen, R., Lu, J., Xie, S., Wang, Z., Huang, H., Li, Xiaowei, Xiong, J., Xia, R., Wei, M., Zhao, D., Zhang, Y., Li, J., Yang, H., Wang, X., Deng, Y., Sun, Ying, Li, H., Zhang, L., Chu, Q., Li, Xinwei, Aqeel, M., Manan, A., Akram, M.A., Liu, X., Li, R., Li, F., Hou, C., Liu, J., He, J.-S., An, L., Bardgett, R.D., Schmid, B., Deng, J., 2021. Aridity-driven shift in

biodiversity–soil multifunctionality relationships. Nat. Commun. 12, 5350. https://doi.org/10.1038/s41467-021-25641-0.
Ines, M.-M., Polonca, S., van Dirk, E.J., 2015. Ecology of Bacillaceae. Microbiol. Spectr. 3. https://doi.org/10.1128/microbiolspec.TBS-0017-2013 3.2.16.
Isbell, F., Calcagno, V., Hector, A., et al., 2011. High plant diversity is needed to maintain ecosystem services. Nature 477, 199–202. https://doi.org/10.1038/nature10282.
Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. Ecol. Appl. 13, 1164–1176.
Jian, S., Li, J., Chen, J.I., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D., Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: a meta-analysis. Soil Biol. Biochem. 101, 32–43.
Kim, M., Heo, E., Kang, H., Adams, J., 2013. Changes in soil bacterial community structure with increasing disturbance frequency. Microb. Ecol. 66, 171–181. https://doi.org/10.1007/s00248-013-0237-9.
Kong, Y., Ling, N., Xue, C., Chen, H., Ruan, Y., Guo, J., Zhu, C., Wang, M., Shen, Q., Guo, S., 2019. Long-term fertilization regimes change soil nitrification potential by impacting active autotrophic ammonia oxidizers and nitrite oxidizers as assessed by DNA stable isotope probing. Environ. Microbiol. 21, 1224–1240. https://doi.org/10.1111/1462-2920.14553.
Lekberg, Y., Arnillas, C.A., Borer, E.T., Bullington, L.S., Fierer, N., Kennedy, P.G., Leff, J.W., Luis, A.D., Seabloom, E.W., Henning, J.A., 2021. Nitrogen and phosphorus fertilization consistently favor pathogenic over mutualistic fungi in grassland soils. Nat. Commun. 12, 1–8.
Li, J., Delgado-Baquerizo, M., Wang, J.-T., Hu, H.-W., Cai, Z.-J., Zhu, Y.-N., Singh, B.K., 2019. Fungal richness contributes to multifunctionality in boreal forest soil. Soil Biol. Biochem. 136, 107526. https://doi.org/10.1016/j.soilbio.2019.107526.
Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O'Connor, M.I., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. Nat. Ecol. Evol. 2, 936–943. https://doi.org/10.1038/s41559-018-0519-1.
Maestre, F.T., Quero, J.L., Gotelli, N.J., Escudero, A., Ochoa, V., Delgado-Baquerizo, M., García-Gómez, M., Bowker, M.A., Soliveres, S., Escolar, C., 2012. Plant species richness and ecosystem multifunctionality in global drylands. Science 1979 (335), 214–218.
McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol. 115, 495–501.
Milleret, R., Le Bayon, R.-C., Gobat, J.-M., 2009. Root, mycorrhiza and earthworm interactions: their effects on soil structuring processes, plant and soil nutrient concentration and plant biomass. Plant Soil 316, 1–12. https://doi.org/10.1007/s11104-008-9753-7.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.
Paungfoo-Lonhienne, C., Yeoh, Y.K., Kasinadhuni, N.R.P., Lonhienne, T.G.A., Robinson, N., Hugenholtz, P., Ragan, M.A., Schmidt, S., 2015. Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. Sci. Rep. 5, 1–6.
Pierre-Alain, M., Amadou, S., Aurore, K., Jean, L., Olivier, M., Julien, G., Battle, K., Laetitia, B., Samuel, D., Sébastien, T., Abad, C., Lionel, R., 2018. High microbial diversity promotes soil ecosystem functioning. Appl. Environ. Microbiol. 84. https://doi.org/10.1128/AEM.02738-17 e02738-17.
Qi, D., Wieneke, X., Xue, P., He, L., DeSilva, U., 2021. Total nitrogen is the main soil property associated with soil fungal community in karst rocky desertification regions in Southwest China. Sci. Rep. 11, 1–12.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596.
Reinprecht, Y., Schram, L., Marsolais, F., Smith, T.H., Hill, B., Pauls, K.P., 2020. Effects of nitrogen application on nitrogen fixation in common bean production. Front. Plant Sci. 11.
Ren, H., Qin, J., Yan, B., 2018. Mass loss and nutrient dynamics during litter decomposition in response to warming and nitrogen addition in a desert steppe. Front. Agric. Sci. Eng. 5, 64–70.
Roger Anderson, O., 2017. Amoebozoan lobose amoebae (Tubulinea, flabellinea, and Others). In: Archibald, J.M., Simpson, A.G.B., Slamovits, C.H., Margulis, L., Melkonian, M., Chapman, D.J., Corliss, J.O. (Eds.), Handbook of the Protists. Springer International Publishing, Cham, pp. 1–31 https://doi.org/10.1007/978-3-319-32669-6_2-1.
Salomon, M.J., Watts-Williams, S.J., McLaughlin, M.J., Bücking, H., Singh, B.K., Hutter, I., Schneider, C., Martin, F.M., Vosatka, M., Guo, L., Ezawa, T., Saito, M., Declerck, S., Zhu, Y.-G., Bowles, T., Abbott, L.K., Smith, F.A., Cavagnaro, T.R., van der Heijden, M.G.A., 2022. Establishing a quality management framework for commercial inoculants containing arbuscular mycorrhizal fungi. iScience 25, 104636. https://doi.org/10.1016/j.isci.2022.104636.
Santillan, E., Seshan, H., Constancias, F., Drautz-Moses, D.I., Wuertz, S., 2019. Frequency of disturbance alters diversity, function, and underlying assembly mechanisms of complex bacterial communities. NPJ Biofilms Microbiomes 5, 8. https://doi.org/10.1038/s41522-019-0079-4.
Shi, Y., Delgado-Baquerizo, M., Li, Y., Yang, Y., Zhu, Y.-G., Peñuelas, J., Chu, H., 2020. Abundance of kinless hubs within soil microbial networks are associated with high functional potential in agricultural ecosystems. Environ. Int. 142, 105869. https://doi.org/10.1016/j.envint.2020.105869.
Stefan, L., Hartmann, M., Engbersen, N., Six, J., Schöb, C., 2021. Positive effects of crop diversity on productivity driven by changes in soil microbial composition. Front. Microbiol. 12, 660749.
Thiele-Bruhn, S., Bloem, J., de Vries, F.T., Kalbitz, K., Wagg, C., 2012. Linking soil biodiversity and agricultural soil management. Curr. Opin. Environ. Sustain. 4, 523–528.
Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., de Ruiter, P.C., van der Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jørgensen, H.B., Christensen, S., Hertefeldt, T.D., Hotes, S., Gera Hol, W.H., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, V., Hedlund, K., 2015. Intensive agriculture reduces soil biodiversity across Europe. Glob Change Biol 21, 973–985. https://doi.org/10.1111/gcb.12752.
Vierheilig, H., Coughlan, A.P., Wyss, U.R.S., Piché, Y., 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl. Environ. Microbiol. 64, 5004–5007.
Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G.A., 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc. Natl. Acad. Sci. 111, 5266–5270.
Wagg, C., Dudenhöffer, J., Widmer, F., van der Heijden, M.G.A., 2018. Linking diversity, synchrony and stability in soil microbial communities. Funct. Ecol. 32, 1280–1292.
Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., van der Heijden, M.G.A., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nat. Commun. 10, 1–10.
Wagg, C., Hautier, Y., Pellkofer, S., Banerjee, S., Schmid, B., van der Heijden, M.G.A., 2021. Diversity and asynchrony in soil microbial communities stabilizes ecosystem functioning. elife 10.
Wang, J., Shi, X., Zheng, C., Suter, H., Huang, Z., 2021. Different responses of soil bacterial and fungal communities to nitrogen deposition in a subtropical forest. Sci. Total Environ. 755, 142449.
Wang, Y.-F., Chen, P., Wang, F.-H., Han, W.-X., Qiao, M., Dong, W.-X., Hu, C.-S., Zhu, D., Chu, H.-Y., Zhu, Y.-G., 2022. The ecological clusters of soil organisms drive the ecosystem multifunctionality under long-term fertilization. Environ. Int. 161, 107133. https://doi.org/10.1016/j.envint.2022.107133.
Ward, D., Kirkman, K., Hagenah, N., Tsvuura, Z., 2017. Soil respiration declines with increasing nitrogen fertilization and is not related to productivity in long-term grassland experiments. Soil Biol. Biochem. 115, 415–422.
Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Soil Sci. Soc. Am. J. 29, 677–678.
Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Guillaumaud, N., le Roux, X., 2007. Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. Environ. Microbiol. 9, 2211–2219. https://doi.org/10.1111/j.1462-2920.2007.01335.x.
Winkler, K., Fuchs, R., Rounsevell, M., et al., 2021. Global land use changes are four times greater than previously estimated. Nat Commun 12, 2501. https://doi.org/10.1038/s41467-021-22702-2.
Wittwer, R.A., Bender, S.F., Hartman, K., Hydbom, S., Lima, R.A.A., Loaiza, V., Nemecek, T., Oehl, F., Olsson, P.A., Petchey, O., 2021. Organic and conservation agriculture promote ecosystem multifunctionality. Sci. Adv. 7, eabg6995.
You, M., Zhu-Barker, X., Hao, X.-X., Li, L.-J., 2021. Profile distribution of soil organic carbon and its isotopic value following long term land-use changes. Catena (Amst) 207, 105623.
Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., Chu, H., 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biol. Biochem. 92, 41–49.
Zhao, Z.-B., He, J.-Z., Geisen, S., Han, L.-L., Wang, J.-T., Shen, J.-P., Wei, W.-X., Fang, Y.-T., Li, P.-P., Zhang, L.-M., 2019. Protist communities are more sensitive to nitrogen fertilization than other microorganisms in diverse agricultural soils. Microbiome 7, 1–16.
Zhou, S., Huang, C., Han, B., Xiao, Y., Tang, J., Xiang, Y., Luo, C., 2017. Simulated nitrogen deposition significantly suppresses the decomposition of forest litter in a natural evergreen broad-leaved forest in the rainy area of Western China. Plant Soil 420, 135–145.
Zhou, Z., Wang, C., Luo, Y., 2020. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nat Commun 11, 3072. https://doi.org/10.1038/s41467-020-16881-7.