



Soil function-microbial diversity relationship is impacted by plant functional groups under climate change



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ABSTRACT

Understanding the interactions between plant and soil microbial diversity is crucial for predicting ecosystem responses to environmental changes. While the individual roles of plant and microbial diversity in driving ecosystem functions have been widely investigated, their interplay especially under stress conditions remains largely underexplored. This study investigated how interactions between plant and microbial diversity affect key soil functions during and after drought. We simultaneously manipulated soil microbial diversity and plant species richness, while also considering the influence of plant functional groups (PFGs), to investigate their interactions and effects on key soil functions. Our results revealed independent and interactive effects of plant and microbial diversity in shaping soil functions. Microbial diversity loss significantly altered microbial community structure and impacted microbially-driven soil N and P pools and processes such as N-mineralization. These effects were modulated by plant species richness and varied across different PFGs. The relative influence of plant and microbial diversity on soil functions was context-dependent. Microbial diversity showed stronger effects on specific functions, such as phosphatase activity, and under the drought condition. Plant diversity, particularly through PFGs (e.g. legumes), played an independent role in shaping the microbial-driven soil functions. These findings advance mechanistic insights and highlight the importance of considering both above- and belowground biodiversity, along with their interactions, in shaping soil functions and ecosystem resilience, particularly under environmental stress. Further, it emphasizes the need to explicitly consider PFGs, along with above- and belowground biodiversity, as a strategy for preserving essential belowground functions in the face of ongoing environmental changes.

1. Introduction

The relationship between plant diversity and ecosystem functioning (BEF) has been a long-standing interest in ecology, driven by the need to understand the consequences of biodiversity decline in the face of global change on ecosystem functions, services, and human well-being (Cardinale et al. 2012; Isbell et al. 2015; Reich et al. 2012). Recent

studies have also highlighted the role of soil biodiversity in supporting multiple ecosystem functions and services (Delgado-Baquerizo et al. 2016; Trivedi et al. 2019; Wagg et al. 2014a), including soil nutrient cycling, carbon storage, and erosion prevention (Liu et al. 2018; Wall et al. 2015). Furthermore, several factors influencing belowground functioning have gained substantial attention within the scientific, geopolitical, and public attention (Amundson et al. 2015; Averill et al.

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2021; Colombo et al. 2016; Delgado-Baquerizo et al. 2019; Soliveres et al. 2016; Thakur et al. 2019; Wagg et al. 2014a). Studies have also highlighted the influence of plant diversity in increasing soil microbial activity and soil carbon storage and nutrient supply, which in turn enhances soil fertility and plant productivity (Furey and Tilman 2021; Lamb et al. 2011; Prommer et al. 2020). Recent evidence suggests that the relationship between soil and plant biodiversity and their function is context-dependent (Canessa et al. 2022; Guerrero-Ramírez and Eisenhauer 2017; Luo et al. 2017; Martins et al., 2024; Stefan et al. 2021), however the biotic and environmental factors shaping these relationships are not well understood. For instance, the influence of soil biodiversity on function may depend on ecosystem water availability (Hu et al. 2021). Additionally, plant species impact on soil bacterial communities appears to depend on plant community diversity and is mediated through the effects of plant-derived resources on antagonistic soil microbes (Schlatter et al. 2015). The complementarity between plant and soil microbial diversity contributes to multifunctionality in grasslands, but these contributions are subject to contextual variations (Martins et al., 2024). Growing evidence underscores the significant role of microbial diversity in shaping the positive relationship between plant diversity and ecosystem function (Delgado-Baquerizo et al. 2017b; Isobe et al. 2020; Wu et al. 2019; Yang et al. 2021b). These studies highlight the context-dependent nature of soil and plant diversity interactions, influenced by factors such as, precipitation history, productivity, and soil microbial community structure. However, experimental evidence that comprehensively consider the interplay between plant and soil biodiversity under varying climatic conditions, particularly water availability, remain scarce. Therefore, incorporating above-ground interactions into BEF research is essential for deepening our understanding of BEF relationships in the context of climate change.

The interplay between plants and microbes involves numerous above- and belowground interactions (e.g., mutualisms and pathogenesis), which have substantial implications for BEF relationships (Eisenhauer et al., 2010, 2019; Hu et al., 2015; Singh and Gupta, 2018; Weisser et al., 2017; Yang et al., 2021b; Yuan et al., 2020). On one hand, soil microbial diversity can independently have a positive impact on plant nutrient uptake by promoting nutrient cycling (Trivedi et al. 2020). However, the relative contribution of soil microbial diversity to nutrient availability may depend on the functional groups within the plant community. Indeed, plant functional groups (PFGs) sharing similar ecological traits, such as nitrogen fixation or photosynthetic pathways, are known to influence the soil microbiome and associated soil functions (Cornwell et al., 2008; Faucon et al., 2017; Gou et al., 2023; Reich et al., 2004; Singh and Gupta, 2018; Trivedi et al., 2020; Xiao et al., 2017). For instance, nitrogen (N)-fixing plants are known to promote keystone microbial taxa, such as *Rhizobium* spp. and *Frankia* spp. (Zheng et al. 2021). Similarly, grasses and forbs, exhibit discernible traits and occupy specific ecological niches (Herz et al. 2017; Šmilauer and Šmilauerová 2013). Compared to forbs, perennial grasses have a higher belowground biomass leading to dense root systems (Ravenek et al. 2016). As a result, grass-dominated communities exhibit accelerated rates of litter decomposition and enhanced soil nutrient cycling (Wu et al. 2011). Consequently, variations in PFGs are likely to influence the composition of associated microbial communities (Xiao et al. 2017), raising the question of how PFGs regulate soil microbial communities and their functions, and how this relationship varies across gradients of microbial diversity. However, the effects of plant functional richness and individual functional groups on soil microbial diversity and ecosystem processes remain unclear. Further, global change drivers and human activities threaten biodiversity and functioning, including potentially the relationships between plant and microbial diversity and soil processes in complex but unknown ways (Rillig et al. 2019; Trivedi et al. 2016). Yet, there is a critical paucity of studies on the biotic interactions when assessing the effects of global change drivers on ecosystem functions (Maestre et al. 2015; Rillig et al. 2019). Lack of understanding of

the relative contributions of plant functional richness, PFGs, and/or microbial diversity, and their interactions with environmental stresses on soil functions (Wei et al. 2019) is a critical knowledge gap that constrains our predictive capacity and informed management strategies to mitigate the negative impacts of global change.

To bridge these knowledge gaps, we conducted an integrative experiment, simultaneously manipulating plant and soil microbial diversity while explicitly considering PFGs and plant functional diversity. We established a microcosm experiment with six plant species, categorized into three functional types (C3 grasses, C4 grasses, and legumes) in three levels of richness (1, 3, 6 species) and three levels of microbial diversity (high, intermediate, low), all under induced drought conditions. We formulated two primary hypotheses: (1) Soil microbial diversity loss would weaken the positive relationship between plant diversity and ecosystem functions, particularly under drought conditions. This hypothesis is based on the fact that soil microbes play an important role in mediating plant diversity-ecosystem function relationships (Delgado-Baquerizo et al. 2016; Eisenhauer et al. 2012) and the microbial communities are susceptible to drought (Sheik et al. 2011). (2) Plant functional groups will play a more significant role in buffering soil functions during drought than plant diversity. This effect arises from the varying capacity of different functional groups (C3 vs. C4 vs. legumes) to withstand abiotic stresses associated with drought conditions. Overall, this study investigates the complex interplay between soil microbial diversity, plant diversity, and PFGs, seeking to decipher their critical role in shaping plant-soil microbiome interactions under drought stress.

2. Material and methods

2.1. Experimental design

We employed a factorial design to investigate the interactive effects of soil microbial diversity, plant functional diversity, and PFGs on various soil N and P pools and processes prior, during and after induced drought. Briefly, the design involved three dilution levels of soil microbial diversity (D0, D2, and D6) and three levels of plant species richness (1, 3, and 6 species). The plant species used were *Chloris gayana*, *Digitaria eriantha* (C4 grasses), *Lolium perenne*, *Phalaris aquatica* (C3 grasses), *Biserrula pelecinus* and *Medicago sativa* (legumes) (Fig. S1). This design is considered incomplete because not all combinations of 3 species with all functional groups were included for logistic reasons. The plant diversity treatments were replicated six times and were identical for D0 (10^{-0} -High), D2 (10^{-2} -Intermediate) and D6 (10^{-6} -Low) microbial diversity treatments, with no-plant controls included for each microbial diversity level. To account for airborne contamination throughout the experiment, the study also included five pots with sterile soil inoculated only with phosphate buffer saline (PBS) solution. In total, there were 33 treatments, for a total of 203 microcosms and five sampling events (Fig. 1).

2.2. Soil microbial diversity manipulation

To manipulate indigenous soil microflora, we utilized the robust dilution-to-extinction approach, following well-established procedures (Delgado-Baquerizo et al. 2020b; Trivedi et al. 2019). In brief, we collected soil from the top 15 cm of a regional grassland. Full site description and soil characteristics can be found in Churchill et al. (2022) (Table S1). Soil was sieved with a 5-mm sieve and aliquots of approx. 1.8 kg of fresh soil in plastic bags were sterilized using gamma-irradiation (50 kGy each) at ANSTO Life Sciences facilities, Sydney, Australia. Soil microbial inoculums representing high, intermediate, and low microbial diversity were created using a 10-fold dilution approach with phosphate buffer saline (PBS) as the buffer. Sterile soil was inoculated with a 9:1 ratio of soil to microbial inoculum, and a portion of the sterile soil was maintained with no microbial

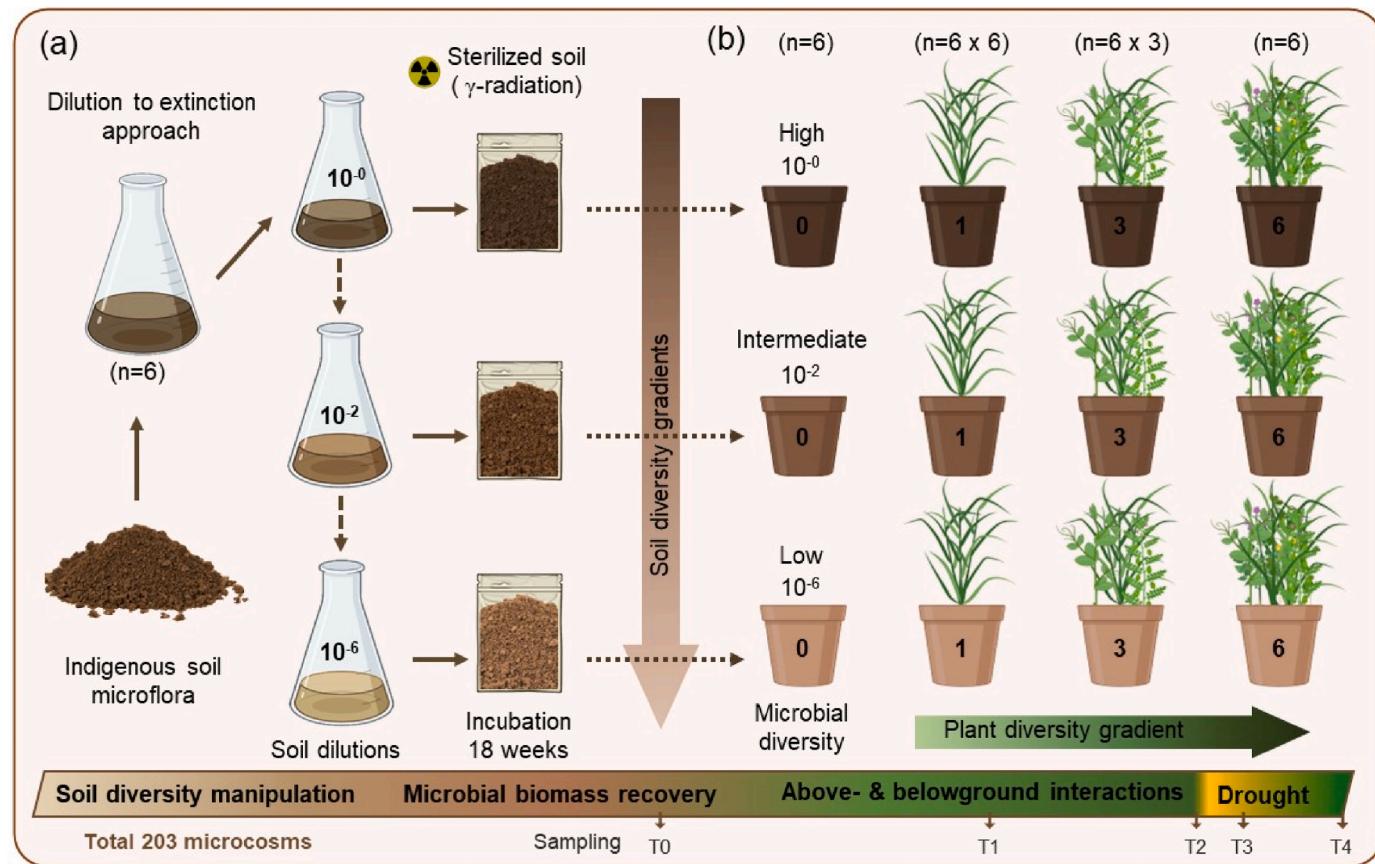


Fig. 1. Schematic illustration of the factorial design of the microcosm glasshouse experiment to manipulate both soil and plant diversity simultaneously. (a) Dilution to extinction approach to create three soils diversity levels (10^{-0} – High, 10^{-2} – Intermediate, 10^{-6} – Low). (b) Plant diversity gradients – from a pool of six plant species with different functional groups (C₃, C₄ and N-fixing). The experiment timeline consists of Soil diversity manipulation, microbial biomass recovery, preparation of microcosms, plant diversity establishment, two weeks of drought treatment and recovery for four weeks after rewetting. There were total 203 microcosms and five sampling events.

diversity (PBS solution only). Since the dilution-to-extinction approach creates differences in inoculum biomass which can affect the rates of soil process (Singh and Gupta 2018), we pre-incubated the microcosms to allow the microbial biomass recovery. After 18 weeks of incubation, microbial colonization and biomass recovery were monitored and validated using amplicon sequencing and qPCR for bacterial 16S rRNA gene and fungal internal transcribed spacer (ITS) (Martins et al. 2024).

2.3. Microcosms and plant diversity establishment

To create the grassland microcosms, pots with an inner diameter of 14 cm and a height of 15 cm (1.9 L) were filled with (~1.9 kg) soil from the respective microbial diversity levels and subsampled (prior to planting, T0). Monoculture, three-species, and six-species microcosms were established using ~5 weeks old seedlings grown in sterile conditions. The allocation of seedlings was executed according to a pre-designed plant diversity pattern, ensuring consistency and replication across the experiment (Fig. S1). All microcosms were arranged randomly in a climate-controlled glasshouse (temperature of 20/15 °C day/night, with a day length of 12 h day/night) (Martins et al., 2024).

2.4. Drought treatment and sampling

The microcosms were kept well-watered, at initial plant establishment period (T1) and early flowering stage (pre-drought, T2), and intermediate soil sampling was done using one soil core taken from each pot. The experiment was carried out in a glasshouse room with corresponding Spring and Summer temperatures from monthly averages up to

28/18 °C day/night, from September to January, based on the last 10-year monthly average data from Meteorological Bureau Station 067021 (<http://www.bom.gov.au>). After 16 weeks of plant establishment, a 2-week drought treatment was applied to half of the pots by reducing watering to 30% of water holding capacity (WHC), while the remaining pots were watered to maintain 60% of WHC. Gravimetric moisture was monitored daily during the drought period, and soil samples were collected (drought, T3) after 2 weeks of drought to assess the effects on soil functions. The final harvest of plant and soil samples (after-recovery, T4) was performed after a 4-week recovery period. This approach allowed us to assess the resilience of belowground functions to drought, providing realistic scenarios of areas with increasing dry periods (Delgado-Baquerizo et al., 2017a).

2.5. Soil physiochemical, functional and diversity measurements

Measurements of soil properties (see Note S1 for methodological details) included moisture content, pH, extractable nitrate (NO₃), ammonium (NH₄), extractable phosphate (PO₄), total carbon (C), and total nitrogen (N). Additionally, we determined the rate of mineralized N and potential acid phosphatase (PHOS) enzyme activity. Total genomic DNA was extracted from soil samples, and microbial biomass was quantified prior to planting (T0), by measuring bacterial and fungal RNA copy number (bacteria: 16S rRNA; fungi: ITS rRNA) using qPCR (see Note S2 for methodological details) and amplicon sequencing for 16S rRNA gene and ITS (see Note S3 for methodological details). This allowed us to validate the dilution-to-extinction approach (Note S3) and obtain insights into the community structure of microbial communities

in response to our experimental manipulations. In addition, plant parameters such as plant biomass, leaf carbon (C), leaf nitrogen (N), and leaf phosphorus (P) were measured as described earlier (Martins et al., 2024).

2.6. Statistical analysis

Two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to assess the effects of dilution-to-extinction approach on microbial abundance, and microbial richness (observed OTUs and Chao1) and diversity (Shannon index). A Šidák's multiple comparison test was used to compare between sampling points or otherwise mentioned in the figure legends. Permutational multivariate analysis of variance (PERMANOVA) was employed to test the significance of changes in microbial composition (number of permutations: 999) based on Bray-Curtis distances using the web-based MicrobiomeAnalyst platform (Chong et al. 2020). Spearman correlation coefficients (ρ) were used to determine the positive or negative relationships between microbial and plant diversity with soil functions across soil diversity and plant richness levels. Additionally, to test for the influence of aboveground plant parameters such as plant biomass, leaf carbon (C), leaf nitrogen (N), and leaf phosphorus (P), we conducted partial correlation analysis between soil functions and plant parameters, while accounting for microbial diversity and community structure (main axis of a NMDS analysis) as control variables at T4 (After Recovery). To evaluate the relative contributions of these variables as drivers of soil functions, we quantified the explained variance (%) for each predictor based on separate linear models. Specifically, we extracted R-squared values and assessed the variance explained by each predictor variable using separate linear regression models for each response variable. Multiple linear regression models (R function, *lm()*) were fitted for soil N and P nutrient pools (NH_4 , NO_3 and PO_4) and processes (N mineralization and Phosphatase activity) using combination of predictor variable such as soil microbial diversity and community structure, plant species richness, PFGs, and drought individually, using nested *lapply()* functions to iterate over all combinations of response and predictor variables. To assess the independent contribution of each predictor, separate models were also run, where each predictor was included individually. In addition, we conducted a multifactorial ANOVA to assess the independent and interactive effects of plant species richness, PFGs, microbial

diversity, and drought on soil nutrient pools and processes. A value of $p < 0.05$ was considered to be statistically significant. All tests and statistical models were constructed and visualized by R 4.1.2 (R Core Team 2018) and GraphPad Prism 9.0 software (Boston, Massachusetts USA, www.graphpad.com). This approach allowed us to quantify the importance of each predictor in explaining the variation in soil N and P pools and processes.

3. Results

3.1. Impact of dilution on microbial biomass recovery and community structure

The dilution-to-extinction approach successfully achieved a microbial diversity gradient, with significant reductions in bacterial and fungal richness at higher dilutions. Quantitative PCR measurements indicated that microbial communities recovered to comparable biomass levels across all dilutions, with undiluted and diluted treatments showing similar bacterial (~9 log copies/g) and fungal (~8 log copies/g) abundances (Fig. S2, Note S2). Soil microbiome analysis revealed significant changes in microbial community structure, with distinct shifts in bacterial and fungal richness and composition observed between dilutions (Fig. 2a, 2b, S3, Note S3), particularly in low-abundance taxa such as Cyanobacteria and Basidiomycota (Fig. 2c and d). The presence of plants had a notable impact on microbial biomass but did not significantly alter community composition across plant functional groups or richness treatments (Fig. S4).

3.2. Effects of soil microbial diversity loss on soil functions

The consequences of microbial diversity loss had a profound impact on the observed soil functions (pools + processes) including soil N and P pools, particularly on ammonium ($F_{(2,195)} = 65.2, p < 0.001$) and nitrate ($F_{(2,195)} = 35.9, p < 0.001$) concentrations. In soils with reduced microbial diversity (D6), ammonium concentrations were approximately 40 times higher compared to those in high (D0) and intermediate (D2) diversity soils, which exhibited similar levels of ammonium (~1.8 mg kg⁻¹) and nitrate (~80 mg kg⁻¹) (Figure S5(i), and S5(ii)). Consequently, ammonium had a significant negative correlation with microbial richness (bacterial; $\rho = -0.61, p < 0.001$ and fungal; $\rho = -0.62, p <$

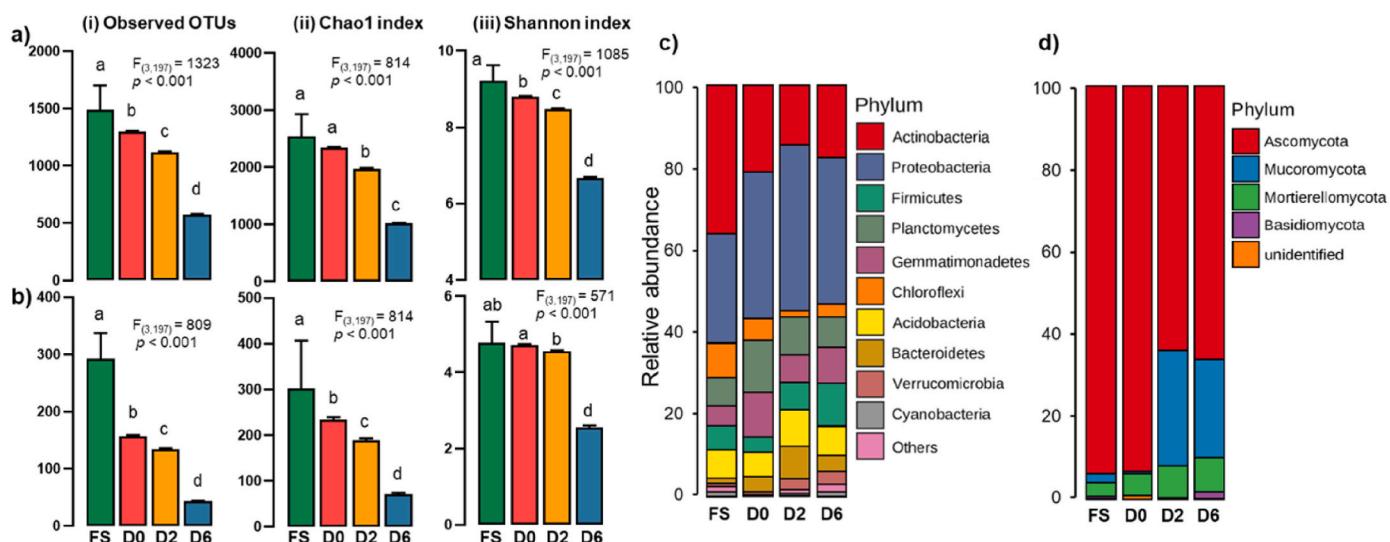


Fig. 2. Effects of dilution on (a) bacterial and (b) fungal observed richness (i), Chao1 index (ii) and Shannon diversity index (iii) measured after microbial biomass recovery (T0; pre-planting) at different dilution levels D0 (10^{-0} –High), D2 (10^{-2} –Intermediate) and D6 (10^{-6} –Low), in comparison with fresh soil (FS). Significant differences between soil dilutions and fresh soil evaluated by one-way ANOVA followed by Tukey's multiple comparison test indicated by small letters significant at $p < 0.05$. Impacts of dilution-to-extinction approach on the relative abundance of (c) bacterial and (d) fungal community composition at Phylum level in different dilutions with comparison to fresh soil.

0.001) (Table S2), while nitrate displayed a strong positive correlation (bacterial; $\rho = 0.77$, $p < 0.001$ and fungal; $\rho = 0.74$, $p < 0.001$). Although phosphate levels did not significantly differ among soil dilutions (Figure S5(iii)), they exhibited a significant negative correlation with microbial richness (bacterial; $\rho = -0.43$, $p < 0.001$ and fungal; $\rho = -0.48$, $p < 0.001$) (Table S2). We also observed that low-diversity soils (D6) had increased the rates of N mineralization, although this difference was not statistically significant ($F_{(2,195)} = 0.34$, $p = 0.711$; Figure S5(iv)), resulting in a negative correlation with microbial richness (bacterial; $\rho = -0.42$, $p < 0.001$ and fungal; $\rho = -0.38$, $p < 0.001$). Similarly, while no significant differences were found in Phosphatase activity among soil diversity levels (Fig. S5(v)), it showed a positive relationship with microbial richness (bacterial; $\rho = 0.27$, $p < 0.001$ and fungal; $\rho = 0.28$, $p < 0.001$) (Table S2).

3.3. Predictors of soil functions and aboveground-belowground interactions

Plant functional groups (PFGs) emerged as the dominant predictors of soil functions, explaining up to 87% of total variance. Plant species richness and PFGs collectively accounted for the majority of the explained variance across four of the five soil functions we examined. However, microbial diversity and community structure primarily explained variance (41.5% and 46.7% respectively) in PHOS activity in pre-drought measurements (Fig. 3a). These findings were robustly supported by our statistical models (Table 1), underscoring the predominant role of PFGs in driving all soil functions. Conversely, microbial diversity exerted a comparatively low influence on soil functions associated with nitrogen cycling during pre-drought sampling. However, its importance increased notably during the drought period, where it

showed higher explained variance than PFGs (Fig. 3b). These results were validated by regression models, which identified the individual and combined effects PFGs and other predictor variables (Table 1). While drought had a negligible direct effect, accounting for less than 1% of the variance, it had a significant impact on microbially driven PHOS activity explaining 5.5% of variance. Following a month of recovery from the drought, we observed the soil functions displaying signs of recuperation. An average of 78% of the variance in these recovered soil functions was attributed to PFGs, with plant species richness, microbial diversity and community structure contributing to ~12.7%, 4.1%, and 4.4% respectively (Fig. 3c).

3.4. Relationship between soil functions and plant species richness across microbial dilution gradient

We examined the intricate relationships between microbial and plant diversity by assessing correlations between plant species richness and soil functions within individual soil dilutions. Additionally, we explored associations between microbial communities and soil functions at various levels of plant richness. Notably, we observed a negative correlation between plant species richness and nitrogen concentrations, except ammonium in D6 (Fig. 4). Conversely, plant species richness exhibited a consistent negative relationship with both ammonium and nitrate levels across all soil diversity levels (Fig. 5i). The impact of drought on nitrogen cycling processes was particularly pronounced in D6. This resulted in a shift in the relationship between plant richness and nitrogen levels, with higher microbial diversity in D0 and D2 stabilizing the effect (Fig. 5(ii)). Importantly, after the recovery period, the relationships largely returned to their pre-drought negative associations (Fig. 5(iii)). Plant species richness showed a significant positive

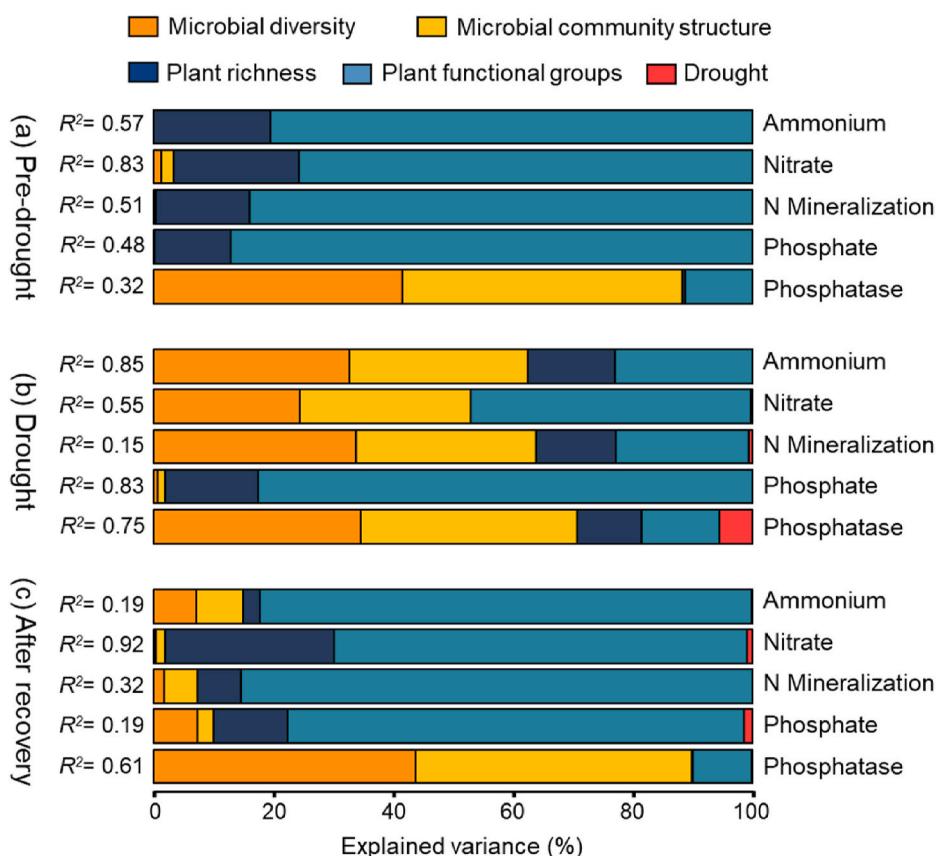


Fig. 3. The relative contribution of microbial diversity and community structure, plant richness, plant functional groups, and induced drought to individual soil pools and processes. The importance of predictor variables is explained as the percentage of explained variance (R^2 represents total variances), taken from the absolute values of their regression coefficients.

Table 1

Linear regression models to predict the effects of microbial (bacterial and fungal) diversity and community structure, plant richness, plant functional groups (PFGs), and drought on belowground pools and processes. All predictor variables were used in ‘combined’ multiple linear regression model. Significant relationships at $p < 0.001$ (***) $, p < 0.01$ (**), $p < 0.05$ (*) are in bold.

Function	Predictor var.	Pre-drought		Drought		After recovery	
		R ²	F	R ²	F	R ²	F
Ammonium	Bacterial diversity	0.00	0.04	0.31	85.29 ***	0.02	4.99 *
	Bacterial composition	0.00	0.07	0.23	55.72 ***	0.00	0.85
	Fungal diversity	0.00	0.00	0.26	69.39 ***	0.02	3.43
	Fungal composition	0.00	0.01	0.23	57.21 ***	0.02	3.47
	Plant richness	0.11	22.59 ***	0.13	28.06 ***	0.01	1.56
	PFGs	0.46	22.26 ***	0.20	6.50 ***	0.16	5.03 ***
	Drought			0.00	0.00	0.00	0.00
	Combined	0.56	18.28 ***	0.57	18.2 ***	0.21	3.64 ***
Nitrate	Bacterial diversity	0.02	2.92	0.10	21.24 ***	0.00	0.19
	Bacterial composition	0.01	2.01	0.15	33.33 ***	0.00	0.52
	Fungal diversity	0.02	4.12 *	0.14	30.33 ***	0.01	2.64
	Fungal composition	0.02	4.22 *	0.17	37.40 ***	0.03	5.17 *
	Plant richness	0.19	44.49 ***	0.00	0.01	0.27	72.91 ***
	PFGs	0.59	37.69 ***	0.25	8.67 ***	0.56	34.89 ***
	Drought			0.00	0.20	0.01	2.75
	Combined	0.63	25.64 ***	0.52	14.67 ***	0.75	43.19 ***
N Mineralization	Bacterial diversity	0.00	0.04	0.31	84.29 ***	0.01	1.37
	Bacterial composition	0.00	0.10	0.23	54.02 ***	0.00	0.04
	Fungal diversity	0.00	0.15	0.26	67.20 ***	0.01	2.78
	Fungal composition	0.00	0.10	0.22	53.23 ***	0.01	2.22
	Plant richness	0.06	13.36 ***	0.11	24.26 ***	0.02	3.50
	PFGs	0.41	18.77 ***	0.18	5.86 ***	0.17	5.37 ***
	Drought			0.00	0.90	0.00	0.02
	Combined	0.24	3.56 ***	0.31	4.19 ***	0.29	4.01 ***
Phosphate	Bacterial diversity	0.00	0.04	0.00	0.13	0.02	3.03
	Bacterial composition	0.00	0.12 nm	0.00	0.18	0.03	5.41 *
	Fungal diversity	0.00	0.00	0.00	0.76	0.01	2.59
	Fungal composition	0.00	0.21	0.00	0.01	0.00	0.13
	Plant richness	0.07	15.69 ***	0.02	4.46 *	0.04	8.92 **
	PFGs	0.43	20.20 ***	0.12	3.78 **	0.24	8.42 ***
	Drought			0.00	0.02	0.01	1.15
	Combined	0.52	15.99 ***	0.12	1.98 *	0.26	4.79 ***
Phosphatase activity	Bacterial diversity	0.12	25.42 ***	0.28	76.42 ***	0.28	74.85 ***
	Bacterial composition	0.13	28.02 ***	0.25	62.12 ***	0.25	65.03 ***
	Fungal diversity	0.14	29.73 ***	0.28	75.73 ***	0.29	81.35 ***
	Fungal composition	0.14	29.77 ***	0.27	68.98 ***	0.26	68.14 ***
	Plant richness	0.00	0.00	0.08	17.39 ***	0.00	0.20
	PFGs	0.03	0.92	0.10	2.90 **	0.06	1.79
	Drought			0.04	7.63 **	0.00	0.09
	Combined	0.19	3.47 ***	0.47	12.1 ***	0.39	9.09 ***

correlation with phosphate only in soils with higher microbial diversity (D0 and D2). However, this relationship was absent in D6 soils ($R^2 = 0.02$, $p = 0.24$) (Fig. 5c(i)). While plant species richness had no discernible effect on PHOS activity during the plant establishment stage ($\rho = -0.04$, $p > 0.05$; Fig. 4i and 5e(i)), the drought phase resulted in a significant positive correlation across all soil diversity levels (Fig. 5e(ii)). In case of N mineralization, increasing plant species richness had a positive influence ($\rho = 0.35$, $p < 0.05$; Fig. 4i and 5d(i)) during pre-drought conditions. However, the drought period led to a decoupling of this relationship, which was further influenced by the soil biodiversity level (Fig. 5d(ii)). Given that higher plant species richness likely corresponds with increased nutrient demand, a negative correlation ($\rho = -0.37$, $p < 0.05$) with nitrate levels was observed (Fig. 4i). Overall, the presence of plants significantly influenced the relationships between microbial diversity and soil functions during the plant establishment stage (pre-drought). Components of the nutrient pools were notably affected with increasing plant species richness (Figs. 4i and 5).

3.5. Plant functional groups as the main predictor of soil functions during drought and recovery

Individual PFGs played a significant role in shaping the intricate relationships between microbial diversity and soil functions (Fig. 4 and

Table 1). To gain a more nuanced understanding of their contributions, we employed multiple linear regression models to dissect the unique roles of each PFG in multitrophic plant-microbial interactions (Table 2, and Fig. 6). During the early flowering stage (pre-drought), all PFGs and their combinations exhibited similar impacts on soil functions (Fig. 4). However, when drought was induced, extractable NH₄, NO₃, and N mineralization became significantly influenced by PFGs (Table 1). This influence was primarily driven by the presence of N-fixing plants, both in isolation ($p < 0.05$) and in combination with other species ($p < 0.05$) (Fig. 6). Notably, NH₄ exhibited a negative correlation with N-fixing plants, whereas NO₃ and N mineralization displayed a positive relationship with N-fixing plants (Table 2 and Fig. 6). Following the recovery from drought, many of these effects of PFGs on soil functions also rebounded. The relationships between soil extractable phosphate and PHOS activity with microbial richness remained largely unaffected by PFGs, with the exception of the presence of C4 plants during the recovery phase (Table 2 and Fig. 6). Partial correlation analysis revealed correlation between aboveground plant parameters and soil functions, was largely independent of microbial community structure, except for specific cases. For instance, a significant positive correlation was observed between plant biomass and soil ammonium ($\rho = 0.13$, $p < 0.05$) and nitrate levels ($\rho = 0.26$, $p < 0.001$), suggesting a potential influence of plant biomass on soil nitrogen dynamics (Table S3).

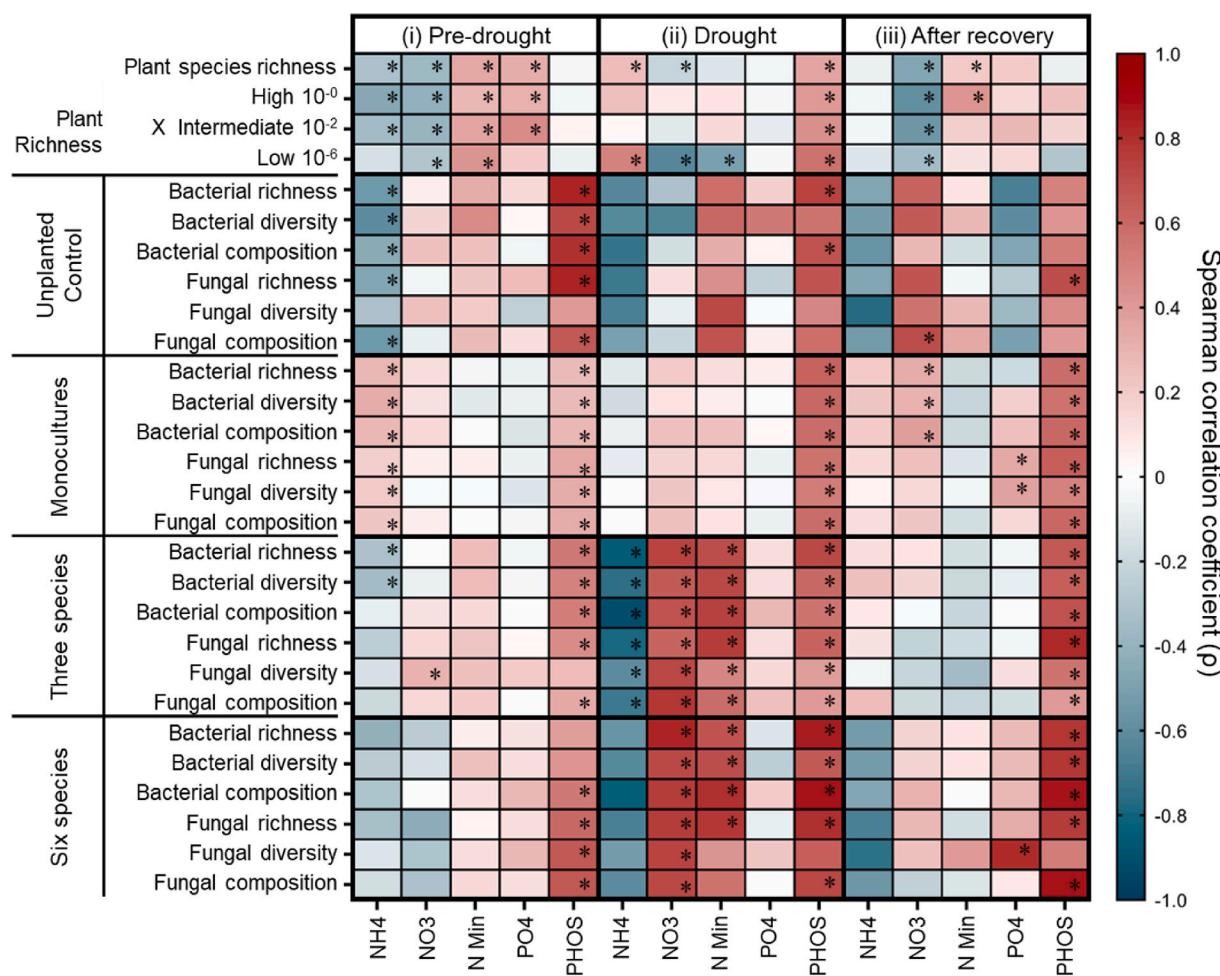


Fig. 4. Links between key soil functions and plant and microbial diversity. Correlation (spearman ρ) between five soil functions and microbial diversity across plant diversity treatments, measured at (i) pre -drought, (ii) drought, and (iii) after recovery. Soil functions were correlated against plant species richness and in individual soil dilution levels. Further, bacterial, and fungal richness, diversity and community structure were correlated with soil functions in each plant richness level. Significant correlations are indicated by * ($p < 0.05$).

Additionally, significant positive correlations were observed between plant biomass and soil phosphate levels ($\rho = 0.12$, $p < 0.05$), and phosphatase activity ($\rho = 0.57$, $p < 0.001$), indicating that aboveground vegetation may contribute to these soil processes. However, in the case of N mineralization, a significant negative correlation was detected with all plant parameters ($\rho = -0.22$, $p < 0.001$) when controlling for microbial diversity, rather than microbial community structure. This suggests that microbial diversity plays a strong role in regulating this process, and the similarity in correlation coefficients across all different plant parameters likely reflects strong intercorrelations among plant parameters themselves (Table S3). In addition, MANOVA results revealed significant main effects and interactions of plant species richness, PFGs, and microbial diversity across the experimental phases (Table S4). During the drought and after recovery phase, plant species richness, PFGs, microbial diversity, and drought all had significant effects. Additionally, several interactions between plant species richness, PFGs, and microbial diversity were significant (Table S4).

4. Discussion

This study represents a substantial advance in our understanding of the intricate relationships among microbial and plant diversity, particularly of plant functional groups (PFGs) and their impacts on essential soil functions under water-limiting conditions. Our results emphasize the potential adverse effects of microbial and plant diversity loss on

ecosystem functions (Delgado-Baquerizo et al. 2016; Trivedi et al. 2019), while advancing the discipline by revealing that the composition of plant communities, particularly their functional groups, could be a dominant predictor of soil functions that could also affect microbial response to environmental stressors (Martins et al. 2024). Crucially, our findings contribute further evidence to the growing body of research that highlights the sensitivity of biotic interactions between plant and microbial diversity to biodiversity loss (Liu et al. 2020; Yang et al. 2021b). Of note, our results underline that the composition of plant communities, with a special focus on functional groups, significantly impacts the rate and resilience of key soil functions (Eisenhauer et al. 2019; Gravuer et al. 2020; Reese et al. 2018; Wei et al. 2017). This highlights the imperative need for holistic evaluations that encompass both above- and belowground biodiversity loss, coupled with explicit consideration of functional groups, in the development of effective management and conservation policies.

One of the intriguing aspects we observed is the dynamic nature of the effects of PFGs and diversity on soil functions over time and in relation to various microbial processes. At the early pre-planting stage, we detected a negative impact of soil microbial diversity loss on soil nitrogen cycling and inorganic nitrogen concentrations. Soil biodiversity loss can either decrease or increase soil functions, depending on microbial diversity and community structure (Wagg et al. 2014b), as less abundant species have greater risk of extinction than the abundant taxa under global change conditions (Chen et al. 2020; Maron et al. 2018).

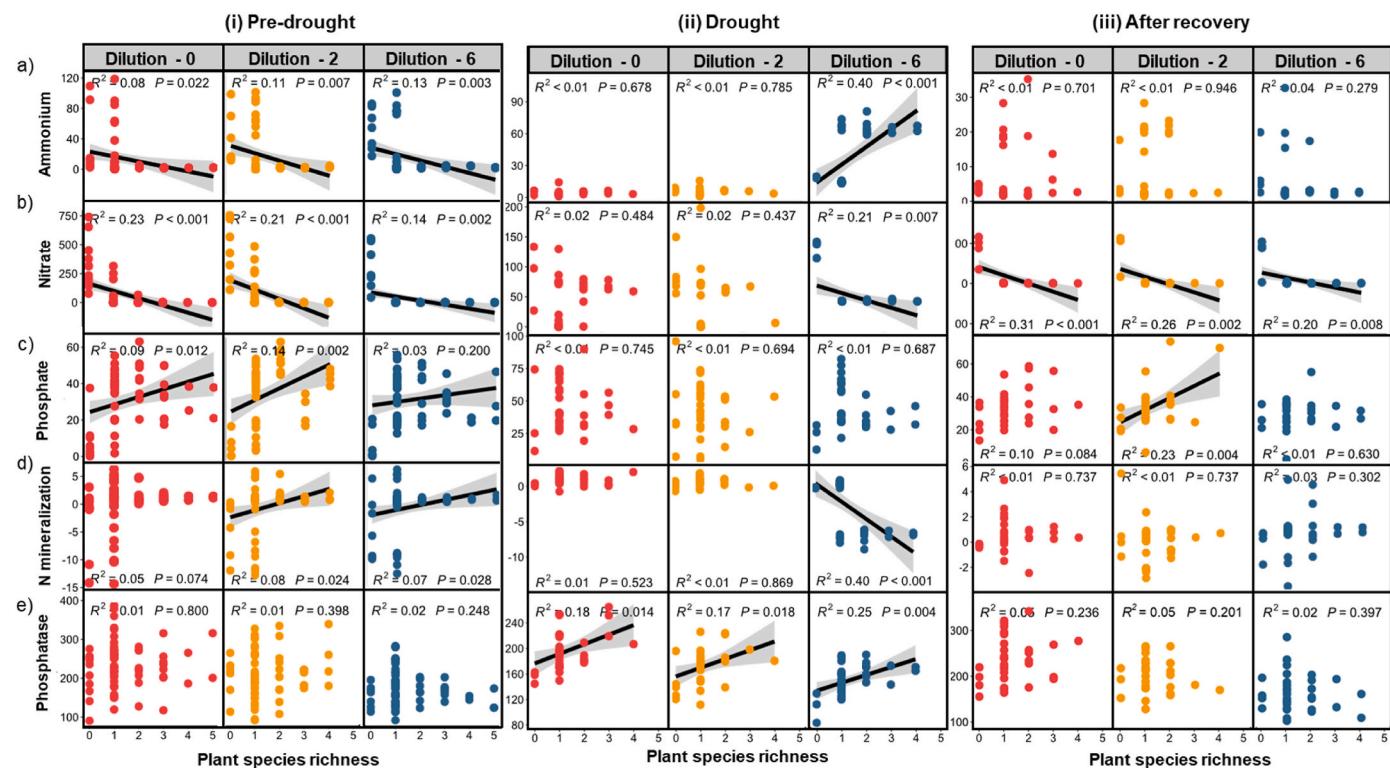


Fig. 5. Effects of plant species richness and soil diversity on soil pools and processes. Relationship between soil functions and observed plant species richness were explored in each soil diversity levels at early flowering stage (pre-drought) (i), during drought (ii), and after recovery (iii). The relationship between observed plant richness and (a) ammonium, (b) nitrate, (c) phosphate, (d) rate of N mineralization, and (e) acid phosphatase activity. Significant plant richness effects were shown with regression lines.

The decrease in soil bacterial diversity was associated with a significant reduction in nitrate accumulation in low-diversity soil (D6), suggesting a direct positive relationship between the rate of nitrification and microbial diversity (Cai et al. 2020). The loss of the nitrifier bacterial community, responsible for converting ammonium into nitrate, coincided with declining microbial diversity (Isobe et al. 2020; Trivedi et al. 2019). This effect was likely amplified in the absence of plants at the pre-planting stage. Similarly, we observed low mineralization rates due to the higher microbial consumption of ammonium and excess accumulation of nitrate, where no competition occurred between different microbial communities and no plant nutrient demand was present (Lama et al. 2020).

During the early flowering stage (pre-drought), the impact of plants on soil functions, particularly the N cycle, was predominant, explaining approximately 98% of the variance. This effect was not surprising, as it was primarily attributed to the presence of N₂-fixing plants, known for their influence on N pool dynamics (Lama et al., 2020; Xu et al., 2019; Gou et al., 2023). More novel however was identifying the fundamental role of PFGs in elucidating how ecosystem functions respond to climate change (Wei et al. 2019). For example, PFGs emerged as the primary factor accounting for the majority of the effects of plant species richness on soil functions during drought. In our statistical analyses, it is important to acknowledge the potential confounding effect between PFGs and plant species richness, where the presence of both variables in the model may make it challenging to disentangle their individual contributions to observed outcomes.

Specifically, our findings align with the concept that the influence of plant diversity on ecosystem functions is intricately linked to the presence of greater plant functional diversity (Eisenhauer et al., 2018). This is exemplified by a positive correlation we observed between the rate of N mineralization and microbial richness in the presence of legumes during drought conditions. Legumes increase soil N availability by N₂ fixation with microbial symbionts, and the mineralization of N-rich

legume litter (Cong et al. 2014). Therefore, presence of legumes increases N mineralization rates, but soil biodiversity loss may lead to decrease in legume performance and hence soil functions (Yang et al. 2021a). For example, a recent study reports the detrimental effect of soil biodiversity loss, which decreased the plant yield under drought conditions (Prudent et al. 2020). However, a different pattern emerged in the case of the P-cycle. Plant species richness had minimal impact on phosphate across all microbial diversity levels. Still, it did strengthen the relationship of PHOS enzyme activity with plant species richness and showed a significant positive correlation with microbial richness. Notably, plants themselves play a role in P cycling as plant roots exude phosphatases as well as carboxylic acids that can solubilize PO₄ (Hacker et al. 2015). In particular, N₂-fixers have a high P demand, possibly explaining why an increase in PHOS activity was observed, without an increase in P. This finding is consistent with a previous study which observed that biodiversity strengthens plant and soil feedback and hence increases P cycling (Wu et al. 2019). These findings significantly advance our understanding of how environmental stressors alter the relative contributions of microbial communities to soil functions (Delgado-Baquerizo et al. 2017a, 2020a; Gravuer et al. 2020; Ye et al. 2019). Moreover, global change drivers, including warming, drought, land use intensification, and other anthropogenic activities, pose threats to both plant and soil biodiversity (Elrys et al. 2021; Gossner et al. 2016; Rillig et al. 2019; Risch et al. 2020; De Vries et al. 2012; Yang et al. 2021b). In line with our hypothesis, the results indicate that the strength and direction of plant diversity's effects on soil functions are influenced by microbial diversity during drought.

Specifically, under drought conditions, microbial diversity and community structure emerge as the most critical drivers of soil functions. The likely reduction in plant metabolic and growth rates during drought, combined with the microbial ability to sustain these functions under stress, can elucidate this shift in relative contribution (Chieb and Gachomo 2023). Microbes engage in dynamic interactions with plants

Table 2

Influence of plant functional groups on the soil function-microbial diversity relationships at early flowering stage (a), during drought (b), and after recovery (c) as explained by multiple linear regression models. Significant influences of PFGs are presented in bold as indicated by *p*-values.

Microbial richness ~ plant functions groups		Bacterial observed species			Fungal observed species		
Function	Predictor Var.	(a) Pre-drought <i>p</i> -value	(b) Drought <i>p</i> -value	(c) After Recovery <i>p</i> -value	(a) Pre-drought <i>p</i> -value	(b) Drought <i>p</i> -value	(c) After Recovery <i>p</i> -value
Ammonium	Intercept	<0.001	0.388	<0.001	<0.001	0.372	<0.001
	C4	0.087	0.722	0.004	0.068	0.778	0.006
	C3	0.001	0.942	<0.001	0.001	0.970	<0.001
	Nfix	0.052	<0.001	0.008	0.044	<0.001	0.008
	C4-C3-Nfix	0.104	<0.001	0.011	0.089	<0.001	0.510
	C3-C3-Nfix	0.102	<0.001	<0.001	0.106	<0.001	<0.001
	C4-Nfix-Nfix	0.110	<0.001	0.004	0.119	<0.001	0.009
Nitrate	Intercept	0.003	<0.001	0.012	<0.001	<0.001	<0.001
	C4	0.889	0.086	0.154	0.887	0.413	0.119
	C3	0.166	0.129	0.168	0.273	0.878	0.147
	Nfix	0.766	<0.001	0.850	0.744	0.003	0.053
	C4-C3-Nfix	0.247	<0.001	0.236	0.335	0.009	0.203
	C3-C3-Nfix	0.222	<0.001	0.220	0.324	0.009	0.227
	C4-Nfix-Nfix	0.229	<0.001	0.221	0.351	<0.001	0.232
Phosphate	Intercept	0.785	0.407	<0.001	0.669	<0.001	<0.001
	C4	0.179	0.550	0.005	0.189	0.930	0.008
	C3	0.992	0.311	0.077	0.753	0.919	0.101
	Nfix	0.772	0.026	0.373	0.528	0.351	0.690
	C4-C3-Nfix	0.657	0.618	0.063	0.904	0.868	0.118
	C3-C3-Nfix	0.887	0.176	0.115	0.721	0.823	0.232
	C4-Nfix-Nfix	0.565	0.290	0.305	0.642	0.979	0.727
N Mineralization	Intercept	0.002	0.978	0.004	<0.001	0.931	0.081
	C4	0.081	0.964	0.110	0.262	0.948	0.302
	C3	0.006	0.909	0.003	0.010	0.973	0.031
	Nfix	0.324	<0.001	<0.001	0.275	<0.001	0.004
	C4-C3-Nfix	0.412	<0.001	0.056	0.466	<0.001	0.379
	C3-C3-Nfix	0.189	<0.001	0.021	0.217	<0.001	0.188
	C4-Nfix-Nfix	0.272	<0.001	<0.001	0.325	<0.001	<0.001
Phosphatase activity	Intercept	0.220	0.114	0.357	<0.001	<0.001	<0.001
	C4	0.3836	0.725	0.833	0.309	0.628	0.638
	C3	0.7241	0.814	0.596	0.545	0.298	0.558
	Nfix	0.1918	0.431	0.394	0.255	0.415	0.869
	C4-C3-Nfix	0.6574	0.093	0.501	0.327	0.249	0.507
	C3-C3-Nfix	0.4454	0.945	0.585	0.359	0.526	0.320
	C4-Nfix-Nfix	0.9188	0.939	0.630	0.768	0.795	0.880

and enhance their resilience during changing environmental stress. As an example, microbes are known to release extracellular polymeric substances into the soil, enhancing water retention capacity (Trivedi et al. 2020). While a previous study reported no significant impact of microbial diversity loss on primary production (Yang et al. 2021b), our findings in this study indicate a positive influence of microbial diversity in reinforcing the effects of plant diversity on soil functions. These effects are relative and context-dependent. Therefore, these varying responses could be attributed to differences in resource availability and other abiotic conditions (Stefan et al. 2021), indicating a context-dependent relationship between plant-soil biodiversity and functions under climate change. This is further supported by the finding that fungal richness may have been indirectly influencing plant composition importance in predicting aboveground functions (Martins et al., 2024).

Most importantly, our results suggest that PFGs play a pivotal role in driving context dependency in the BEF relationships supporting and helping explain prior observations. For example, our results are consistent with the few studies that examine plant functional group identity and richness independently of species richness (Martins et al., 2024; Reich et al. 2004; Wei et al. 2019). Our findings also imply that the effects of biodiversity on ecosystem processes exhibit variation based on the types and traits of plants involved both within and among PFGs (Martins et al., 2024). This is consistent with evidence that increasing the number of PFGs in grassland plots increased primary productivity (Hoover et al. 2014; Tilman et al. 1997), especially if the PFGs differed

in their resource use strategies, such as nitrogen fixation or rooting depth and even when holding species richness constant (Reich et al. 2004). Similarly, a meta-analysis by (Hooper et al. 2005) showed that the impact of biodiversity on ecosystem functioning was stronger when PFGs were defined based on traits related to resource acquisition and allocation, rather than taxonomic affiliation. These reports exemplify how PFGs can modulate the BEF relationship by influencing the complementarity and redundancy of species within ecosystems.

The observed correlations between soil nutrient dynamics and plant biomass highlight the direct influence of aboveground vegetation on certain soil processes. The negative correlation between plant biomass and N mineralization levels indicates that microbial diversity plays a key role in regulating nitrogen transformations in the soil. This suggests that variations in plant biomass may indirectly influence nitrogen dynamics by altering microbial diversity, which in turn drives N mineralization processes. This aligns with the notion that higher plant biomass may lead to increased nitrogen uptake or altered nitrogen cycling dynamics (Xu et al. 2012). Moreover, the positive correlations of soil phosphate levels, and phosphatase activity with plant biomass highlight the multifaceted roles of aboveground plant characteristics in shaping key soil processes. While our regression and correlation analyses explored gradient responses, the MANOVA revealed that plant species richness, PFGs, and microbial diversity independently contributed to changes in soil nutrient dynamics. Furthermore, significant interactions, particularly during and after-recovery phase, indicated complex relationships between above- and belowground factors, suggests that the functional

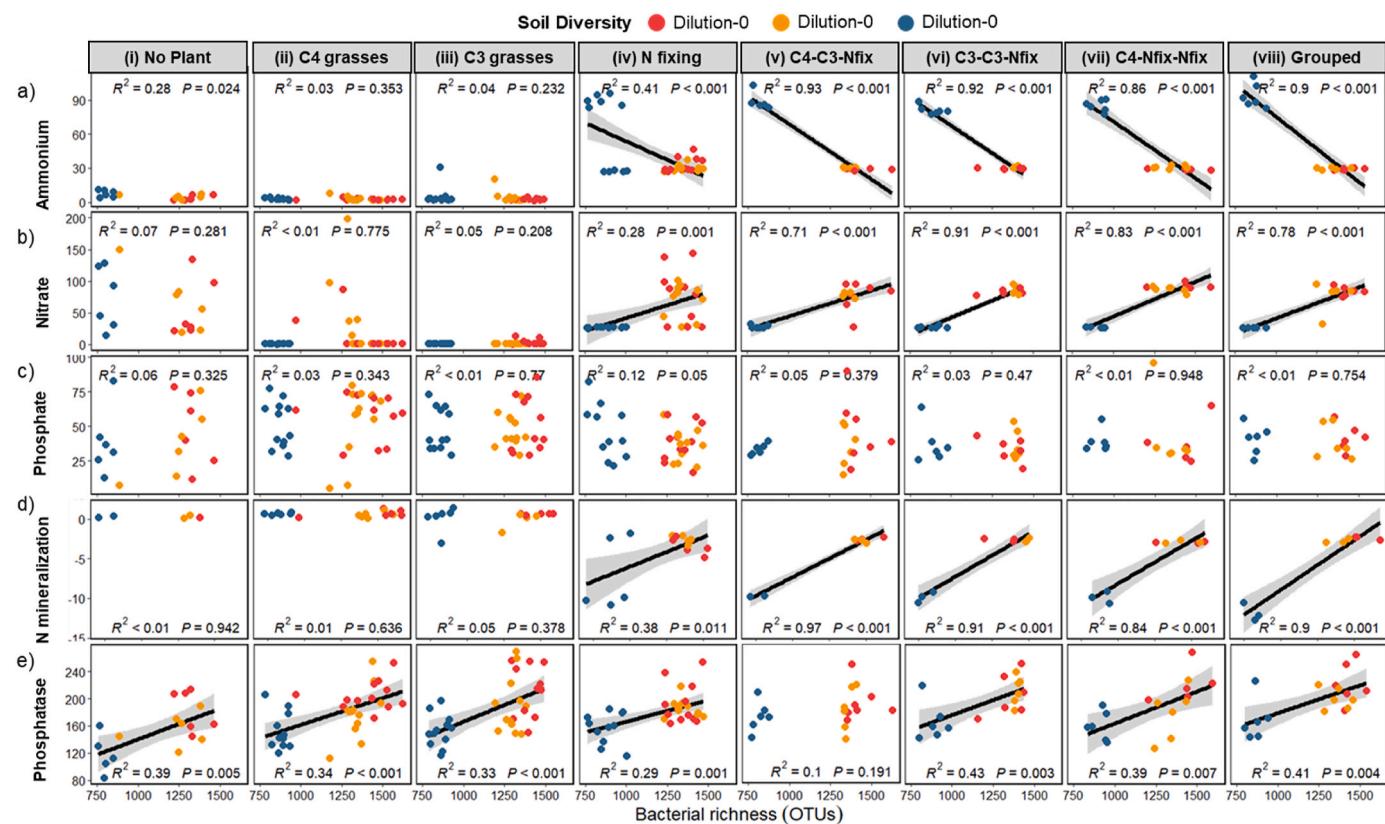


Fig. 6. Influence of PFGs on the relationship between microbial richness (bacterial observed species) and soil pools such as ammonium (a), nitrate (b), phosphate (c), and processes such as rate of N mineralization (d), and acid phosphatase activity (e), during drought. Significant relationships were shown with regression lines.

diversity of plant communities played a crucial role in shaping microbial-driven soil processes. These findings support that plant diversity and PFGs can influence not only aboveground dynamics but also play a significant role in belowground processes, contributing to nutrient cycling and enzyme activities (Craven et al. 2016; Eisenhauer et al. 2018; Martins et al., 2024; Steinauer et al. 2015). Our study thus provides empirical evidence to link between soil functions and plant-microbial interactions, offering a more comprehensive understanding of the intricate interplay between above and belowground diversity. This nuanced knowledge advances the current understanding on the complex relationships between plant-microbial diversity and soil functions, enhancing the relevance and applicability of our findings in the context of broader ecological studies and ecosystem management.

In conclusion, this study employed an innovative experimental approach that allowed improved understanding of the factors driving the variation in soil N and P pools and processes and the relative significance of soil microbial diversity, plant species richness, PFGs, and their interplay in shaping essential soil functions under environmental stressors. The results suggest that while plant diversity can enhance soil functions independently, other functions (e.g. phosphatase activity) is largely linked to microbial diversity. Furthermore, the relationship between microbial diversity and soil functions, other than phosphatase activity, becomes more evident in the presence of legumes and under drought conditions. These findings underscore the context-specific interactions between plant and microbial diversity in regulating soil functions. Soil microbial diversity proved particularly significant under drought stress, with PFGs mediate microbial responses to drought. Our results emphasize the critical importance of conserving both soil microbial and plant diversity, with special attention to PFGs, to maintain the stability of multiple soil functions in a changing world. This work advances our understanding of the intricate interactions between above- and belowground biodiversity and their combined effects on soil

functions. It provides a new line of future investigation to unravel the specific mechanisms governing these interactions and elucidate their broader implications for sustainable agriculture and ecosystem management. Such knowledge is essential for the development of effective strategies to preserve both above- and belowground biodiversity, ensuring the long-term health and productivity of our ecosystems.

CRediT authorship contribution statement

Ramesha H. Jayaramaiah: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Catarina S.C. Martins:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Eleonora Egidi:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Catriona A. Macdonald:** Writing – review & editing, Methodology, Investigation, Data curation. **Jun-Tao Wang:** Data curation, Formal analysis, Methodology, Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Hongwei Liu:** Writing – review & editing, Methodology, Investigation, Data curation. **Peter B. Reich:** Methodology, Investigation, Funding acquisition, Conceptualization. **Manuel Delgado-Baquerizo:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Brajesh K. Singh:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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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.soilbio.2024.109623>.

Data availability

All data will be made available after publication

References

- Amundson, R., Berhe, A.A., Hopmans, J.W., Olson, C., Szein, A.E., Sparks, D.L., 2015. Soil and human security in the 21st century. *Science* 348, 1979.
- Averill, C., Werbin, Z.R., Atherton, K.F., Bhatnagar, J.M., Dietze, M.C., 2021. Soil microbiome predictability increases with spatial and taxonomic scale. *Nature Ecology & Evolution* 5, 747–756. <https://doi.org/10.1038/s41559-021-01445-9>.
- Cai, Y., Shen, J.P., Di, H.J., Zhang, L.M., Zhang, C., He, J.Z., 2020. Variation of soil nitrate and bacterial diversity along soil profiles in manure disposal maize field and adjacent woodland. *Journal of Soils and Sediments* 20, 3557–3568.
- Canessa, R., van den Brink, L., Berdugo, M.B., Hättenschwiler, S., Rios, R.S., Saldaña, A., et al., 2022. Trait functional diversity explains mixture effects on litter decomposition at the arid end of a climate gradient. *Journal of Ecology*.
- Cardinale, B.J.B., Duffy, J.E., Gonzalez, A., Hooper, D.U.D.U., Perrings, C., Venail, P., et al., 2012. Biodiversity loss and its impact on humanity. *Nature* 486, 59–67.
- 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. *Environment International* 140.
- Chieb, M., Gachomo, E.W., 2023. The role of plant growth promoting rhizobacteria in plant drought stress responses. *BMC Plant Biology* 23 (1), 1–23, 2023 23.
- Chong, J., Liu, P., Zhou, G., Xia, J., 2020. Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. *Nature Protocols* 15, 799–821.
- Churchill, A.C., Zhang, H., Fuller, K.J., Amiji, B., Anderson, I.C., Barton, C.V.M., et al., 2022. Pastures and climate extremes: impacts of cool season warming and drought on the productivity of key pasture species in a field experiment. *Frontiers of Plant Science* 0, 484.
- Colombo, F., Macdonald, C.A., Jeffries, T.C., Powell, J.R., Singh, B.K., 2016. Impact of forest management practices on soil bacterial diversity and consequences for soil processes. *Soil Biology and Biochemistry* 94, 200–210.
- Cong, W.-F.F., van Ruijven, J., Mommer, L., De Deyn, G.B., Berendse, F., Hoffland, E., 2014. Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *Journal of Ecology* 102, 1163–1170.
- Cornwell, W.K., Cornelissen, J.H.C.C., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O., et al., 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters* 11, 1065–1071.
- Craven, D., Isbell, F., Manning, P., Connolly, J., Bruelheide, H., Ebeling, A., et al., 2016. Plant diversity effects on grassland productivity are robust to both nutrient enrichment and drought. *Philosophical Transactions of the Royal Society B: Biological Sciences* 371.
- Delgado-Baquerizo, M., Bardgett, R.D., Vitousek, P.M., Maestre, F.T., Williams, M.A., Eldridge, D.J., et al., 2019. Changes in belowground biodiversity during ecosystem development. *Proceedings of the National Academy of Sciences* 116, 6891–6896.
- Delgado-Baquerizo, M., Doulcier, G., Eldridge, D.J., Stouffer, D.B., Maestre, F.T., Wang, J., et al., 2020a. Increases in aridity lead to drastic shifts in the assembly of dryland complex microbial networks. *Land Degradation & Development* 31, 346–355.
- Delgado-Baquerizo, M., Eldridge, D.J., Ochoa, V., Gozalo, B., Singh, B.K., Maestre, F.T., 2017a. Soil microbial communities drive the resistance of ecosystem multifunctionality to global change in drylands across the globe. *Ecology Letters* 20, 1295–1305.
- Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., et al., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications* 7.
- Delgado-Baquerizo, M., Powell, J.R., Hamonts, K., Reith, F., Mele, P., Brown, M.V., et al., 2017b. Circular linkages between soil biodiversity, fertility and plant productivity are limited to topsoil at the continental scale. *New Phytologist* 215, 1186–1196.
- Delgado-Baquerizo, M., Reich, P.B., Trivedi, C., Eldridge, D.J., Abades, S., Alfaro, F.D., et al., 2020b. Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Natural Ecology Evolution* 4, 210–220.
- Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., et al., 2010. Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* 91, 485–496.
- Eisenhauer, N., Hines, J., Isbell, F., van der Plas, F., Hobbie, S.E., Kazanski, C.E., et al., 2018. Plant diversity maintains multiple soil functions in future environments. *Elife* 7.
- Eisenhauer, N., Reich, P.B., Scheu, S., 2012. Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic and Applied Ecology* 13, 571–578.
- Eisenhauer, N., Schielzeth, H., Barnes, A.D., Barry, K.E., Bonn, A., Brose, U., et al., 2019. A multitrophic perspective on biodiversity–ecosystem functioning research. *Advances in Ecological Research* 61, 1–54.
- Elrys, A.S., Ali, A., Zhang, H., Cheng, Y., Zhang, J., Cai, Z.C., et al., 2021. Patterns and drivers of global gross nitrogen mineralization in soils. *Global Change Biology* 27, 5950–5962.
- Faucon, M.P., Houben, D., Lambers, H., 2017. Plant functional traits: soil and ecosystem services. *Trends in Plant Science* 22, 385–394.
- Furey, G.N., Tilman, D., 2021. Plant biodiversity and the regeneration of soil fertility. *Proceedings of the National Academy of Sciences of the U.S.A.* 118.
- Gossner, M.M., Lewinsohn, T.M., Kahl, T., Grassein, F., Boch, S., Prati, D., et al., 2016. Land-use intensification causes multitrophic homogenization of grassland communities. *Nature* 540, 266–269.
- Gou, X., Reich, P.B., Qiu, L., Shao, M., Wei, G., Wang, J., et al., 2023. Leguminous plants significantly increase soil nitrogen cycling across global climates and ecosystem types. *Global Change Biology* 29, 4028–4043.
- Gravuer, K., Eskelinen, A., Winbourne, J.B., Harrison, S.P., 2020. Vulnerability and resistance in the spatial heterogeneity of soil microbial communities under resource additions. *Proceedings of the National Academy of Sciences* 117, 7263–7270.
- Guerrero-Ramírez, N.R., Eisenhauer, N., 2017. Trophic and non-trophic interactions influence the mechanisms underlying biodiversity–ecosystem functioning relationships under different abiotic conditions. *Oikos* 126, 1748–1759.
- Hacker, N., Ebeling, A., Gessler, A., Gleixner, G., González Mace, O., de Kroon, H., et al., 2015. Plant diversity shapes microbe–rhizosphere effects on P mobilisation from organic matter in soil. *Ecology Letters* 18, 1356–1365.
- Herz, K., Dietz, S., Haider, S., Jandt, U., Scheel, D., Bruelheide, H., 2017. Drivers of intraspecific trait variation of grass and forb species in German meadows and pastures. *Journal of Vegetation Science* 28, 705–716.
- Hooper, D.U.D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., et al., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75, 3–35.
- Hoover, D.L., Knapp, A.K., Smith, M.D., 2014. Resistance and resilience of a grassland ecosystem to climate extremes. *Ecology* 95, 2646–2656.
- Hu, H.-W.W., Macdonald, C.A., Trivedi, P., Holmes, B., Bodrossy, L., He, J.-Z.Z., et al., 2015. Water addition regulates the metabolic activity of ammonia oxidizers responding to environmental perturbations in dry subhumid ecosystems. *Environmental Microbiology* 17, 444–461.
- Hu, W., Ran, J., Dong, L., Du, Q., Ji, M., Yao, S., et al., 2021. Aridity-driven shift in biodiversity–soil multifunctionality relationships. *Nature Communications* 12.
- Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., et al., 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. *Nature* 526, 574–577.
- Isobe, K., Ise, Y., Kato, H., Oda, T., Vincenot, C.E., Koba, K., et al., 2020. Consequences of microbial diversity in forest nitrogen cycling: diverse ammonifiers and specialized ammonia oxidizers. *ISME Journal* 14, 12–25.
- Lama, S., Velescu, A., Leimer, S., Weigelt, A., Chen, H., Eisenhauer, N., et al., 2020. Plant diversity influenced gross nitrogen mineralization, microbial ammonium consumption and gross inorganic N immobilization in a grassland experiment. *Oecologia* 193, 731–748.
- Lamb, E.G., Kennedy, N., Siciliano, S.D., 2011. Effects of plant species richness and evenness on soil microbial community diversity and function. *Plant and Soil* 338, 483–495.
- Liu, L., Zhu, K., Wurzburger, N., Zhang, J., 2020. Relationships between plant diversity and soil microbial diversity vary across taxonomic groups and spatial scales. *Ecosphere* 11, e02999.
- Liu, Y.-R., Delgado-Baquerizo, M., Wang, J.-T., Hu, H.-W., Yang, Z., He, J.-Z., 2018. New insights into the role of microbial community composition in driving soil respiration rates. *Soil Biology and Biochemistry* 118, 35–41.

- Luo, S., De Deyn, G.B., Jiang, B., Yu, S., 2017. Soil biota suppress positive plant diversity effects on productivity at high but not low soil fertility. *Journal of Ecology* 105, 1766–1774.
- Maestre, F.T., Delgado-Baquerizo, M., Jeffries, T.C., Eldridge, D.J., Ochoa, V., Gozalo, B., et al., 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences of the U.S.A.* 112, 15684–15689.
- Maron, P.-A., Sarr, A., Kaisermann, A., Lévéque, J., Mathieu, O., Guiguer, J., et al., 2018. High microbial diversity promotes soil ecosystem functioning. *Applied and Environmental Microbiology* 84.
- Martins, C.S.C., Delgado-Baquerizo, M., Jayaramaiah, R.H., Tao, D., Wang, J.-T., Sáez-Sandino, T., et al., 2024. Aboveground and belowground biodiversity have complementary effects on ecosystem functions across global grasslands. *PLoS Biology* 22, e3002736.
- Prommer, J., Walker, T.W.N., Wanek, W., Braun, J., Zezula, D., Hu, Y., et al., 2020. Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. *Global Change Biology* 26, 669–681.
- Prudent, M., Dequiedt, S., Sorin, C., Girodet, S., Nowak, V., Duc, G., et al., 2020. The diversity of soil microbial communities matters when legumes face drought. *Plant, Cell and Environment* 43, 1023–1035.
- R Core Team, 2018. *Radiokhimiya: A language and environment for statistical computing*.
- Ravenek, J.M., Mommer, L., Visser, E.J.W., van Ruijven, J., van der Paauw, J.W., Smit-Tiekstra, A., et al., 2016. Linking root traits and competitive success in grassland species. *Plant and Soil* 407, 39–53.
- Reese, A.T., Luoluo, K., David, L.A., Wright, J.P., 2018. Plant community and soil conditions individually affect soil microbial community assembly in experimental mesocosms. *Ecology and Evolution* 8, 1196–1205.
- Reich, P.B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S.E., Flynn, D.F.B.B., et al., 2012. Impacts of biodiversity loss escalate through time as redundancy fades. *Science* 336, 589–592, 1979.
- Reich, P.B., Tilman, D., Naeem, S., Ellsworth, D.S., Knops, J., Craine, J., et al., 2004. Species and functional group diversity independently influence biomass accumulation and its response to CO₂ and N. *Proceedings of the National Academy of Sciences of the U.S.A.* 101, 10101–10106.
- Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., et al., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science* 366, 886–890.
- Risch, A.C., Zimmermann, S., Moser, B., Schütz, M., Hagedorn, F., Firn, J., et al., 2020. Global impacts of fertilization and herbivore removal on soil net nitrogen mineralization are modulated by local climate and soil properties. *Global Change Biology* 26, 7173–7185.
- Schlatter, D.C., Bakker, M.G., Bradeen, J.M., Kinkel, L.L., 2015. Plant community richness and microbial interactions structure bacterial communities in soil. *Ecology* 96, 134–142.
- Sheik, C.S., Beasley, W.H., Elshahed, M.S., Zhou, X., Luo, Y., Krumholz, L.R., 2011. Effect of warming and drought on grassland microbial communities. *The ISME Journal* 5 (10), 1692–1700, 2011.5.
- Singh, J.S., Gupta, V.K., 2018. Soil microbial biomass: a key soil driver in management of ecosystem functioning. *Science of the Total Environment* 634, 497–500.
- Šmilauer, P., Šmilauerová, M., 2013. Asymmetric relationship between grasses and forbs: results from a field experiment under nutrient limitation. *Grass and Forage Science* 68, 186–198.
- Soliveres, S., Van Der Plas, F., Manning, P., Prati, D., Gossner, M.M., Renner, S.C., et al., 2016. Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature* 536, 456–459.
- 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. *Frontiers in Microbiology* 12.
- Steinauer, K., Tilman, D., Wragg, P.D., Cesarz, S., Cowles, J.M., Pritsch, K., et al., 2015. Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment. *Ecology*.
- Thakur, M.P., Del Real, I.M., Cesarz, S., Steinauer, K., Reich, P.B., Hobbie, S., et al., 2019. Soil microbial, nematode, and enzymatic responses to elevated CO₂, N fertilization, warming, and reduced precipitation. *Soil Biology and Biochemistry* 135, 184–193.
- Tilman, D., Lehman, C.L., Thomson, K.T., Carroll, I.T., Hector, A., Srivastava, D.S., et al., 1997. *Plant Diversity and Ecosystem Productivity: Theoretical Considerations*, vol. 94. Proceedings of the National Academy of Sciences, pp. 1857–1861.
- Trivedi, C., Delgado-Baquerizo, M., Hamonts, K., Lai, K., Reich, P.B., Singh, B.K., 2019. Losses in microbial functional diversity reduce the rate of key soil processes. *Soil Biology and Biochemistry* 135, 267–274.
- Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., et al., 2016. Microbial regulation of the soil carbon cycle: evidence from gene-enzyme relationships. *ISME Journal* 10, 2593–2604.
- Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant-microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology* 18 (11), 607–621, 2020.18.
- De Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M., et al., 2012. Land use alters the resistance and resilience of soil food webs to drought. *Nature Climate Change* 2, 276–280.
- Wagg, C., Bender, S.F., Widmer, F., van der Heijden, M.G.A., 2014a. Soil Biodiversity and Soil Community Composition Determine Ecosystem Multifunctionality, vol. 111. *Proceedings of the National Academy of Sciences*, pp. 5266–5270.
- Wagg, C., Bender, S.F., Widmer, F., Van Der Heijden, M.G.A., 2014b. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the U.S.A.* 111, 5266–5270.
- Wall, D.H., Nielsen, U.N., Six, J., 2015. Soil biodiversity and human health. *Nature* 528, 69–76.
- Wei, X., Reich, P.B., Hobbie, S.E., 2019. Legumes regulate grassland soil N cycling and its response to variation in species diversity and N supply but not CO₂. *Global Change Biology* 25, 2396–2409.
- Wei, X., Reich, P.B., Hobbie, S.E., Kazanski, C.E., 2017. Disentangling species and functional group richness effects on soil N cycling in a grassland ecosystem. *Global Change Biology* 23, 4717–4727.
- Weisser, W.W., Roscher, C., Meyer, S.T., Ebeling, A., Luo, G., Allan, E., et al., 2017. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: patterns, mechanisms, and open questions. *Basic and Applied Ecology* 23, 1–73.
- Wu, H., Xiang, W., Ouyang, S., Forrester, D.I., Zhou, B., Chen, L., et al., 2019. Linkage between tree species richness and soil microbial diversity improves phosphorus bioavailability. *Functional Ecology* 33, 1549–1560.
- Wu, T., Ayres, E., Bardgett, R.D., Wall, D.H., Garey, J.R., 2011. Molecular study of worldwide distribution and diversity of soil animals. *Proceedings of the National Academy of Sciences* 108, 17720–17725.
- Xiao, C., Zhou, Y., Su, J., Yang, F., 2017. Effects of plant functional group loss on soil microbial community and litter decomposition in a steppe vegetation. *Frontiers of Plant Science* 8, 2040.
- Xu, G., Fan, X., Miller, A.J., 2012. Plant Nitrogen Assimilation and Use Efficiency. *Annual Review of Plant Biology* 63, 153–182. <https://doi.org/10.1146/annurev-arplant-042811-105532>.
- Xu, H., Detto, M., Li, Y., Li, Y., He, F., Fang, S., 2019. Do N-fixing legumes promote neighbouring diversity in the tropics? *Journal of Ecology* 107, 229–239.
- Yang, G., Roy, J., Veresoglou, S.D., Rillig, M.C., 2021a. Soil biodiversity enhances the persistence of legumes under climate change. *New Phytologist* 229, 2945–2956.
- Yang, G., Ryo, M., Roy, J., Hempel, S., Rillig, M.C., 2021b. Plant and soil biodiversity have non-substitutable stabilising effects on biomass production. *Ecology Letters* 24, 1582–1593.
- Ye, J.S., Delgado-Baquerizo, M., Soliveres, S., Maestre, F.T., 2019. Multifunctionality debt in global drylands linked to past biome and climate. *Global Change Biology* 25, 2152–2161.
- Yuan, Z., Ali, A., Ruiz-Benito, P., Jucker, T., Mori, A.S., Wang, S., et al., 2020. Above- and below-ground biodiversity jointly regulate temperate forest multifunctionality along a local-scale environmental gradient. *Journal of Ecology* 108, 2012–2024.
- Zheng, H., Yang, T., Bao, Y., He, P., Yang, K., Mei, X., et al., 2021. Network analysis and subsequent culturing reveal keystone taxa involved in microbial litter decomposition dynamics. *Soil Biology and Biochemistry* 157.