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Research paper

# Composition outweighs abundance: Soil bacterial communities mediate plant performance under drought and post-recovery

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ABSTRACT

Within the biodiversity-ecosystem functioning framework, soil biodiversity is linked to stability under environmental stress. However, in intensively managed cropping systems, empirical tests of this assumption remain limited, especially under drought and post-drought recovery. For this, we used dilution-to-extinction (DTE) approach to create gradients that simultaneously reduced microbial diversity and abundance, mimicking community depletion in soil. Barley plants were grown in these soils, exposed to a 21-day drought, followed by a 15-day recovery. The DTE successfully reduced bacterial ASV richness, Shannon diversity, and Pielou's evenness, and drought further reduced these indices. These effects persisted after recovery. Shoot and root biomass increased with community depletion at peak drought. Shoot biomass was unaffected by drought, whereas root biomass declined by 24%. After recovery, microbial communities continued to influence shoot biomass, whereas no effects of either drought or community depletion were observed on root biomass. We further observed that depleted soils were enriched with beneficial bacterial taxa, including *Arthrobacter*, *Burkholderia* s.l., *Massilia*, and *Mucilaginibacter*. Bacterial genome copy number and litter decomposition rates increased, indicating enhanced nutrient cycling and colonization by fast-growing, r-strategist bacteria. These shifts may have reduced plant-microbial competition and improved plant growth. Overall, our results suggest a decoupling of soil biodiversity from crop productivity and drought mitigation. Importantly, our results should not be interpreted as evidence that microbial diversity is not important for agroecosystem functioning nor that decreasing microbial diversity would benefit plant productivity. Instead, these findings indicate that community composition may have significantly mediated plant productivity in our experimental context.

## 1. Introduction

Ongoing climate change is projected to increase the intensity and frequency of drought events in the future, with consequences for productivity and functioning of agroecosystems (Rosenzweig et al., 2014). Drought significantly influences soil biological processes and crop productivity worldwide by disrupting plant-water relationships and microbial processes in soil, with cascading influences on nutrient cycling, rhizosphere functioning, and crop performance (Azarbad et al., 2018; Saraswat et al., 2025). Although plant physiological responses (such as

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hormonal signaling, water-use efficiency, gene regulation, etc.) to drought have been extensively studied (Gupta et al., 2020; Koehler et al., 2023), growing evidence also suggests that these responses may be strongly mediated by soil microbial communities via altered biogeochemical processes including litter decomposition and nutrient mobilization (de Vries et al., 2020; Dijkstra et al., 2015).

Additionally, drought stress often reduces soil biodiversity and selects for a few taxa that can withstand water limitation (Bérard et al., 2012; Gremion and Thakur, 2025; Xu et al., 2021). Moreover, bacterial communities characterized by rapid growth (r-strategists) respond quickly to environmental changes, whereas fungal communities take longer to establish and influence plant performance with their more K-selected strategies (Schimel et al., 2007; Rousk and Bååth, 2011). Therefore, in short term studies, quickly-responding bacterial communities would primarily mediate plant performance under environmental variability (de Vries et al., 2018). Such compositional shifts in microbial communities not only feedback to plant performance under drought stress but can persist in soil after rewetting and generate soil legacies. These soil legacies can persist in soil and permanently affect plant performance (Bakker et al., 2018; Lozano et al., 2022). Reduced functional microbial biodiversity in soils with reduced complementarity may lead to incomplete and/or slow recovery after rewetting, ultimately influencing crop productivity. Therefore, it becomes crucial to investigate to what extent soil biodiversity affects plant performance during peak drought and after soil rewetting (recovery) to understand microbial-mediated resistance and resilience mechanisms. Such drought-related responses highlight the broader importance of soil microbial diversity in maintaining agroecosystem functioning under environmental stress.

Generally, microbial communities in soils are extremely diverse and collectively mediate soil organic matter (SOM) decomposition, carbon (C) and nutrient cycling, and also contribute to soil structural properties (Dang and Morrissey, 2024; Rillig et al., 2019a). Ecological theories predict that biodiversity can stabilize ecosystem functions under environmental stresses. Such stabilization is often linked to functional complementarity and insurance effects, in which diverse communities buffer ecosystem functions through species that are less impacted by environmental stressors (Dee et al., 2023; Loreau and Hector, 2001; Yachi and Loreau, 1999). Recent empirical tests increasingly support these predictions in soil, where ecosystem multifunctionality and stability under multiple environmental stressors have been linked to soil biodiversity (Delgado-Baquerizo et al., 2016; Rillig et al., 2019b).

Nevertheless, the importance of soil microbial communities should be considered within the context of cropping systems where management practices strongly regulate their composition and functioning (Kumar et al., 2026). For instance, increasing intensification is linked to reduced complexity of soil food webs and microbial network complexity (Edlinger et al., 2022; Hartmann et al., 2015; Tsiafouli et al., 2015). Moreover, agricultural intensification has been shown to reduce microbial network complexity as well as abundance of keystone microbial taxa (Banerjee et al., 2019). However, changes in soil properties, including pH, nutrient availability, SOM content, covary with microbial communities under distinct management practices (Hartman et al., 2018; Liu et al., 2022), thereby leading to confounding influences on crop productivity. Therefore, it remains difficult to disentangle biotic (microbial-mediated) and abiotic (physico-chemical properties-mediated) influences on crop performance, especially under environmental stresses.

Manipulation of soil biodiversity through dilution-to-extinction (DTE) has been widely used (Yan et al., 2015; Ossowicki et al., 2021; Yang et al., 2021a, 2021b) to generate controlled gradients of microbial communities by serially diluting soils and reintroducing them into sterilized substrates. This creates simplified subsets of the original community, reducing both diversity and abundance and thereby enabling tests of how microbial community depletion affects ecosystem functioning. Empirical studies show that such depletion can influence ecosystem multifunctionality and demonstrate the importance of soil biodiversity (Philippot et al., 2013; Wagg et al., 2014). In cropping systems, such co-occurring reductions in both microbial diversity and abundance are generally observed under intensive management practices including inputs of various agrochemicals and frequent tillage (Banerjee et al., 2019; Tsiafouli et al., 2015). Therefore, we define DTE-manipulated microbial communities as depletion of original communities, reflecting management-induced reduction of microbial community complexity and abundance. However, because taxa are removed in a non-targeted manner with the DTE approach, outcomes depend on which groups are lost. For example, the unintended removal of rare but functionally important taxa can alter key processes (Mao et al., 2023). As a result, findings are often context-dependent and difficult to generalize (Hol et al., 2015; Radujković et al., 2025), and relatively very few studies have directly linked microbial community depletion to crop productivity and stability under fluctuating conditions (Yachi and Loreau, 1999).

Here, we investigated whether soil microbial biodiversity modulates the response of barley to drought and its legacy during the recovery period, using an experimental manipulation of microbial communities via DTE. We proposed the following hypotheses: H1: Barley biomass and its recovery after soil rewetting will decrease with decreasing microbial community integrity, with plants growing in depleted communities showing reduced plant biomass, particularly under drought, compared to well-watered conditions. H2: Plant physiological performance (N uptake, $\delta^{13}C$) and post-drought recovery will be higher with higher microbial community integrity, indicating reduced stress and faster nutrient acquisition. H3: Less depleted microbial communities will show greater drought resistance and resilience after rewetting, reflected by smaller decline during drought and faster recovery of biomass, diversity, and community structure after rewetting.

## 2. Materials and methods

### 2.1. Soil sampling

The soil was sampled from an agricultural field in Niederhaverbeck, Germany (53.144272° N, 9.912668° E). The soil was collected from the top 20 cm, passed through a 2-mm sieve, and gamma-sterilized with 30 kGy (Fig. 1). The soil texture was sandy (93% sand), with the following properties: organic matter (2.3%), total C (0.98%), total N (0.07%), and pH ($CaCl_2$) of 4.9. The agricultural field had been under conventional use, with application of 1.2 t $ha^{-1}$ lime marl 85/5 and 200 kg $ha^{-1}$ potash magnesia. Prior to soil sampling, a mixture of grass-legume for hay production was cultivated on the field. The grass-legume mixture consisted of *Trifolium incarnatum* (Incarnate clover), *Trifolium repens* (white clover), *Trifolium palustre* (Red clover), *Lolium perenne* (rye grass), *Festulolium sp* (cross between Festuca and Lolium).

### 2.2. Soil inoculum preparation

Fresh soil samples, which served as the inoculum, were collected from the same agricultural field. Briefly, we collected soil from seven locations across the agricultural field, denoting them as replicates. For each replicate, five soil cores (diameter 10 cm and height 20 cm) were collected to make one composite replicate. These seven replicates were at least 20 m apart from each other. Thereafter, soil for inoculum retrieval was brought to the laboratory, passed through a 2-mm sieve and stored at room temperature overnight before preparing the soil dilution the next day. A DTE approach was used to deplete microbial communities of inocula (Domeignoz-Horta et al., 2020; Philippot et al., 2013) (Fig. 1). Unlike target approaches that change specific microbial communities, DTE is non-targeted and alters both the diversity and abundance of microbes non-specifically. Therefore, we defined microbial manipulation through DTE as a shift in microbial community integrity, where increasing dilution levels resulted in more depleted communities with lesser interactions (Fig. 1). Briefly, serial dilutions

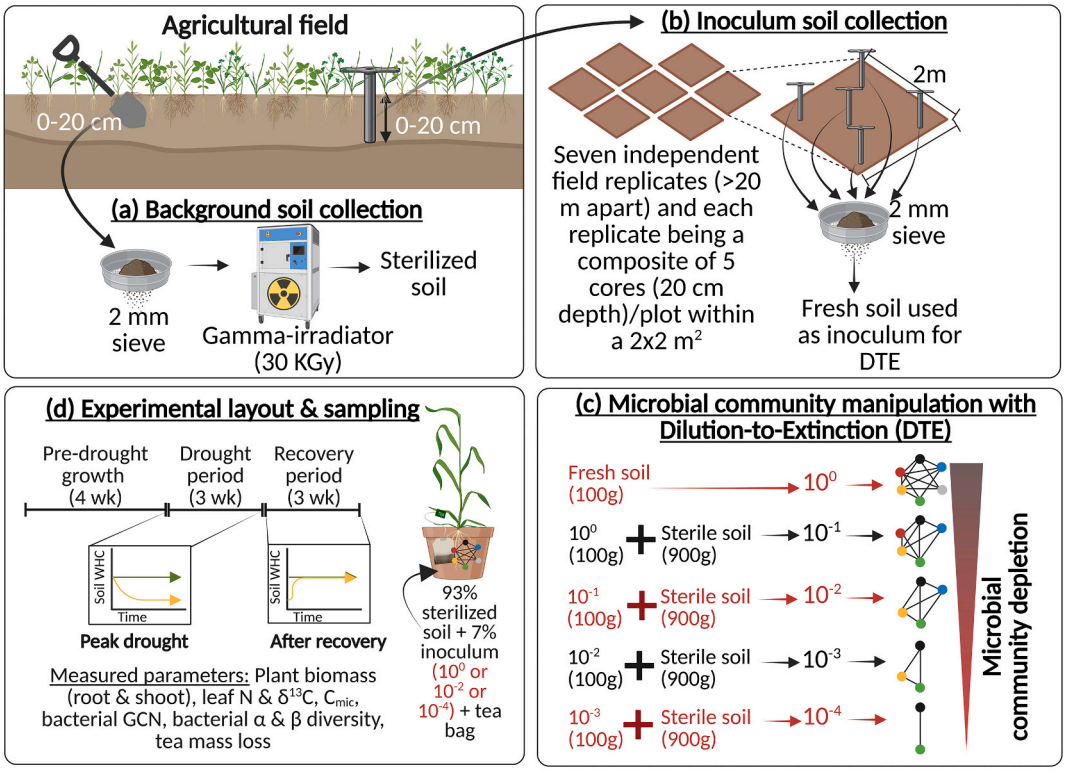



**Fig. 1.** The figure illustrates the structure of the experimental design employed in this study. (a) Background soil collection, (b) inoculum soil collection, (c) manipulation of soil microbial communities with dilution-to-extinction approach, and (d) controlled environment (in a climate chamber) experimental setup and sampling campaigns. wk. refers to weeks.

were prepared by mixing fresh soil (100 g dry weight equivalent) with gamma-sterilized (30 kGy) soil (900 g dry weight equivalent), thereby creating a 1:10 ($10^{-1}$) dilution. Thereafter, 100 g of $10^{-1}$ dilution was mixed with 900 g of sterilized soil, and this process was repeated to reach 1:100 ($10^{-2}$), 1:1000 ($10^{-3}$), and 1:10,000 ($10^{-4}$) dilution. From these serial dilutions, we selected $10^{-2}$ and $10^{-4}$ for the inoculation experiment. 100 g of fresh soil (without sterilization) represented the original community integrity with high diversity and abundance ($10^{0}$) (Fig. 1). We selected two endpoints (0 and $10^{-4}$) and an intermediate point ($10^{-2}$) of the dilution gradient, which allowed us to maintain a tractable experimental design and provided well-separated contrasts. This approach has previously shown to progressively change microbial diversity and community composition with increasing dilution (Delgado-Baquerizo et al., 2016; Yang et al., 2021a, 2021b).

To account for field heterogeneity, the same procedure was followed separately for all seven field replicates. It should be noted that the microbial dilutions were prepared by mixing fresh and sterilized soils instead of making soil slurries by serially diluting soil with water or buffer solutions as described previously (Liu et al., 2023; Yang et al., 2021a). We particularly chose this approach to better preserve microbial viability by maintaining a similar physical environment as opposed to the soil slurry method, since many microbes may experience stress in soil slurries or die due to access water. This could be true especially for microbes that are sensitive to changes in soil water levels.

To validate the effectiveness of the DTE approach in manipulating soil biodiversity, we measured total microbial biomass (overall change in microbial community), bacterial gene copy number (estimate of changes in bacterial abundance), and bacterial 16S rRNA GCN per genome (bacterial community-level shifts in life-history functional traits). We quantified 16S rRNA in the inocula after serial dilutions to reflect the active microbial fraction. We chose this approach because soil used for dilution was gamma-sterilized. Although gamma-sterilization can effectively kill active microbes, their DNA can persist in soil. This relic DNA can introduce artefacts by artificially increasing bacterial gene copy number. In contrast, RNA is less stable and quickly degrades after cell death. Therefore, we aimed to more accurately analyze the active microbial proportion across dilution levels using RNA quantification.

As dilution was applied to whole-soil inoculum, we used 'microbial-mediated' effects when referring to the inoculum manipulation as a whole, whereas 'bacterial-mediated' was specifically used to support 16S rRNA-based community data.

### 2.3. Experimental setup

The experiment was conducted in a climate chamber under controlled conditions (light phase: 21.6 ± 1.1°C; dark phase: 17.2 ± 1 °C; 16 h light/8 h dark; lamps: SANlight P4-serie, 400–760 nm; PAR: 302 ± 21 μmol $m^{-2}$ $s^{-1}$) at the University of Lüneburg. For this, each pot (volume: 2 L) received 1.7 kg soil, of which 93% was sterilized (30 kGy) soil and the remaining 7% was inoculated with the microbial community inoculum obtained with DTE approach. By filling a majority of the pot with one common sterilized soil and adding a small amount of soil inoculum characterized by different microbial communities allowed us to keep the abiotic soil properties similar and focus on the effects of soil microbes. It is important to note that gamma-irradiation likely led to dead-cell/extracellular DNA in soil and 16S rRNA sequencing therefore represents total amplifiable bacterial DNA. Because all DTE treatments received the same amount of gamma-irradiated soil and were later processed using the same extraction, amplification, and bioinformatics analyses, any residual soil-derived DNA was considered as a shared methodological background across the treatments.

Pre-weighed and oven dried rooibos tea bags (EAN: 8722700188438, Lipton, Unilever) were buried at the center of each pot (one tea bag per pot) to quantify decomposition rate of a standard litter. Thereafter, one seedling of barley (*Hordeum vulgare* L. Barke) was grown in each pot. Seeds were surface-sterilized with 70% ethanol and

thoroughly rinsed with distilled water. To facilitate germination, seeds were soaked in distilled water over night after the sterilization. Then, seeds were transferred to plastic petri dishes consisting of a moist filter paper and allowed to germinate in a climate chamber under optimal conditions. After germination (three days), seedlings were transferred to the pots (one seedling per pot). All pots were maintained at 70% maximal water holding capacity (mWHC) for 30 days before imposing drought. For inducing drought conditions, we ceased watering in one set of pots from each soil diversity gradient and maintained them at 30% mWHC for three weeks, whereas the other set of pots was kept at 70% mWHC. As we did not determine the permanent wilting point from a plant-based wilting assay or a soil-water retention curve, 30% mWHC was considered as an operational drought stress. After three weeks, one set of droughted and control pots were destructively harvested (peak drought harvest). For the second set, the droughted pots were carefully rewetted to 70% mWHC and allowed to recover for three weeks before harvesting (recovery harvest). Pots were randomized every week throughout the experimental period to avoid position-specific effects in the growth chamber (Saraswat et al., 2026; van Duijnen et al., 2021). With seven replicates for each treatment combination, we had 84 experimental units (three levels of soil biodiversity x two treatments (drought and control) x two harvests (peak drought and after the recovery) x 7 replicates).

### 2.4. Harvests

At both peak drought and after the recovery phase, shoots were cut at the base and oven-dried at 70 °C until a constant weight. Thereafter, the pots were carefully opened and the tea bags were retrieved, cleaned, and dried at 70 °C until a constant weight. The tea bag mass loss was used as a proxy for litter decomposition rate. Roots were collected from the soil and shaken vigorously to detach loosely bound soil. The soil that remained attached to the roots was collected in 2-ml Eppendorf tubes and considered as rhizosphere soil, and directly frozen at −80 °C for DNA extraction and bacterial community analysis. All the roots were cleaned with deionized water and stored at −20 °C for root trait measurements.

### 2.5. Plant and soil analyses

Dried leaves were ball-milled to measure total carbon (C), nitrogen (N), and $\delta^{13}C$ by using an isotopic ratio mass spectrometer (Isoprime Ltd., Cheadle Hulme, UK) coupled to an elemental analyzer (Vario el cube, Elementar, Hanau, Germany). Dried roots were measured for total C and N by using a C/N analyzer (Vario EL Cube, Elementar, Langenselbold, Germany).

Soil microbial biomass C ($C_{mic}$) was determined using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, 10 g non-fumigated soil was dissolved in 40 ml of 0.05 M $K_2SO_4$ and continuously shaken for 1 h before filtering the soil suspension. The filtrate was measured for total organic C (DOC) (iso TOC cube, Elementar, Langenselbold, Germany). The same extraction procedure was followed for fumigated soils, which were fumigated in a desiccator with ethanol-free chloroform for 24 h. The difference in DOC between the fumigated and non-fumigated samples was used to calculate $C_{mic}$. An extraction efficiency factor of 0.45 was used for $C_{mic}$ (Wu et al., 1990).

### 2.6. DNA extraction and bacterial metabarcoding

The V4 region of the 16S rRNA gene was amplified from 500 mg of soil using the primer pair 515FB-806RB (Apprill et al., 2015; Parada et al., 2016) and sequenced on an Illumina MiSeq (2 × 300 bp). Reads were processed using DADA2 v1.30.0 (Callahan et al., 2016), taxonomically assigned with SILVA v138.1 (Quast et al., 2013), and decontaminated using decontam v1.22.0 (Davis et al., 2018). After rarefaction to 39,236 reads in 6700 ASVs (6671 from bacteria and 29 from archaea) were retained. Detailed protocols for DNA extraction, PCR, library preparation, and bioinformatic processing are provided in supporting information. The read counts throughout the DADA2 pipeline are provided in Supporting Table S1 (DADA2 reads). We also predicted 16S GCN to evaluate the relative abundance of fast-growing and slow-growing bacterial groups in the microbiome during peak drought and after recovery.

### 2.7. Statistics

Subsequent analyses were performed using the package *Phyloseq* v1.46.0 (McMurdie & Holmes, 2013) and visualized using *ggplot2* v3.5.1 (Wickham, 2016) unless specified differently. Alpha diversity indices were calculated using the package *microbiome* v1.24.0 (Lahti & Shetty, 2017). The 16S rRNA gene copy number per genome (GCN) prediction was performed using the software *RasperGade16S* v0.0.1.0000 (Gao & Wu, 2023).

For all response variables, data were analyzed using linear models that included the main effects of microbial dilution (log-transformed) and treatment (control vs drought), as well as their interaction, for each sampling time:

$$Response\ variable \sim \log(dilution\ gradient) \times treatment \quad (1)$$

Model assumptions of normality and homogeneity of variance were assessed using residual plots, Shapiro–Wilk tests, and heteroscedasticity checks (R Core Team, 2023). Type III ANOVA (car package; Fox & Weisberg, 2019) was used to test the significance of main effects and interactions. If the interaction term was not significant, a simpler model without the interaction was fitted to assess main effects. To examine treatment-specific responses, slopes of each response variable across microbial dilution were estimated using the *emtrends* function (emmeans package; Lenth, 2024), which calculates estimated marginal trends for each treatment. Slopes were tested for significance (slope $\neq 0$), and pairwise comparisons of slopes between treatments were conducted to evaluate whether the slopes differed significantly, reflecting interaction effects. For visualization, treatment-specific regression lines were plotted: solid lines indicate a significant slope (microbial dilution had an effect), and dashed lines indicate a non-significant slope.

Microbial community compositional shifts were visualized through Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarities calculated at the ASV level. To test for significant differences, permutational multivariate analysis of variance (PERMANOVA) was performed separately for each time point using the adonis2 function (vegan package; 999 permutations). We applied a sequential model (by = "terms") to evaluate the main effects of drought treatment and microbial dilution, as well as their interaction. Homogeneity of variances was ensured to be equal throughout groups beforehand. To quantify the magnitude of the drought impact, Euclidean distances between group centroids were calculated for each dilution level and time point. For this, we utilized the first five principal coordinate axes, which cumulatively explained 50.2% of variation.

To identify bacterial genera significantly responding to the microbial dilution, abundance data was aggregated at the genus level and filtered to a minimum mean relative abundance of 0.5% across all samples. Raw counts were Centred Log-Ratio (CLR)-transformed after adding a pseudo-count of 1. For each genus and time point, the full factorial model was used to test for potential interactions between the dilution level and the drought treatment. Since no significant interaction effects were observed for any of the tested taxa, an additive linear model was used to assess the main effect of the dilution while controlling for treatment:

$$CLR\ abundance \sim log(dilution\ gradient) + treatment \quad (2)$$

Results were extracted from the additive models via Type I ANOVA. Resulting *p*-values for the dilution effect were adjusted using the Benjamini-Hochberg (FDR) method to account for multiple testing.

Genera with an adjusted $p$-value <0.05 at the peak drought and after the recovery phase were selected as significant responders. For visualization, mean CLR abundances were standardized to $Z$-scores.

## 3. Results

### 3.1. Microbial manipulation and drought impact on plant parameters at peak drought and after recovery

Shoot biomass increased with serially depleted microbial communities at both peak drought ($F_{1,35} = 29.48$, $P < 0.001$) and after recovery period ($F_{1,36} = 13.45$, $P < 0.001$), whereas effects of drought on shoot biomass were absent (Fig. 2a). Root biomass also increased with serially depleted microbial communities, but only at peak drought ($F_{1,35} = 19.33$, $P < 0.001$) (Fig. 2b). Drought caused an overall 24% reduction in root biomass at peak drought, whereas no effect of depleted microbial communities and drought legacies were observed after the recovery period. The effects of microbial communities on leaf N content depended on drought at both peak drought and after the recovery period (Fig. 2c). At peak drought, we observed that drought ($F_{1,34} = 22.6$, $P < 0.001$) and its interaction with microbial community manipulation ($F_{1,34} = 6.8$, $P = 0.013$) led to variation in leaf N content, where leaf N content decreased with increasing microbial community depletion only under drought. After the recovery period, the leaf N content was impacted by drought legacies ($F_{1,35} = 4.86$, $P = 0.034$) and its interaction with the manipulation of microbial communities ($F_{1,35} = 5.6$, $P = 0.023$). We found that increasing microbial community depletion led to a reduced leaf N content only under control conditions, whereas drought legacy effects were not evident. Drought ($F_{1,33} = 9.93$, $P = 0.003$) and its interaction with microbial communities ($F_{1,33} = 15.88$, $P < 0.001$) affected leaf $\delta^{13}C$, i.e., $\delta^{13}C$ values decreased (became more negative) with depleting microbial communities at peak drought (Fig. 2d). After the recovery period, leaf $\delta^{13}C$ values decreased with depletion of microbial communities ($F_{1,35} = 5.51$, $P = 0.025$), while drought legacy and its interaction with soil biodiversity were not significant.

### 3.2. Microbial manipulation and drought effects on standard litter decomposition, microbial biomass, and bacterial community dynamics at peak drought and after recovery

At peak drought, litter mass loss increased with depleting microbial communities ($F_{1,34} = 10.2$, $P = 0.003$), whereas drought effects were not significant ($F_{1,34} = 3$, $P = 0.09$) (Fig. 3a). Interestingly, the effect of microbial community manipulation disappeared after the recovery phase. At peak drought, $C_{mic}$ decreased with depleting microbial communities ($F_{1,35} = 25.36$ $P < 0.001$). Moreover, $C_{mic}$ decreased by 31% under drought as compared to control ($F_{1,35} = 14.14$, $P < 0.001$) (Fig. 3b). After the recovery period, $C_{mic}$ was neither impacted by drought legacies nor by microbial community manipulation.

At peak drought, average GCN significantly increased with microbial community depletion ($F_{1,27} = 25.52$, $P < 0.001$). Furthermore, average GCN was 10% higher in the drought treatment compared to the control ($F_{1,27} = 15.1$, $P < 0.001$) (Fig. 3c). In contrast, after the recovery period, the average GCN was not significantly influenced by the manipulation of microbial communities, drought legacies, or their interaction.

Bacterial alpha diversity consistently declined with microbial community depletion at both peak drought and after the recovery period, including ASV richness (peak drought: $F_{1,26} = 51.98$, $P < 0.001$; recovery: $F_{1,26} = 9.4$, $P = 0.005$) (Fig. 3d), Shannon diversity (peak drought: $F_{1,27} = 56.6$, $p < 0.001$; recovery: $F_{1,27} = 15.6$, $P < 0.001$) (Fig. 3e), and Pielou's evenness (peak drought: $F_{1,27} = 25.6$, $P < 0.001$; recovery: $F_{1,27} = 14.1$, $P < 0.001$) (Fig. 3f). Additionally, the drought treatment reduced ASV richness (by 17% at peak drought, 11% after recovery) and Shannon diversity (by 4.3% at peak drought, recovery 3.6%,). In contrast, drought negatively impacted Pielou's evenness (by 1.9%) only after the recovery phase.

At peak drought, bacterial community composition was significantly driven by both drought (PERMANOVA: $F_{1,18} = 3.85$, $R^2 = 0.11$, $P = 0.001$) and microbial community manipulation ($F_{2,18} = 4.71$, $R^2 = 0.28$, $P = 0.001$), with no significant interaction (Fig. 4a). Correspondingly, compositional dissimilarity between drought and control treatments increased with microbial community depletion, rising from a centroid distance of 0.18 (dilution 0) to 0.33 (dilution 4) (Fig. 4b). After the

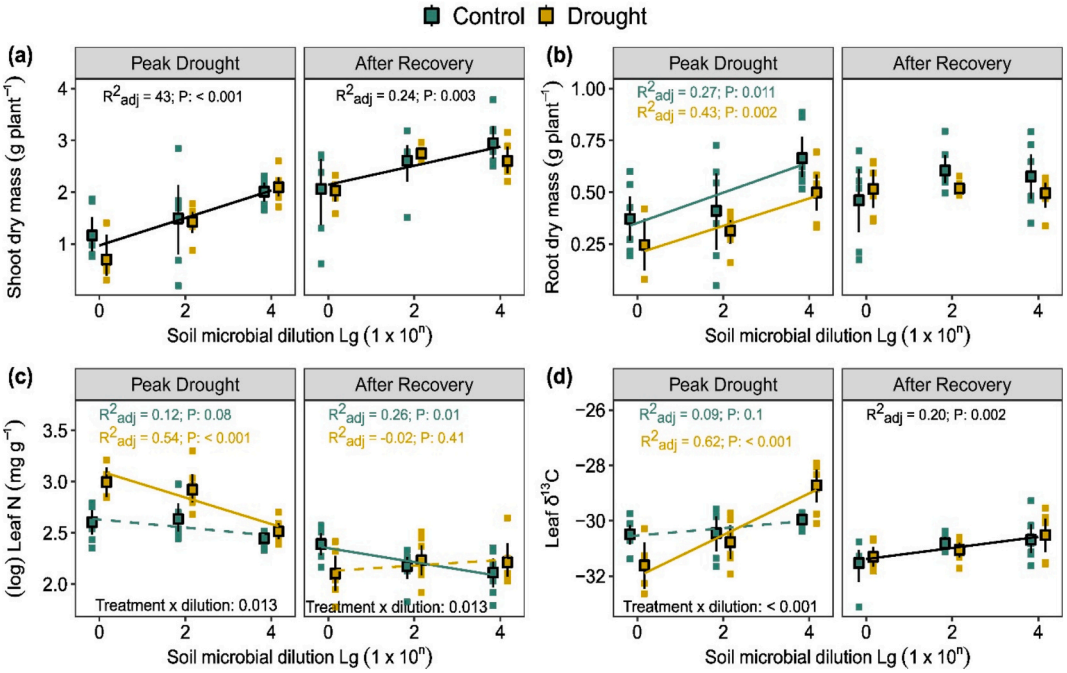



**Fig. 2.** (a) Shoot biomass, (b) root biomass, (c) leaf N content, and (d) leaf $\delta^{13}C$ responses to a soil microbial dilution under control and drought at two phases: peak drought and after recovery. Regression lines illustrate the effect of soil biodiversity level (dilution gradient of 0, 2, and 4), with solid lines indicating statistically significant relationships and dashed lines indicating non-significant effects.

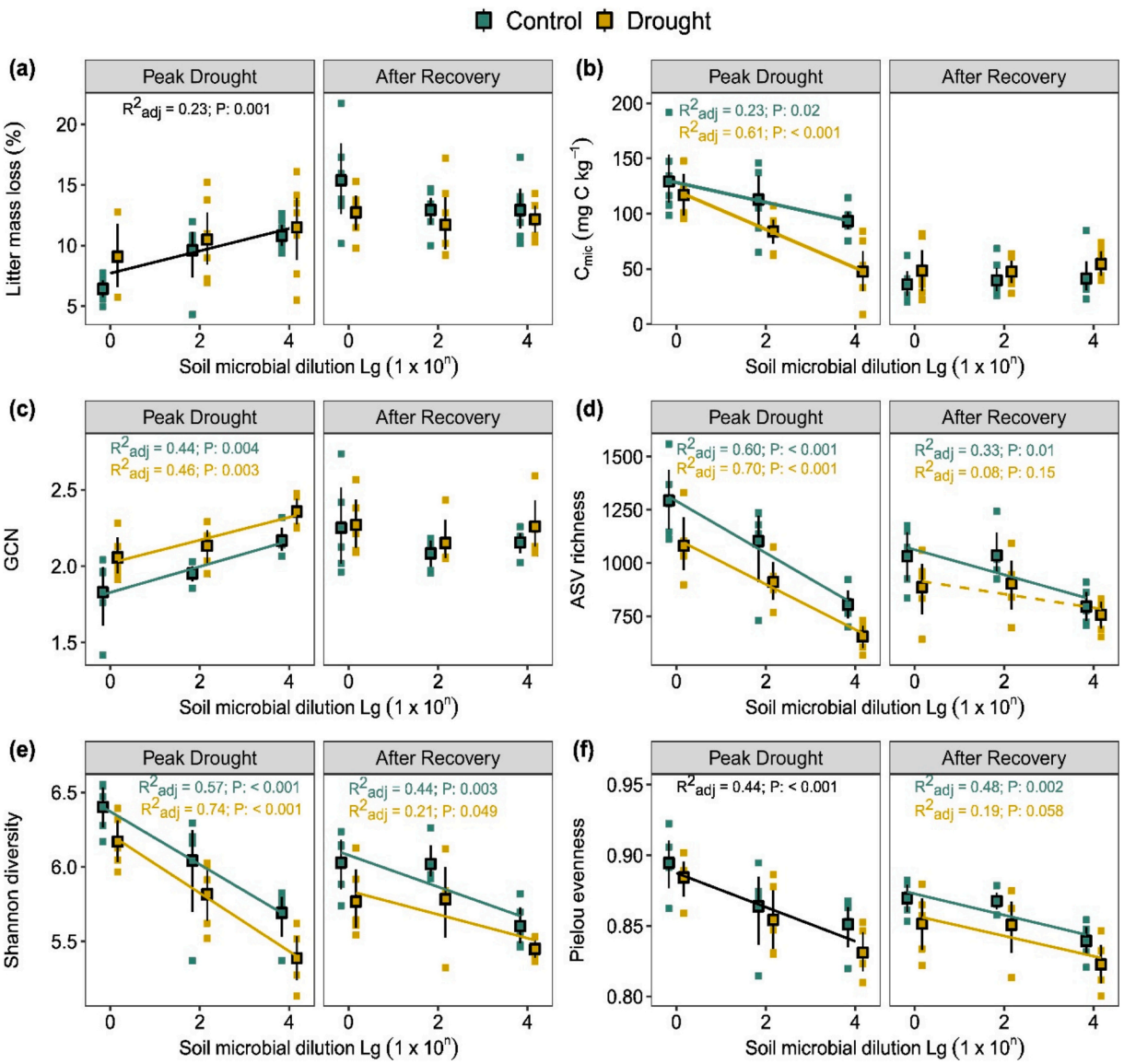



**Fig. 3.** (a) Standard litter (tea bag) mass loss, (b) microbial biomass C, (c) bacterial GCN, (d) bacterial ASV richness, (e) bacterial Shannon diversity, and (f) bacterial Pielou's richness responses to soil microbial dilution under control and drought at two phases: peak drought and after-recovery. Regression lines illustrate the effect of soil biodiversity level (dilution gradient of 0, 2, and 4), with solid lines indicating statistically significant relationships and dashed lines indicating non-significant effects.

recovery period, the effect of drought disappeared, but the soil biodiversity effects were still evident ($F_{2,18} = 2.78$, $R^2 = 0.19$, $P = 0.001$). Additionally, a significant interaction emerged between drought legacy and soil biodiversity ($F_{2,18} = 2.00$, $R^2 = 0.14$, $P = 0.003$), and a reversed dissimilarity pattern emerged. Bacterial community differences between control and drought legacies started to decrease with decreasing soil biodiversity. The ten most abundant bacterial phyla represented 93.2% of the total bacterial community. Across all samples, the bacterial community was dominated by members of the *Pseudomonadota* (37.0 ± 4.9%, mean ± SD), followed by *Actinomycetota* (17.9 ± 3.5%), and *Bacillota* (9.7 ± 6.2%) (Fig. 5).

At peak drought, depletion of microbial communities consistently increased the relative abundance of *Pseudomonadota*, *Actinomycetota*, and *Armatimonadota*, while decreasing the relative abundance of *Acidobacteriota*, *Bacillota*, and *Planctomycetota*. Drought modulated the impact of microbial community manipulation on *Chloroflexota* and *Gemmatimonadota*. In contrast, the abundance of *Bacteroidota* increased strictly under drought conditions regardless of microbial community manipulation (Fig. 5). After recovery, most of the manipulation effects on bacterial community composition persisted. The abundance of *Armatimonadota* and *Pseudomonadota* remained high, while the abundance of *Acidobacteriota* and *Bacillota* remained low. Furthermore, *Verrucomicrobiota* increased alongside microbial community depletion. The interactive effect of drought and microbial community manipulation persisted only for *Chloroflexota* (Fig. 5).

To investigate the specific taxonomic drivers behind the community shifts, we analyzed the differential abundance of bacterial genera along the microbial community manipulation gradient (Fig. 6). At peak drought, several genera showed a significant positive response to depleting microbial communities, independent of treatment, including *Burkholderia* s.l. ($F_{1,21} = 33.97$, $P_{adj} < 0.001$), *Arthrobacter* ($F_{1,21} = 52.17$, $P_{adj} < 0.001$), and *Mucilaginibacter* ($F_{1,21} = 12.75$, $P_{adj} = 0.005$). In contrast, genera decreasing in relative abundance with depletion of microbial communities included *Bacillus* ($F_{1,21} = 11.84$, $P_{adj} = 0.007$), *Clostridium* s.s. ($F_{1,21} = 10.22$, $P_{adj} = 0.010$), and *Mesorhizobium* ($F_{1,21} = 16.17$, $P_{adj} = 0.003$). Additionally, some genera, including *Pseudomonas* ($F_{1,21} = 18.76$, $P_{adj} = 0.002$) and *Massilia* ($F_{1,21} = 12.60$, $P_{adj} = 0.010$), were significantly enriched solely under drought as compared to the

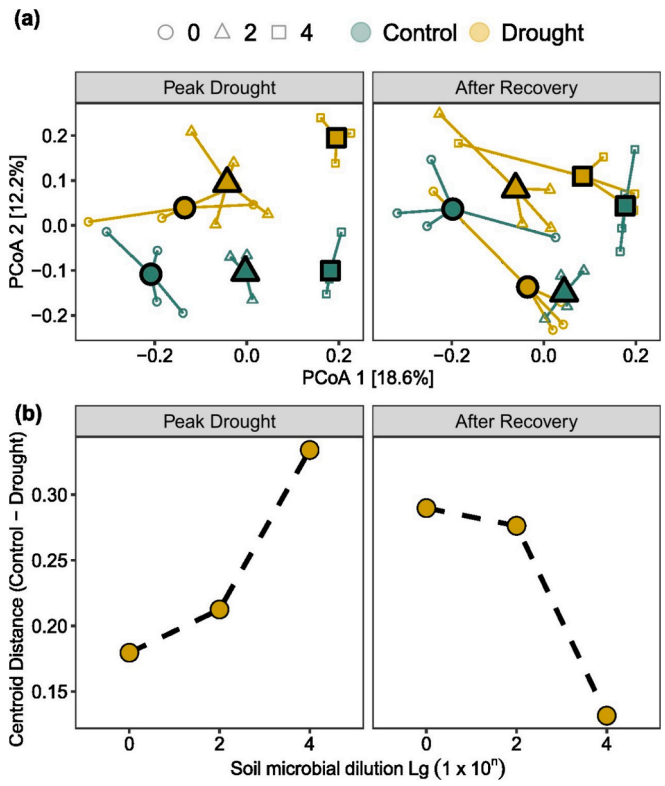



**Fig. 4.** Impact of drought and soil microbial dilution on bacterial community composition. (A) Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarities of the bacterial communities. Large symbols with black outlines represent group centroids, while smaller symbols represent individual samples. (B) Compositional dissimilarity between the control and drought communities across soil biodiversity levels (dilution gradient of 0, 2, and 4). Shown are the Euclidean distances between the respective treatment group centroids, calculated across the first five principal coordinate axes.

control. After the recovery period, the positive influence of microbial community depletion persisted for *Burkholderia* s.l. ($F_{1,21} = 11.15$, $P_{adj} = 0.025$) and *Mucilaginibacter* ($F_{1,21} = 9.93$, $P_{adj} = 0.026$). After the recovery period, *Bacillus* was the only genus that remained significantly depleted with microbial community depletion ($F_{1,21} = 12.95$, $P_{adj} = 0.018$). Detailed model results for all responding taxa can be found in Supporting Table S2 (Responding taxa).

## 4. Discussion

### *4.1. Plant biomass responses to drought and microbial community manipulation*

Our results challenge the paradigm that diverse microbial communities invariably improve ecosystem functions, particularly plant biomass. In contrast, we found that microbial communities depleted with the DTE approach resulted in higher plant biomass (both root and shoot biomass). Moreover, we did not observe any buffering effects of soil biodiversity on plant biomass against drought (Fig. 2a and b). These findings reject our first hypothesis which predicted the opposite, and highlight the context-dependent impact of microbial communities on plant productivity. This negative relationship between plant biomass and microbial communities in soil may be due to microbial "overhead cost" through competition, antagonism, and predation networks in highly diverse communities, whereas simplifying/depleting these interactions via dilution shifts microbial communities toward a functionally optimal state.

In our study, a negative relation between soil biodiversity and plant biomass could be due to two non-mutually exclusive mechanisms. First, depletion of microbial communities to more simplified communities could have reduced plant-microbial competition for limited soil resources (Gannett et al., 2024a, 2024b). In contrast, a diverse community with high biomass may act as a nutrient sink by immobilizing more nutrients, making them less available for plant uptake (Hodge et al., 2000; Zhai et al., 2024). These findings are supported by a strong negative relationship between plant biomass and microbial biomass C (a proxy of overall microbial abundance) at peak drought (supporting information S1. Fig. 1). Moreover, we found that both bacterial GCN and decomposition rate of a standardized litter increased with microbial community depletion. These results suggest that dilution-mediated microbial community depletion favored fast-growing bacterial copiotrophs over slow-growing oligotrophs. This shift may have improved nutrient mineralization and availability to plants, thereby increasing plant growth. Our findings are supported by a recent finding showing how untargeted selection of copiotrophs with dilution-to-extinction can

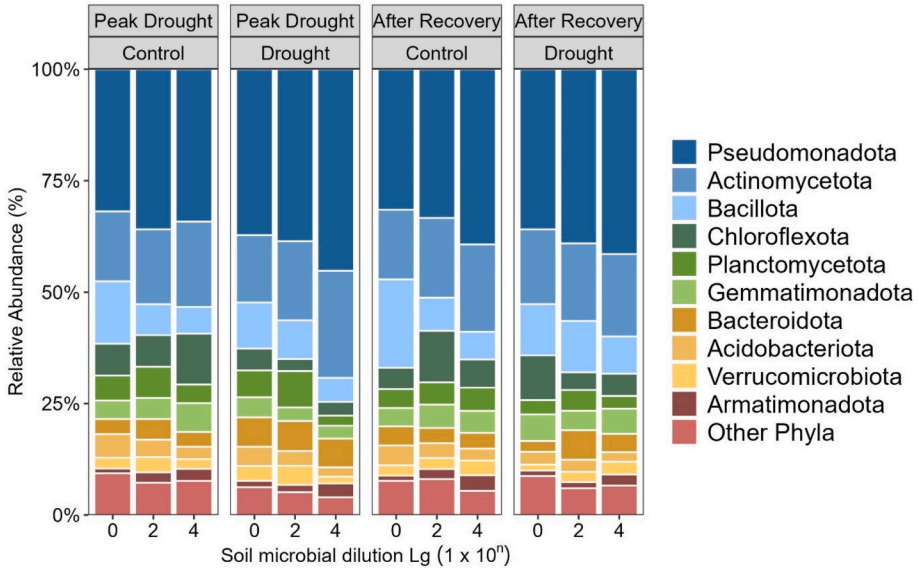



**Fig. 5.** Relative abundance of the top ten most abundant bacterial phyla across soil biodiversity levels (dilution gradient of 0, 2, and 4) under control and drought at two phases: peak drought and after-recovery. Phyla not represented in the top ten are shown as "other phyla".

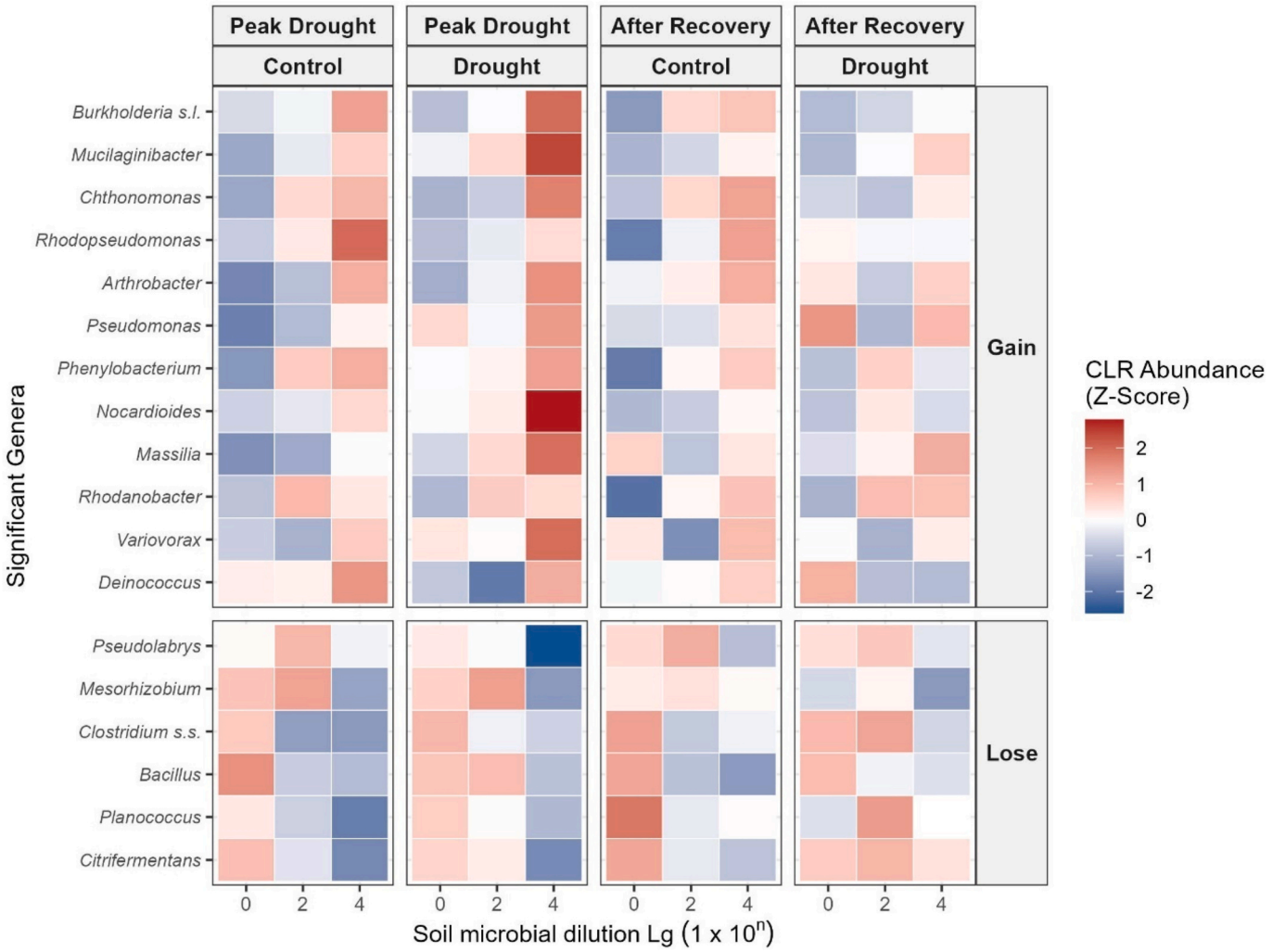



**Fig. 6.** Differential abundance of bacterial genera along the soil microbial dilution gradient. Heatmap showing the standardized (*Z*-score) mean CLR-transformed abundances of bacterial genera that responded significantly to the dilution ($P_{adj} < 0.05$) at peak drought. Taxa are grouped into those increasing ('Gain') or decreasing ('Lose') abundance along the soil biodiversity level (dilution gradient of 0, 2, and 4), based on the slope of the additive linear models.

complicate the diversity-functioning relationship (Mao et al., 2023).

Secondly, more diverse microbial communities may also harbor multiple antagonistic microbes including both generalists and specialist pathogens which negatively affect plant fitness (García-Bayona and Comstock, 2018; Peterson et al., 2020; Wang et al., 2023). As the used DTE approach is taxonomically not selective, serial soil dilutions may have altered or eliminated soil-borne pathogens (protozoa, parasitic nematodes, and fungal and bacterial pathogens). Consequently, soil dilution may have reduced pathogen load, thereby lowering negative impacts on plant fitness at higher dilution levels. As plants invest a significant amount of energy and resources to fight against soil pathogens, these investments can be greater in soils with higher soil biodiversity and decrease with community simplification, and therefore, these resources can be allocated to growth. Our interpretations are supported by shifts in specific bacterial taxa. For instance, reducing soil biodiversity by serially diluting soil inocula enriched bacterial taxa that are beneficial for plant performance. We observed that, among others, the relative abundance of genera *Arthrobacter, Burkholderia* s.l., *Massilia, Mucilaginibacter* increased with increasing soil dilution at both peak drought and this effect persisted until after soil rewetting. *Arthrobacter* and *Burkholderia* are involved in phytohormone production, P and N solubilization, and biofilm formation, especially under drought stress (Chhetri et al., 2022; Han et al., 2024). *Massilia* and *Mucilaginibacter* are linked to auxin and gibberellin signaling modulation and plant stress tolerance through ABA-independent pathways and antioxidant mechanisms (Fan and Smith, 2022). In contrast, the relative abundance of the genus *Bacillus*, well-known for promoting plant growth and enhancing plant defense, significantly decreased with increasing soil dilution at peak drought and after recovery. We believe that this negative relationship between *Bacillus* and plant biomass cannot simply be causal. Instead, decrease in the relative abundance of *Bacillus* can be linked to its competitive disadvantage for soil resources and niches over other taxa. Furthermore, *Mesorhizobium* and *Clostridium*, genera known for $N_2$ fixation (Verma et al., 2013; Zeiller et al., 2015), also declined with dilution. However, considering the higher number and broad spectrum of plant growth promoting taxa and their steeper increase with dilution, the overall net effect of enriched and depleted bacterial taxa may have contributed to the improved plant performance. These results are consistent with recent findings that diversity-mediated functioning relationships are dependent on dominance of certain microbial guilds in the system (Saghaï et al., 2022; Wang et al., 2023).

Given the limitations of the DTE approach to manipulate soil biodiversity (unspecific manipulation of microbial taxa), we cannot quantify the relative contributions of the mentioned mechanisms. The increased plant performance may be the result of additive / synergistic effects between enrichment of beneficial taxa as well as the reduction in pathogen load through the dilution process. Finally, it is unclear whether the observed increase in plant performance persists over time,

or if the dilution-induced loss of beneficial bacteria like *Bacillus* will compromise plant health in the long term. Moreover, although we mainly focused on bacterial communities given the short experimental timescale, other microbes (including fungi and protists) may also influence plant performance. Therefore, future studies considering all microbial groups would be valuable to have a more comprehensive understanding of plant responses.

We also observed that shoot biomass was not affected, whereas root biomass decreased under drought stress. This reduction in root biomass is likely because of drought-induced suppression of root elongation (Zhang et al., 2020) as well as increased fine-root senescence (Harrison Day et al., 2025), whereas short-term conservative adjustments of shoots to growth may have resulted in no change in shoot biomass.

### 4.2. Plant physiological responses to drought and microbial community manipulation

We found a reduction in leaf N content with microbial community depletion under peak drought. These findings are consistent with previous research linking reduced diffusion and transport of N to roots under water shortage (Dijkstra et al., 2015; He and Dijkstra, 2014) and lower abundance of some N-cycling related microbial taxa. In support of this finding, we also observed reduced abundances of *Mesorhizobium* and *Clostridium*, two N-mobilizing genera, which may have led to lower N availability in soil. Since these taxa are not primarily associated with barley in a mutualistic way, their response is likely to be indirect. A decrease in leaf N content accompanied with an increase in plant biomass alongside decreasing soil biodiversity at peak drought suggests that shoot biomass increased more quickly than N accumulation, hinting toward a growth-dilution effect instead of direct N deficiency. Alternatively, reduced microbial biomass with community depletion may have reduced short-term microbial N immobilization, thereby increasing N availability to plants. Interestingly, after recovery, the pattern reversed, with leaf N content declining only in the control plants but not in those that had previously experienced drought. Plants that previously experienced drought have been shown to reallocate N from shoots to roots (Fuchslueger et al., 2016). These findings suggest that soil biodiversity plays a role in plant N uptake under drought stress. However, drought legacies can alter these patterns through reallocation of N within the plant.

Furthermore, the fact that leaf $\delta^{13}C$ values increased (became less negative) with microbial community depletion under drought stress supports improved intrinsic water-use efficiency through reduced stomatal conductance, thereby enhancing carbon gain per unit of water loss as previously found for barley (Chen et al., 2011). The convergence of leaf $\delta^{13}C$ values between control and previously drought-stressed plants suggests a systemic physiological adjustment of plants once water becomes available again. Plants grown in soil with reduced soil biodiversity appear to adjust to a more conservative water-use strategy. This can either be a direct effect of soil microbes by transporting water away from the root-depletion zone, or indirectly through microbial-mediated changes in root system architecture including reduced root branching, or adjusted hormonal signaling in the rhizosphere.

### 4.3. Microbial biomass and bacterial community response to drought and microbial community manipulation

Regarding the overall microbial biomass and bacterial community response, our data revealed that experimental manipulation of microbial communities and drought acted independently. As expected, depletion of microbial communities with DTE approach led to a gradual decrease in overall microbial biomass and bacterial alpha diversity. Drought also negatively impacted both microbial biomass and bacterial alpha diversity, which aligns with previous findings (Preece et al., 2020, 2019; Xie and Shou, 2021). However, we did not observe a greater decline in microbial biomass and bacterial diversity with microbial community depletion under drought stress. These results do not support the insurance hypothesis, which states that a diverse microbial community is better at buffering environmental changes (Yachi and Loreau, 1999).

The distribution of centroid distances between treatment groups revealed a distinct ecological response of bacterial communities to drought, which varied by microbial community manipulation. During peak drought, the centroid distance between drought and control groups increased with microbial community depletion. This suggests that soil dilution increased susceptibility of some bacterial taxa to drought stress. It must be noted, however, that the PERMANOVA did not confirm a statistically significant interaction. Nevertheless, this trend aligns well with previous studies demonstrating that less diverse (depleted) microbial communities exhibit reduced resistance to environmental disturbances through lower network connectivity and functional redundancy (Winterfeldt et al., 2024; Zhang et al., 2024). Interestingly, this trend reversed following the recovery period, supported by a significant interactive effect between drought legacy and microbial community manipulation on the bacterial community composition. These findings suggest that the bacterial communities can quickly recover to resemble the control state. This process is likely driven by the recolonization of opportunistic copiotrophic taxa that were already selected for during the dilution process, as disturbances can favour similar fast-growing taxa through altered soil properties caused by drought stress (Adkins et al., 2022; Mao et al., 2023) and nutrient flushed because of cell death. Conversely, the soil with higher biodiversity exhibited a larger compositional shift, leading to a distinct compositional state after the recovery period, which aligns with recent findings that drying-rewetting cycles can have a larger impact on the microbial community composition compared to reduced precipitation itself (Wang et al., 2022). Taken together, the trend in alpha diversity suggested simple additive effects of drought and microbial community manipulation with DTE. However, shifts in bacterial community composition point toward an ecological trade-off between resilience and initial resistance, which is mediated by diversity and has previously been observed (Shade et al., 2012).

From an agronomic perspective, our findings suggest that bacterial diversity alone may not be a suitable short-term predictor of crop performance in an intensively managed cropping systems including high-input crop production and horticultural systems. Under these conditions, external inputs may partially substitute for functions provided by soil microbes, thereby decoupling biodiversity-productivity outcomes in the short-term. Crop productivity should therefore be also evaluated alongside disease suppression, nutrient retention, carbon and nutrient fluxes, while taking into account the full range of microbial diversity, including other taxonomic groups.

## 5. Conclusions

This research contributes to the biodiversity-ecosystem functioning framework by showing that soil biodiversity and plant productivity can be decoupled under drought and post-drought recovery in cropping systems. Experimental reduction of soil microbial communities and drought stress consistently reduced overall microbial abundance and bacterial diversity, and these effects were evident both at peak drought and after the recovery period. Although our findings show a negative correlation between microbial abundance and plant biomass, we advise caution in the interpretation of these results given the controlled conditions and short duration of the experiment. Strong positive biodiversity-productivity relationships are generally reported in long-term field-based studies, suggesting that environmental complexity and temporal scale may significantly impact the outcomes. Therefore, instead of contradicting these claims, our findings rather align with emerging biodiversity-ecosystem functioning research by adding to the evidence that biodiversity effects can be context-dependent and function-specific. Our results highlight that the microbial capabilities to mediate soil functions can persist under environmental stress conditions

despite significant losses in diversity, as long as key microbial groups are present. We therefore recommend incorporating microbial functional traits when evaluating ecosystem responses to biodiversity loss under climate change.

## CRediT authorship contribution statement

**Amit Kumar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sebastian Bibinger:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis. **Jing Tian:** Writing – review & editing. **Biao Zhu:** Writing – review & editing. **Shuo Jiao:** Writing – review & editing. **Rüdiger Reichel:** Writing – review & editing, Methodology. **Nicolas Brüggemann:** Writing – review & editing, Funding acquisition. **Michael Schloter:** Writing – review & editing, Funding acquisition. **Stefanie Schulz:** Writing – review & editing. **Vicky M. Temperton:** Writing – review & editing, Investigation, Funding acquisition.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2026.107497.

## Data availability

The sequence data obtained in this study are deposited in the short-read archive of NCBI under accession number PRJNA1454034.

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