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RESEARCH ARTICLE

Drought and vegetation restoration lead to shifts in soil microbial diversity and co-occurrence networks in California coastal prairie

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Abstract

Background and aims Both drought and vegetation restoration can have dramatic effects on plant community composition, but how they influence soil microbial community diversity, structure, and co-occurrence networks remain less well known.

Methods To better understand the regulatory mechanisms of drought and vegetation restoration on soil microorganisms, we planted 12 native species in precipitation manipulation experimental plots in an invaded coastal grassland in California, USA. We measured soil bacterial and fungal community composition by amplicon sequencing, and quantified plant species richness and coverage in the third experimental year.

Results Our results showed that drought significantly altered soil bacterial diversity and composition; however, neither drought nor vegetation restoration had significant effects on fungal diversity and composition. The control plots had the most cooperative interactions (greatest number of correlations) among bacterial and/or fungal species, while drought plots yielded the most complex co-occurrence network with the highest modularity and clustering coefficient. Structural equation modeling revealed that plant species richness, net gains, and soil moisture played dominant roles in shaping bacterial community structure. Drought and bacterial community structure directly affected fungal community structure. Plant dominant species cover, common species

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cover, and bacterial diversity were the key drivers in regulating the microbial co-occurrence network complex.

Conclusion We conclude that soil bacterial and fungal communities differ in their responses to abiotic and biotic environmental changes, which may weaken the interspecies interactions among soil microorganisms.

Keywords Aboveground-belowground interactions · Illumina sequencing · Keystone species · Mediterranean-type climate · Plant cover · Precipitation amount

Introduction

Soil microorganisms provide essential ecosystem services (e.g., soil organic matter decomposition, nutrient cycling, aggregate stabilization, and mutualistic and pathogenic interactions with plants) that may be altered by multiple global change factors (Fahey et al. 2020). Soil bacteria and fungi have different resource utilization abilities and survival strategies (Yang et al. 2023), yet the dynamics of how groups of bacteria, fungi, or their co-occurrence relationships develop under environmental changes are poorly understood, due to complex interactions between community members (Mamet et al. 2019). The linkages within plant-microbial communities are even more uncertain when considering interactions between abiotic (e.g., drought) and biotic (e.g., nonnative invasion or native restoration) global change factors.

Ongoing global change is characterized by altered precipitation and drought patterns globally, resulting in more extreme climates, especially for Mediterranean ecosystems (IPCC 2021). Drought decreases soil moisture, which not only reduces plant productivity, but also a limiting factor for microbial growth, and long-term changes in community composition (Meng et al. 2019; Yang et al. 2021c). Many studies have investigated how different groups of the microbial community respond differently to drought, with fungi being generally more resistant, but less resilient than bacteria (de Vries et al. 2018; Ochoa-Hueso et al. 2018). This differential response can be explained by niche selection, whereby taxa that are drought tolerant increase in relative abundance compared to drought sensitive taxa (Evans et al. 2014),

and generally bacteria have a fast-growing and quick-response rate (Engelhardt et al. 2018). Moreover, the response pathways of bacteria and fungi to drought are generally divergent. For example, we have previously shown that in a temperate semi-arid grassland ecosystem, soil moisture was the best predictor for describing bacterial diversity and composition, but the fungal community was also affected by drought-driven changes in plant production allocation and soil nutrient status (Yang et al. 2021c). However, we know less about how species co-occurrence relationships within microbial communities respond to drought and its interaction with different plant species, such as plant invasion or vegetation restoration.

Invasion by exotic species also represents one of the most significant components of global change (Evans et al. 2001; Vitousek et al. 1997). California coastal prairies are one of the most diverse grassland types in North America but are threatened by exotic species invasions (Luong et al. 2021). In order to reduce plant mortality and improve native cover, vegetation restoration (i.e., planted native seedlings) is mandated for disturbed coastal prairies by the California Coastal Act of 1976. However, whether through plant invasion or vegetation restoration, changes in plant species and diversity can affect soil microbial diversity, composition, and species relationships (co-occurrence network) via multiple pathways (Fahey et al. 2020). For example, shoot community composition is a strong determinant of the quantity and quality of litter input to the soil and affect soil organic matter decomposition (Bloor and Bardgett 2012). Changes in root and rhizosphere community composition can also alter the quantity and composition of the root exudates (Zhu et al. 2020). All these changes will undoubtedly influence the diversity and structure of the soil microbial communities. To some extent, drought could modify the effects of plant community composition on soil microbial communities via altered abundance and physiology, and microorganisms might feedback on plant responses to drought (Yang et al. 2009). Altogether, the interactions of plant-microbial communities are likely to be modified by interactive effects of drought and different plant functional group composition, but our ability to predict these changes is still lacking.

Here, we explored the effects of drought and vegetation restoration on soil microbial diversity, composition, and co-occurrence relationships (network structure) in

a coastal prairie of California, which is a rare type of grassland that receives summer fog and winter rainfall (Baguskas et al. 2018). We performed soil bacterial and fungal diversity analyses based on Hiseq sequencing of 16S rRNA and ITS genes (Yang et al. 2024) to illustrate patterns after 3-year treatments. We hypothesized that: (1) soil bacterial diversity and composition are sensitive to drought stress, due to the fact that they are highly reliant on water for movement and substrate diffusion (Yang et al. 2021c); (2) soil fungal diversity and composition will be significantly affected by vegetation restoration, because hyphal growth is controlled by changes in rhizosphere microenvironments (Matsumoto et al. 2005); and (3) soil microbial composition/structure and co-occurrence networks are dependent more on plant richness than soil physical properties under drought and vegetation restoration.

Methods

Study area

The study was performed at the Younger Lagoon UC Natural Reserve near Santa Cruz, California, USA (36°95′N, 122°06′W, 15 m above sea level). The site is characterized as a Mediterranean climate, with dry, warm summers and cool, rainy winters, and a mean annual precipitation (1960–2016) of 752 mm that falls mostly from November through April (Loik et al. 2019). The mean annual temperature (1960–2020) is 14.2 °C. The plant community was dominated by exotic annual grasses (i.e., *Avena barbata* and *Bromus diandrus*) and forbs (i.e., *Medicago polymorpha*) at the start of the experiment in 2015.

Experimental design

The study site was established in July 2015, and was divided it into 20 plots each 4 m×4 m, with at least 3 m between plots. Plots were assigned to one of four treatments: ambient rainfall + exotic vegetation (C), 60% rainfall exclusion + exotic vegetation (D), ambient rainfall + planted native species (P), and 60% rainfall exclusion + planted native species (DP), with five replicates of each. The plots were trenched to 50 cm deep and lined with a 1 mm black plastic prior to the construction of the overhead shelter infrastructure.

The drought treatments were initiated in August 2015 using overhead rainfall interception shelters (Yahdjian and Sala 2002) to reduce each rainfall event passively by 60%. The percent reduction in precipitation was designed to achieve a statistically extreme deviation in annual precipitation representing a 1-in-100 year drought (as determined for the International Drought Experiment; Knapp et al. 2017). Precipitation was intercepted by V-shaped polycarbonate plastic troughs, held over research plots on a support frame. There are three parallel, horizontal bars at 0.6, 1, and 1.5 m height above the ground, with one each on the east and west edges of the plot, and one in the middle. Each horizontal bar was supported by three vertical posts. This design produces a "wedge-shaped" shelter with an upper and lower edge above the plant canopy (Fig. S1). The elasticity of the shelter is sufficient to withstand the strong winds of our research site. The troughs sit above the plant canopy and slope downward and empty into gutters on the lower end. The gutters empty into flexible drain pipes that channel rain away from the plots, and cause minimal aboveground meteorological impacts on plants (Loik et al. 2019).

Twelve plant species (Table S1) were chosen from a list of those that likely occurred historically at Younger Lagoon Reserve. Seeds were collected in August 2015 from local reference sites and were grown in the UCSC Greenhouses. In keeping with restoration practices for California coastal grasslands, planted plots were hand-weeded prior to planting to remove all standing biomass, and then planted with 5-month old seedlings in January 2016. The young plants were randomly assigned to planting positions on a grid for each plot. Exotic plants were removed from the plots by hand once early (January 2016) and once late (April 2016) in the growing season of the first year of the experiment, but not thereafter. Exotic plants were removed by hand from wooden planks suspended above the plots to minimize soil compaction (Luong et al. 2021).

Environmental conditions monitoring

Meteorological data were measured on the roof of a building <500 m from the field site (UT-30, Campbell Scientific Inc., Logan, UT, USA). Soil moisture and temperature (10 cm depth) sensors (METER Environmental GS1, Decagon, Pullman, WA, USA)

were employed to continuously monitor throughout the study period in a representative block.

Plant community composition and coverage

In mid-May 2018, we conducted a vegetation survey (plant species composition, percent aboveground cover) for each plot. Species richness was measured as the total number of species present in each plot. Net gains and losses in species richness were determined by comparison to April 2016. We randomly selected and permanently marked six locations within 25 cm × 100 cm quadrats and estimated cover of all species. We estimated absolute cover at the ground level and at multiple leaf canopy heights to ensure all species were represented. We classified all species into three different groups (dominant, common, and rare species). The abundance curve for aboveground cover vs. species rank across all the plots was used to define dominant species with relative abundance >5%, common species with relative abundance ranging from 1% to 5%, and rare species with relative abundance <1% (Yang et al. 2021a). This classification regime yielded 7 dominant species, 10 common species, and 7 rare species (Table S2).

Soil sampling and measurements

Soil samples (3 cm diameter) were collected from each plot after the vegetation survey. Each sample comprised five soil cores at a depth of 0–10 cm. We sampled the soils not directly attached to the roots, which we collected by removing several roots and gently shaking them to release the soils. The samples were placed in individual plastic bags and then immediately transferred to the laboratory using a 4°C portable picnic cooler. The soil samples were sieved through 2 mm mesh to remove roots and small animals and stored at −20°C for microbial DNA extraction as soon as possible.

At sampling time, volumetric soil moisture (SM-V) at 0–10 cm soil depth was measured using a time-domain reflectometry (TDR; TRIME-PICO32) probe (IMKO, Ettlingen, Germany) with single measurement mode and recorded by HD2 Hand-Measurement Device at three points in each plot. The soil temperatures were also measured three times using an electronic thermometer (GSP-8 digital thermometer, Jingchuang Electric Co., Ltd., Jiangsu, China). The

results of soil physical properties are shown in Supplementary Table S3.

DNA extraction, purification, and high-throughput sequencing

DNA was extracted directly from the soil samples using the Power Soil Extraction Kit (Mo Bio Laboratories, San Diego, CA, USA) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were measured using a Nanodrop 2000 spectrometer (Thermo Fisher Scientific, Wilmington, DE, USA). The soil DNA was stored at −20°C until use.

The primers 515F and 806R were used for amplifying the bacterial 16S V4 region (Caporaso et al. 2012), while the primers ITS5-1737F and ITS2-2043R were used for amplifying the fungal ITS1 region (White et al. 1990). For both bacteria and fungi, the polymerase chain reaction (PCR) was carried out with Phusion® High Fidelity PCR Master Mix (Biolabs Inc., CA, USA). The PCR was run three times to reduce PCR bias for each sample.

Three replicated PCR products for each sample were pooled before running amplicons on 2% agarose gels. Samples with a bright main strip between 400 and 450 bp were purified with the Qiagen Gel Extraction Kit (Qiagen, Dusseldorf, Germany). The resultant PCR products were analyzed by an Illumina HiSeq2500 (Illumina Inc., San Diego, CA, USA). The raw sequences were quality-filtered in Quantitative Insights in Microbial Ecology (QIIME) (Caporaso et al. 2010) and MOTHUR (Schloss et al. 2009). The trimmed sequences were grouped into the same operational taxonomic units (OTUs) at 97% similarity. The 16S V4 and ITS1 genes of the remaining reads were aligned to the SILVA and UNITE reference databases to determine their taxonomic classification level (Pruesse et al. 2007). To get the phylogenetic relationship of all OTUs, representative sequences were submitted to analysis with MUSCLE. The OTU matrices were rarefied to 92,866 reads per sample for bacteria, and 86,564 reads per sample for fungi. After quality filtering, a total of 1,857,320 bacterial sequences and 1,731,280 fungal sequences were obtained in this study, and clustered into 9239 and 1896 OTUs for bacterial and fungal communities, respectively. We used the output OTU data for

subsequent analyses of alpha and beta diversity of bacterial and fungal communities.

Data analysis

The Chao1 index (the estimated number of OTUs), which indicates microbial species richness, was calculated using the following equation:

$$\text{Chao1} = S_{obs} + \frac{n_1(n_1 - 1)}{2(n_2 + 1)}$$

where S_{obs} is the observed number of OTUs, n_1 is the number of OTUs with only one sequence, and n_2 is the number of OTUs with only two sequences. We generated non-metric multi-dimensional scaling (NMDS) and "Adonis" analyses based on the Bray-Curtis dissimilarity matrix to explore the significant differences in bacterial and fungal community structure across different treatments. Permutational multivariate analysis of variance (PERMANOVA) was performed to assess the effects of drought and vegetation restoration on the community composition of bacterial and fungal groups using the adonis function in the "vegan" package of R (Oksanen et al. 2017).

To examine the relationship and interactions between different microbial species, co-occurrence network analyses based on Spearman's rank was performed using abundance data of OTU level with the relative abundances greater than 0.05% (Xue et al. 2022), this resulted in 415 bacterial OTUs and 191 fungal OTUs. The co-occurrence patterns were explored based on strong (Spearman's $\rho > |0.8|$) and significant correlations ($P < 0.01$). We adjusted all P values for multiple testing using the false discovery rate (FDR) according to the Benjamini controlling procedure (Benjamini et al. 2006). The nodes with high degree were considered as key species in microbial co-occurrence networks (Banerjee et al. 2018a; Marasco et al. 2018). Networks were visualized in the R library igraph (Csárdi and Nepusz 2006).

A two-way analysis of variance (ANOVA) was conducted to determine the individual and interacting effects of the fixed factors precipitation (ambient precipitation or drought) and plant composition (natural vegetation composition or planting native species) on plant (e.g., richness, cover) and soil microbial (e.g., Chao1 index) diversity. To clarify the significant interaction effects of drought and vegetation restoration,

we conducted the simple effects analysis. All of the data met assumptions of normality and homogeneity of variances. Because we had only five field replicates for each treatment and the site had high spatial heterogeneity, we considered statistically significant difference at $P < 0.1$ in this study. Pearson correlation was calculated to depict the relationship between the plant characteristics, soil physical properties, and microbial diversity and co-occurrence network topological features. All analyses were performed in R version 4.0.2 (R Core Team 2020).

We used structural equation modeling (SEM) to evaluate the direct and indirect effects of drought and vegetation restoration on soil microbial community composition/structure. Our SEM was conducted with AMOS 23 (IBM SPSS, Inc.). In the SEM, drought was binary (0 = no and 1 = yes), and vegetation restoration was binary (0 = no and 1 = yes) (Liu et al. 2022). We quantified direct and indirect effects as standardized path coefficients. The red and blue arrows indicate positive and negative paths, the path widths are scaled proportionally to the path coefficient, and the numbers on the arrows are standardized path coefficients. The R^2 value represents the proportion of total variance explained for the dependent variable of interest. The performances of the SEM were evaluated using a combination of the Chi-square statistic (where $0 \leq \chi^2 \leq 2$ df and $P > 0.05$ indicates a good fitting model), the root mean square error of approximation (RMSEA; where RMSEA ≤ 0.08 indicates a good fitting model), and the Bentler's comparative fit index (CFI, where CFI > 0.95 indicates a good fitting model).

Results

Environmental conditions

The total rainfall was 496 mm (from 24 October 2017 to 23 June 2018) in the third experimental year, and the maximum daily precipitation was 84.6 mm in early-April (Fig. 1a). For the drought plots, the rainfall shelter excluded about 297 mm (estimated value) of precipitation. Drought significantly reduced soil moisture after each major rainfall event (Fig. 1b). Compared with the ambient precipitation plots, soil average temperature was warmer by as much as
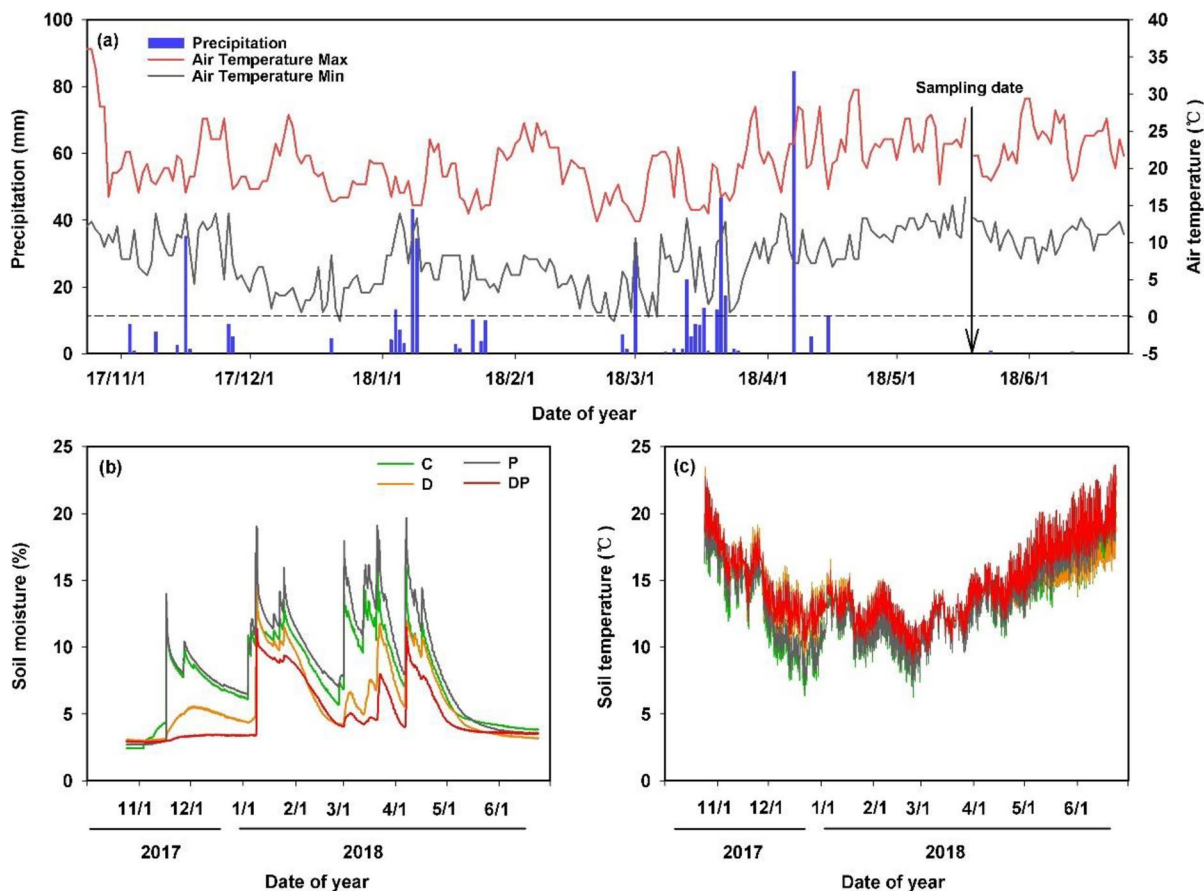
Fig. 1 (**a**) Daily precipitation and air temperature, (**b**) soil moisture, and (**c**) soil temperature under drought and vegetation restoration treatments from 24 October 2017 to 23 June 2018. C = control, D = drought, P = vegetation restoration (planted native species), and DP = drought and vegetation restoration (planted native species)

1.1 °C in the drought plots during the third experimental year (Fig. 1c).

Vegetation characteristics

The characteristics of the plant community are listed in Table 1. Significant interactive effects between drought and vegetation restoration were detected for plant species richness ($F = 15.69$; $P < 0.001$), net losses ($F = 3.28$; $P < 0.1$), dominant richness ($F = 8.1$; $P < 0.05$), common richness ($F = 9.68$; $P < 0.01$), dominant cover ($F = 3.68$; $P < 0.1$), and common cover ($F = 3.28$; $P < 0.1$). There was a significant increase in plant species net gains ($F = 427.78$; $P < 0.001$), rare richness ($F = 235.64$; $P < 0.001$), and rare cover ($F = 42.95$; $P < 0.001$) in planted native species treatments.

Diversity of soil microbial communities

Drought had a significant impact on the bacterial Chao1 index ($F = 7.01$; $P < 0.05$) with the lowest value in the DP plots of 6001 ± 30. Vegetation restoration had no effect on both bacterial ($F = 0.11$; $P > 0.1$) or fungal diversity ($F = 0.03$; $P > 0.1$; Fig. 2). Interactions between drought and vegetation restoration were significant for the fungal Chao1 index ($F = 3.11$; $P < 0.1$). In the planted plots, fungal Chao1 index was reduced 16.44% by drought ($F = 5.01$; $P < 0.1$; Fig. 2b).

Similar to the alpha diversity results, drought significantly altered the composition of the soil bacterial communities ($P < 0.05$; Fig. 3a and Table S4), but did not affect the composition of the fungal communities ($P > 0.05$; Fig. 3b and Table S4). In

Table 1 Vegetation characteristics under drought and vegetation restoration treatments

| Treatment	Richness	Net losses	Net gains	Dominant richness	Common richness	Rare richness	Dominant cover (%)	Common cover (%)	Rare cover (%)
C	9.4 ± 0.6	1.2 ± 0.5	2.8 ± 0.9	4.6 ± 0.2	4.4 ± 0.7	0.4 ± 0.2	70.1 ± 5.9	29.1 ± 6.3	0.8 ± 0.5
D	5.4 ± 0.8	1.2 ± 0.5	1.8 ± 0.7	2.8 ± 0.6	2.2 ± 0.2	0.4 ± 0.4	81.7 ± 2.3	17.1 ± 2.8	1.1 ± 1.1
P	19.0 ± 0.0	7.0 ± 0.0	14.0 ± 0.0	6.0 ± 0.0	9.0 ± 0.0	4.0 ± 0.0	70.5 ± 1.1	24.3 ± 1.2	5.2 ± 0.3
DP	19.0 ± 0.0	7.0 ± 0.0	14.0 ± 0.0	6.0 ± 0.0	9.0 ± 0.0	4.0 ± 0.0	69.9 ± 0.3	25.0 ± 0.2	5.2 ± 0.1
Significance									
D	15.69^{***}	$3.28†$	0.78^{ns}	8.10^*	9.68^{**}	0.00^{ns}	2.95^{ns}	2.60^{ns}	0.04^{ns}
P	527.69^{***}	128.21^{***}	427.78^{***}	52.90^{***}	259.92^{***}	235.64^{***}	$3.17†$	0.18^{ns}	42.95^{***}
D × P	15.69^{***}	$3.28†$	0.78^{ns}	8.10^*	9.68^{**}	0.00^{ns}	$3.68†$	$3.28†$	0.08^{ns}

C control, *D* drought, *P* vegetation restoration (planted native species), and *DP* drought and vegetation restoration (planted native species)

Data are means ± 1 SE ($n = 5$). *F* statistics for two-way ANOVA are provided. Levels of significance are indicated as – ns: $P > 0.1$, †: $P < 0.1$, *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$

Fig. 2 (**a**) Bacterial and (**b**) fungal Chao 1 diversity index under drought and vegetation restoration treatments. C = control, D = drought, P = vegetation restoration (planted native species), and NoP = exotic vegetation (non-planted native species). Levels of significance are indicated as – ns: $P > 0.1$, †: $P < 0.1$, *: $P < 0.05$. Data are means ± 1 SE ($n = 5$)
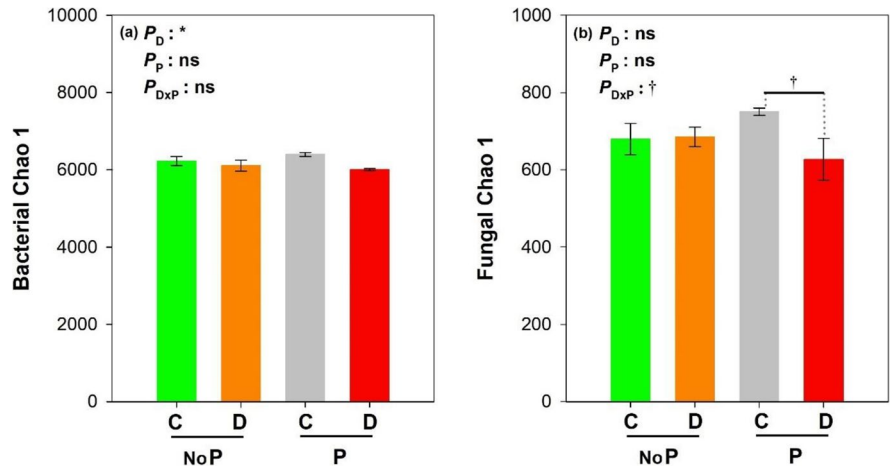
Fig. 3 (**a**) Bacterial and (**b**) fungal community composition indicated by non-metric multi-dimensional scaling (NMDS) under drought and vegetation restoration treatments. C = control, D = drought, P = vegetation restoration (planted native species), and DP = drought and vegetation restoration (planted native species)
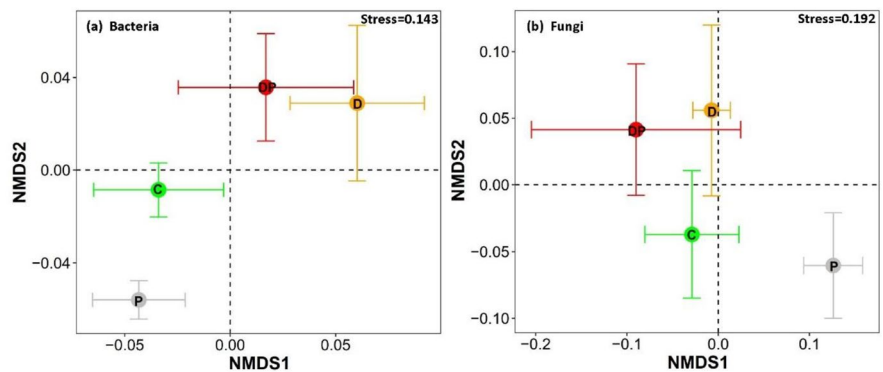
addition, PERMANOVA analysis showed differences in bacterial communities between C and P plots ($R^2 = 0.14$, $P = 0.08$), and between D and P plots ($R^2 = 0.23$, $P = 0.01$; Fig. 3a and Table S4). For

the fungal communities, the C ($R^2=0.18$, $P=0.01$), D ($R^2=0.18$, $P=0.01$), and DP ($R^2=0.21$, $P=0.01$) plots differed significantly from the P plots (Fig. 3b and Table S4).

Microbial co-occurrence networks

We constructed co-occurrence networks that included both bacterial and fungal OTUs for four treatments: C, D, P, and DP (Fig. 4). The modularity values of the four co-occurrence networks were 0.871, 0.898, 0.873, and 0.888, respectively; all values are >0.4 suggesting that these co-occurrence networks had a modular structure (Table 2). Multiple network topological features consistently showed that microbial co-occurrence patterns differed between drought and ambient precipitation networks, and between planted and non-planted networks (Fig. 4 and Table 2). Drought formed smaller networks with less edges (D: 3627; DP: 3409) than the ambient precipitation networks (C: 3871; P: 3607); meanwhile the planted networks contained less edges (links) than the non-planted networks, which created less intricate planted network patterns when compared with non-planted plots. Moreover, the clustering coefficient increased in the drought treatments (D: 0.887 & DP: 0.871 vs. C: 0.863 & P: 0.831) and decreased in the vegetation restoration treatments (P & DP vs. C & D). The C network was larger (i.e., more links) and the D network was more complex (i.e., higher clustering coefficient) among four co-occurrence networks.

Table 2 Topological features of four co-occurrence networks of soil microbial communities under drought and vegetation restoration treatments (Fig. 4)

Network topology	C	D	P	DP
Number of nodes	591	587	591	594
Number of edges	3871	3627	3607	3409
Number of positive correlations	2162	1989	1997	1801
Number of negative correlations	1709	1638	1610	1608
Modularity	0.871	0.898	0.873	0.888
Clustering coefficient	0.863	0.887	0.831	0.871
Average path length	8.377	6.135	9.381	12.155
Network diameter	22	20	24	28
Average degree	13.100	12.358	12.206	11.478

C control, *D* drought, *P* vegetation restoration (planted native species), and *DP* drought and vegetation restoration (planted native species)

The resulting DP network had an average degree of 11.5 that was lower than the C network with average degree of 13.1 (Table 2).

Keystone species were defined as taxa interacting with many other members (i.e., top 1% of interactions); such taxa were thought to play crucial roles in the overall soil microbial community. Keystone species in four co-occurrence networks were also altered by drought and vegetation restoration (Table 3). The C and DP networks hosted all keystone species belonging to the bacteria phyla (*Verrucomicrobia*,
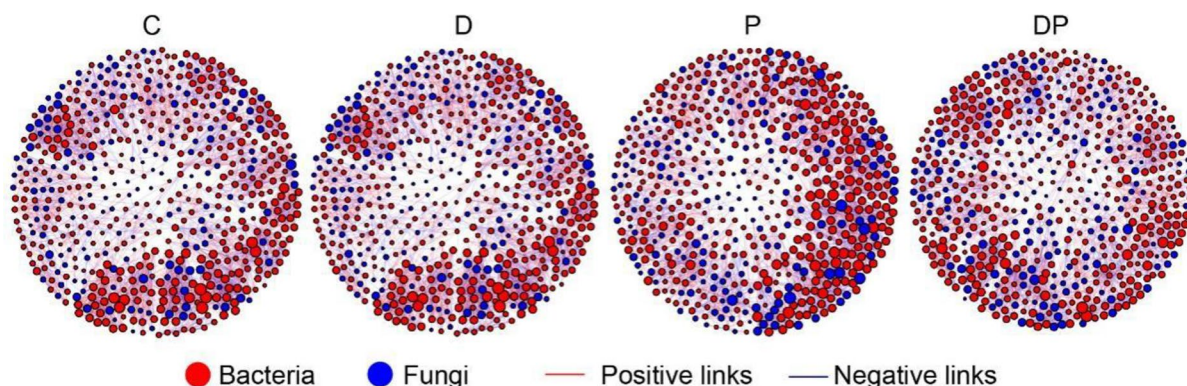
Fig. 4 Microbial network showing co-occurrences of bacterial (red) and fungal (blue) OTUs under drought and vegetation restoration treatments. Red and blue lines represent positive and negative relationships. See Table 2 for co-occurrences network properties. C=control, D=drought, P=vegetation restoration (planted native species), and DP=drought and vegetation restoration (planted native species)

Table 3 Network features and taxonomy of top five keystone taxa under drought and vegetation restoration treatments

Treatment	OTUid	Degree	Kingdom	Phylum
C	Botu2124	40	Bacteria	*Verrucomicrobia*
	Botu2586	40	Bacteria	*Proteobacteria*
	Botu361	40	Bacteria	*Chloroflexi*
	Botu26	37	Bacteria	*Cyanobacteria*
	Botu966	37	Bacteria	*Planctomycetes*
D	Botu275	34	Bacteria	*Chloroflexi*
	Botu884	32	Bacteria	*Planctomycetes*
	Botu112	31	Bacteria	*Chloroflexi*
	Fotu85	31	Fungi	*Ascomycota*
	Fotu108	31	Fungi	*Ascomycota*
P	Botu228	29	Bacteria	*Verrucomicrobia*
	Fotu130	29	Fungi	*Ascomycota*
	Fotu186	28	Fungi	*Ascomycota*
	Botu415	28	Bacteria	*Planctomycetes*
	Fotu270	28	Fungi	*Ascomycota*
DP	Botu786	30	Bacteria	*Proteobacteria*
	Botu1263	30	Bacteria	*Proteobacteria*
	Botu406	28	Bacteria	*Proteobacteria*
	Botu263	27	Bacteria	*Planctomycetes*
	Botu6608	27	Bacteria	*Acidobacteria*

C control, *D* drought, *P* vegetation restoration (planted native species), and *DP* drought and vegetation restoration (planted native species)

Proteobacteria, *Chloroflexi*, *Cyanobacteria*, *Planctomycetes*, and *Acidobacteria*). However, the D and P network analysis showed 2–3 fungal keystone species replaced the bacterial keystone species, and they all belonging to the phyla *Ascomycota*.

Relationships among plant, soil, and microbial properties

We used structural equation modeling (SEM) to examine the effects of soil physical properties and vegetation characteristics on bacterial and fungal community composition/structure. Our SEM showed that drought and vegetation restoration had no significant direct effects on bacterial community composition. There were direct negative effects of plant species net gains ($P < 0.001$) and soil moisture ($P < 0.1$), and a positive effect of plant richness ($P < 0.01$) on soil bacterial community composition/structure. Meanwhile, drought showed significant direct and indirect effects by bacterial community composition
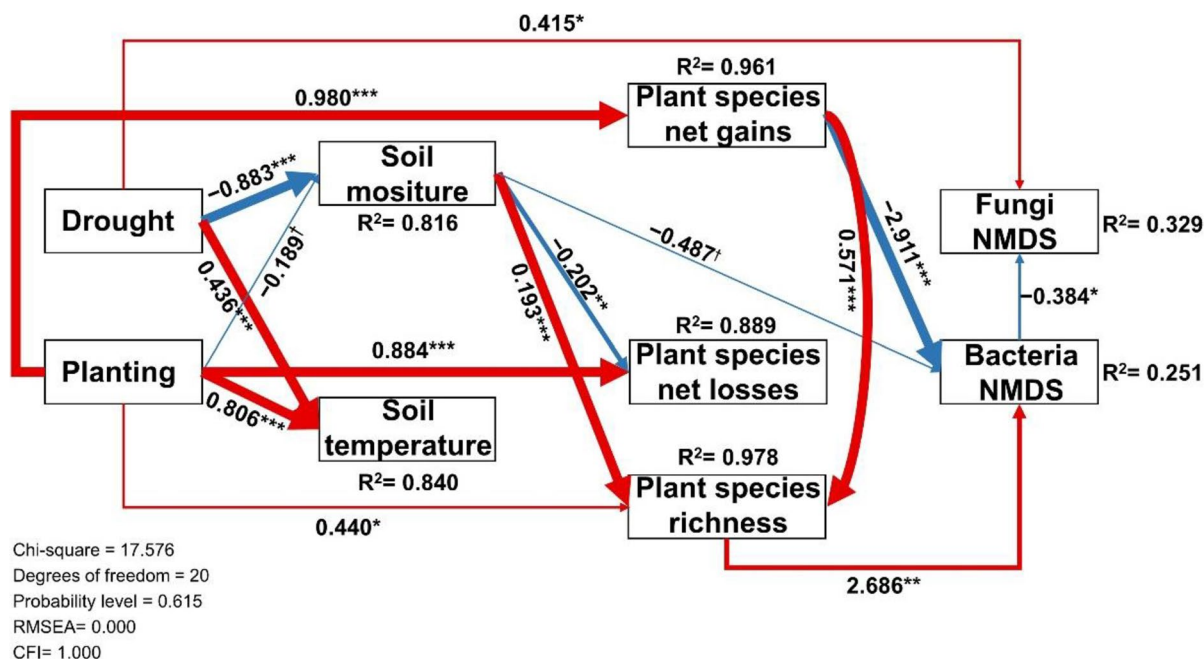
on fungal community composition (both $P < 0.05$). Notably, both drought and vegetation restoration had direct positive effects on soil temperature ($P < 0.001$), which did not affect soil microbial community composition (Fig. 5).

Pearson correlation analysis revealed that dominant plant species cover, common species cover, and bacterial diversity were the dominant predictors, which had significant correlations with the clustering coefficient (complexity) of microbial co-occurrence networks in response to drought and vegetation restoration (all $P < 0.05$). Plant dominant species cover was negatively associated with the clustering coefficient; however, plant common species cover and bacterial diversity had positive relationships with the clustering coefficient. By contrast, neither network nodes nor edges (co-occurrence network size) were correlated with any of the plant and soil factors ($P > 0.05$; Fig. 6).

Discussion

Effects of drought and vegetation restoration on microbial diversity and composition

Consistent with our first hypothesis, we found that drought significantly reduced soil bacterial diversity and altered bacterial community composition compared with soils receiving ambient precipitation, especially for plots on which native species had been planted (Figs. 2a and 3a). Sheik et al. (2011) reported that effects of changes in precipitation on bacterial communities could be associated directly with the close association among soil moisture, temperature, and bacterial diversity and community structure. For example, interannual variability in bacterial communities in Tibetan Plateau grassland soils were correlated significantly with fluctuations in MAP and MAT (Shi et al. 2020). Our previous results demonstrated that ambient precipitation had the highest bacterial diversity, as decreases of 30% and 50% precipitation both profoundly reduced bacterial alpha diversity, likely due to the low soil moisture that likely greatly constrained bacterial growth (Yang et al. 2021c). In other studies bacteria were more strongly driven by instantaneous soil moisture (Hawkes et al. 2011). Additionally, drought could also affect bacteria due to certain side effects, such as increases in

Fig. 5 Structural equation modeling depicting the effects of drought and vegetation restoration on the microbial community composition/structure (bacterial and fungal communities, represented by the first axis of their NMDS). Significant positive and negative paths are shown as red and blue arrows, respectively. The width of the paths (arrows) is proportional to the strength of the path coefficients. The path coefficients are the numbers on the arrows, and R^2 indicates the proportion of variance explained. Levels of significance are indicated as – †: $P < 0.1$, *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$

soil temperature. Between direct and indirect mechanisms, the former usually played a more important role (Yang et al. 2021b).

By contrast to the bacterial patterns, the diversity and composition of fungi were relatively stable and not affected by drought or vegetation restoration. Drought slightly reduced fungal Chao1 diversity and altered fungal composition in vegetation restoration plots (Fig. 2b and Table S4). Our finding differs from a study in the southeast US that indicated that changes in plant functional groups and drought together had less of an effect on fungal richness and composition than either treatment alone (Fahey et al. 2020). Vegetation restoration could result in more competition between exotic and native species, so plant richness and biomass might either decrease or increase, while the plant communities would be vulnerable under drought stress (Table 1). As the decomposers and plant symbionts, soil fungal species interact with plant health (Paungfoo-Lonhienne et al. 2015). Changes in plant richness, productivity, and carbon allocation induced by vegetation restoration

plus drought may potentially influence the diversity of fungal communities, especially if given a longer period of treatment.

The shift of microbial co-occurrence networks in response to drought and vegetation restoration

Previous studies using microbial co-occurrence network analysis have often assessed archaeal, bacterial, or fungal communities (Sun et al. 2017; Wang et al. 2023), but the roles of their interactions among these groups have been underrepresented in microbial co-occurrence network analyses (Banerjee et al. 2018b). In microbial ecology, the positive interactions could be mutualistic and/or cooperative for communities, whereas the negative interactions have been attributed to amensalism and/or competition (Faust and Raes 2012; Yang et al. 2022). The numbers of positive links in all co-occurrence networks were greater than the negative links (Table 2), indicating more interspecies cooperation than competition in our studied grassland. As indicated by the interactions

Fig. 6 Pearson correlations among the vegetation characteristics, soil physical properties, and soil microbial diversity and co-occurrences network. Levels of significance are indicated as – *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$
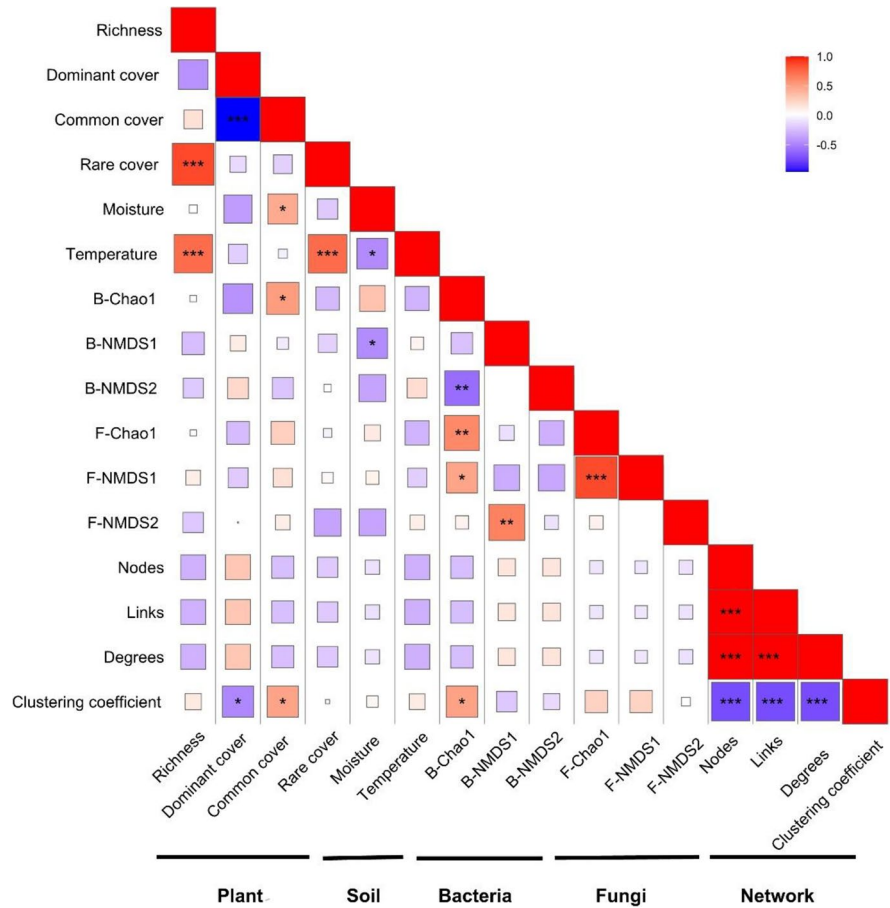
among interspecies, a larger network size is accompanied by a greater network connectivity (Fan et al. 2018). The control network formed the largest bacterial-fungal network size, because it possessed more edges and higher average degree than the other networks. A possible explanation is that the lifestyles of soil microorganisms are more universal and adapt to more ecological niches along with environmental changes; however, different microbial species possibly result in stronger competition for similar niches under the drought or vegetation restoration treatments (Wan et al. 2020). Additionally, microorganisms can also form complex association networks, it has been shown that complex networks are more robust to environmental perturbations than simple networks (Santolini and Barabási 2018). We demonstrated that the co-occurrence network structure of drought plots had a more complex (higher modularity and clustering coefficient) interspecies interactions than the other three treatments, its co-occurrence network

had typical small-world features. This was consistent with those reported by Wang et al. (2022), who found that microbial co-occurrence networks become more complex as precipitation decreases or increases. de Vries et al. (2018) have shown that some microbial taxa were sensitive to changes in precipitation and soil water availability, while this sensitivity produced an increase in microbial co-occurrence network complexity to adverse water stress.

Keystone species are often strongly connected taxa that exert a substantial influence on other members of microbial communities and play a critical role in maintaining ecosystem function and stability (Faust and Raes 2012; Wan et al. 2020; Wang et al. 2022). The phylum *Planctomycetes* was identified as the most prominent keystone taxon detected in the four treatment co-occurrence networks, suggesting this group may thrive in the studied grassland soils. Notably, it is also prevalent in European grasslands (Karimi et al. 2019). We found that some keystone

species that belonged to the *Verrucomicrobia* OTUs disappeared in the drought (D & DP) networks. Previous studies have suggested that members of this phylum are important in soil carbon cycling, because they can utilize multiple carbon compounds, such as cellulose, xylan, and pectin (Dunfield et al. 2007; Gu et al. 2019; Pol et al. 2007). The *Chloroflexi* OTUs were identified in the treatments without vegetation restoration. Wang et al. (2018) showed that they were acidogenic bacteria whose presence was involved in the anaerobic digestion process. *Proteobacteria*, known as copiotrophic *r*-strategists, have a positive effect on plant health that is well documented (Fernández-González et al. 2020). These species play vital roles in energy flow and nutrient cycling, which might be active utilizers of fresh photosynthate and identified as the most stress-tolerant microorganisms (Bastida et al. 2016; Cheng et al. 2020; Wang et al. 2019) under DP treatment. In the present study, the majority of keystone species came from bacterial communities whereas *Ascomycota* were important in the drought and planted networks. *Ascomycota* are particularly dominant fungi in soils (Wu et al. 2021), because of their saprotrophic activity in degrading refractory organic carbon (Hu et al. 2020; Xiao et al. 2017). Changes in the soil microenvironment induced by drought or vegetation restoration may enhance the potential carbon use efficiency of fungi.

The biotic factors determining microbial community composition and co-occurrence networks

The effects of the drought and vegetation restoration treatments on bacterial composition were offset by the indirect effects through alterations to plant diversity (richness and net gains) and soil moisture (Fig. 5). It has been increasingly recognized that changes in the diversity and structure of plant communities occur in parallel to changes in the structure of soil bacterial communities (Liu et al. 2021). Our data indicated that plant communities were the most important drivers of soil bacterial NMDS1 compared with soil moisture, which suggested that bacterial community composition had a higher demand for carbon than water. Exotic plants can accelerate ecosystem carbon loss via photodegradation (Austin and Vivanco 2006), and the negative effect of exotic plants was also reflected in the reduction of soil dissolved organic carbon (Pérez Castro et al. 2019),

which may have been due to carbon limitation of soil microorganisms under nonnative invasion. On the other hand, drought can decrease soil carbon input by reducing plant biomass and cover (Yang et al. 2021c), this can also exacerbate the requirement for photosynthetic carbon by bacterial communities. Interestingly, by contrast to bacteria, we found fungal composition was directly influenced by bacterial diversity, but not plant diversity. The negative correlation between soil bacterial and fungal communities implies a competitive relationship between them by drought and vegetation restoration. Different microbial groups would go through competition to acquire similar resources in order to adapt to environmental changes (Li et al. 2020). Additionally, we further investigated whether soil bacterial diversity exhibited the strongest positive effect in regulating microbial co-occurrence network complexity (Fig. 6), indicating that soil bacterial communities dominated the microbial co-occurrence network structure in this study. However, we still know much less about the interactions among bacterial and/or fungal keystone species in determining microbial co-occurrence network complexity and stability. Metagenomic and meta-transcriptome sequencing techniques can be applied to reveal the ecological functions of soil microorganisms in our future studies, which may contribute to elucidate the mechanisms of plant-microbial interactions under the background of global change.

Conclusions

We revealed that bacterial diversity and composition were sensitive to three years of 60% reduction in precipitation and plant community composition, whereas fungal communities were not. Perhaps the fungi require longer than three years for changes to play out. Our results also showed that plant species richness and soil moisture were the most important factors affecting soil microbial diversity under drought or restoration planting. A larger microbial co-occurrence network with more connections appeared in soils under invasive plant communities (control plots), while both drought and vegetation restoration altered the microbial co-occurrence networks. Although microbial co-occurrence networks were dominated by bacterial communities, drought or vegetation restoration also led to the emergence of fungal

keystone species. This further highlighted the importance of interkingdom associations in soil microbial co-occurrence networks.

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Data availability   The DNA sequences in this study have been deposited in SRA of National Center for Biotechnology Information (NCBI) database under accession No. SRP401226.

Declarations

Competing interests   The authors have no relevant financial or non-financial interests to disclose.

References

Austin AT, Vivanco L (2006) Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. Nature 442:555–558. https://doi.org/10.1038/nature05038

Baguskas SA, Clemesha RES, Loik ME (2018) Coastal low cloudiness and fog enhance crop water use efficiency in a California agricultural system. Agric For Meteorol 252:109–120. https://doi.org/10.1016/j.agrformet.2018.01.015

Banerjee S, Schlaeppi K, van der Heijden MGA (2018a) Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol 16:567–576. https://doi.org/10.1038/s41579-018-0024-1

Banerjee S, Thrall PH, Bissett A et al (2018b) Linking microbial co-occurrences to soil ecological processes across a woodland-grassland ecotone. Ecol Evol 8:8217–8230. https://doi.org/10.1002/ece3.4346

Bastida F, Torres IF, Moreno JL et al (2016) The active microbial diversity drives ecosystem multifunctionality and is physiologically related to carbon availability in Mediterranean semi-arid soils. Mol Ecol 25:4660–4673. https://doi.org/10.1111/mec.13783

Benjamini Y, Krieger AM, Yekutieli D (2006) Adaptive linear step-up procedures that control the false discovery rate. Biometrika 93:491–507. https://doi.org/10.1093/biomet/93.3.491

Bloor JMG, Bardgett RD (2012) Stability of above-ground and below-ground processes to extreme drought in model grassland ecosystems: interactions with plant species diversity and soil nitrogen availability. Perspect Plant Ecol Evol Syst 14:193–204. https://doi.org/10.1016/j.ppees.2011.12.001

Caporaso JG, Kuczynski J, Stombaugh J et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335. https://doi.org/10.1038/nmeth.f.303

Caporaso JG, Lauber CL, Walters WA et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624. https://doi.org/10.1038/ismej.2012.8

Cheng C, Li Y, Long M et al (2020) Moss biocrusts buffer the negative effects of karst rocky desertification on soil properties and soil microbial richness. Plant Soil 475:153–168. https://doi.org/10.1007/s11104-020-04602-4

Csárdi G, Nepusz T (2006) The igraph software package for complex network research. InterJ Complex Syst 1695:1–9

de Vries FT, Griffiths RI, Bailey M et al (2018) Soil bacterial networks are less stable under drought than fungal networks. Nat Commun 9:3033. https://doi.org/10.1007/s11104-020-04602-4

Dunfield PF, Yuryev A, Senin P et al (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature 450:879–882. https://doi.org/10.1038/nature06411

Engelhardt IC, Welty A, Blazewicz SJ et al (2018) Depth matters: effects of precipitation regime on soil microbial activity upon rewetting of a plant-soil

system. ISME J 12:1061–1071. https://doi.org/10.1038/s41396-018-0079-z

Evans RD, Rimer R, Sperry L, Belnap J (2001) Exotic plant invasion alters nitrogen dynamics in an arid grassland. Ecol Appl 11:1301–1310. https://doi.org/10.1890/1051-0761(2001)011[1301:EPIAND]2.0.CO;2

Evans SE, Wallenstein MD, Burke IC (2014) Is bacterial moisture niche a good predictor of shifts in community composition under long-term drought? Ecology 85:110–122. https://doi.org/10.1890/13-0500.1

Fahey C, Koyama A, Antunes PM et al (2020) Plant communities mediate the interactive effects of invasion and drought on soil microbial communities. ISME J 14:1396–1409. https://doi.org/10.1038/s41396-020-0614-6

Fan K, Weisenhorn P, Gilbert JA, Chu H (2018) Wheat rhizosphere harbors a less complex and more stable microbial co-occurrence pattern than bulk soil. Soil Biol Biochem 125:251–260. https://doi.org/10.1016/j.soilbio.2018.07.022

Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10:538. https://doi.org/10.1038/nrmicro2832

Fernández-González AJ, Cardoni M, Cabanás CG-L et al (2020) Linking belowground microbial network changes to different tolerance level towards Verticillium wilt of olive. Microbiome 8:11. https://doi.org/10.1186/s40168-020-0787-2

Gu S, Hu Q, Cheng Y et al (2019) Application of organic fertilizer improves microbial community diversity and alters microbial network structure in tea (*Camellia sinensis*) plantation soils. Soil Tillage Res 195:104356. https://doi.org/10.1016/j.still.2019.104356

Hawkes CV, Kivlin SN, Rocca JD et al (2011) Fungal community responses to precipitation. Glob Chang Biol 17:1637–1645. https://doi.org/10.1111/j.1365-2486.2010.02327.x

Hu X, Liu J, Yu Z et al (2020) Continuous cropping of soybean induced a more fluctuating fungal network and intensive pathogenic fungal interactions in a Mollisol of Northeast China. Soil Sci Soc Am J 84:775–783. https://doi.org/10.1002/saj2.20069

IPCC (2021) Climate Change 2021: the Physical Science Basis Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change

Karimi B, Dequiedt S, Terrat S et al (2019) Biogeography of soil bacterial networks along a gradient of cropping intensity. Sci Rep 9:3812. https://doi.org/10.1038/s41598-019-40422-y

Knapp AK, Avolio ML, Beier C et al (2017) Pushing precipitation to the extremes in distributed experiments: recommendations for simulating wet and dry years. Glob Chang Biol 23:1774–1782. https://doi.org/10.1111/gcb.13504

Li J, Li C, Kou Y et al (2020) Distinct mechanisms shape soil bacterial and fungal co-occurrence networks in a mountain ecosystem. FEMS Microbiol Ecol 96:fiaa030. https://doi.org/10.1093/femsec/fiaa030

Liu W, Liu L, Yang X et al (2021) Long-term nitrogen input alters plant and soil bacterial, but not fungal beta diversity in a semiarid grassland. Glob Chang Biol 27:3939–3950. https://doi.org/10.1111/gcb.15681

Liu J, Isbell F, Ma Q et al (2022) Overgrazing, not haying, decreases grassland topsoil organic carbon by decreasing plant species richness along an aridity gradient in northern China. Agric Ecosyst Environ 332:107935. https://doi.org/10.1016/j.agee.2022.107935

Loik ME, Lesage JC, Brown TM, Hastings DO (2019) Drought-net rainfall shelters did not cause nondrought effects on photosynthesis for California central coast plants. Ecohydrology 12:e2138. https://doi.org/10.1002/eco.2138

Luong JC, Holl KD, Loik ME (2021) Leaf traits and phylogeny explain plant survival and community dynamics in response to extreme drought in a restored coastal grassland. J Appl Ecol 58:1670–1680. https://doi.org/10.1111/1365-2664.13909

Mamet SD, Redlick E, Brabant M et al (2019) Structural equation modeling of a winnowed soil microbiome identifies how invasive plants re-structure microbial networks. ISME J 13:1988–1996. https://doi.org/10.1038/s41396-019-0407-y

Marasco R, Mosqueira MJ, Fusi M et al (2018) Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. Microbiome 6:215. https://doi.org/10.1038/s41396-019-0407-y

Matsumoto LS, Martines AM, Avanzi MA et al (2005) Interactions among functional groups in the cycling of, carbon, nitrogen and phosphorus in the rhizosphere of three successional species of tropical woody trees. Appl Soil Ecol 28:57–65. https://doi.org/10.1016/j.apsoil.2004.06.00

Meng B, Shi B, Zhong S et al (2019) Drought sensitivity of aboveground productivity in *Leymus chinensis* meadow steppe depends on drought timing. Oecologia 191:685–696. https://doi.org/10.1007/s00442-019-04506-w

Ochoa-Hueso R, Collins SL, Delgado-Baquerizo M et al (2018) Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. Glob Chang Biol 24:2818–2827. https://doi.org/10.1111/gcb.14113

Oksanen J, Blanchet FG, Friendly M et al (2017) Vegan: community ecology package. http://CRAN.R-project.org/packagevegan

Paungfoo-Lonhienne C, Yeoh YK, Kasinadhuni NRP et al (2015) Nitrogen fertilizer dose alters fungal communities in sugarcane soil and rhizosphere. Sci Rep 5:8678. https://doi.org/10.1038/srep08678

Pérez Castro S, Cleland EE, Wagner R et al (2019) Soil microbial responses to drought and exotic plants shift carbon metabolism. ISME J 13:1776–1787. https://doi.org/10.1038/s41396-019-0389-9

Pol A, Heijmans K, Harhangi HR et al (2007) Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature 450:874–878. https://doi.org/10.1038/nature06222

Pruesse E, Quast C, Knittel K et al (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188–7196. https://doi.org/10.1093/nar/gkm864

R Core Team (2020). R: A language and environment for statistical. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Santolini M, Barabási A (2018) Predicting perturbation patterns from the topology of biological networks. Proc Natl Acad Sci the U S A 115:E6375–E6383. https://doi.org/10.1101/349324

Schloss PD, Westcott SL, Ryabin T et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/aem.01541-09

Sheik CS, Beasley WH, Elshahed MS et al (2011) Effect of warming and drought on grassland microbial communities. ISME J 5:1692–1700. https://doi.org/10.1038/ismej.2011.32

Shi Y, Zhang K, Li Q et al (2020) Interannual climate variability and altered precipitation influence the soil microbial community structure in a Tibetan plateau grassland. Sci Total Environ 714:136794. https://doi.org/10.1016/j.scitotenv.2020.136794

Sun S, Li S, Avera BN et al (2017) Soil bacterial and fungal communities show distinct recovery patterns during forest ecosystem restoration. Appl Environ Microbiol 83:e00966–e00917. https://doi.org/10.1128/aem.00966-17

Vitousek PM, D'Antonio CM, Loope LL et al (1997) Introduced species: a significant component of human-caused global change. N Z J Ecol 21:1–16

Wan X, Gao Q, Zhao J et al (2020) Biogeographic patterns of microbial association networks in paddy soil within eastern China. Soil Biol Biochem 142:107696. https://doi.org/10.1016/j.soilbio.2019.107696

Wang P, Wang HT, Qiu YQ et al (2018) Microbial characteristics in anaerobic digestion process of food waste for methane production-a review. Bioresour Technol 248:29–36 https://doi.org/10.1016/j.biortech.2017.06.152

Wang M, Chen S, Chen L, Wang D (2019) Responses of soil microbial communities and their network interactions to saline-alkaline stress in cd-contaminated soils. Environ Pollut 252:1609–1621. https://doi.org/10.1016/j.envpol.2019.06.082

Wang J, Wang C, Zhang J et al (2022) Decreased precipitation reduced the complexity and stability of bacterial co-occurrence patterns in a semiarid grassland. Front Microbiol 13:1031496. https://doi.org/10.3389/fmicb.2022.1031496

Wang J, Wang C, Hu M et al (2023) Bacterial co-occurrence patterns are more complex but less stable than archaea in enhanced oil recovery applied oil reservoirs. Process Biochem 130:40–49. https://doi.org/10.1016/j.procbio.2023.04.003

White TJ, Bruns TD, Lee SB, Taylor JW (1990) PCR protocols: a guide to methods and applications. In: Innis MA, Gelfand DH, Sninsky JJ (eds) PCR protocols: a guide to methods and applications. Academic Press, Cambridge, pp 315–322

Wu X, Yang J, Ruan H et al (2021) The diversity and co-occurrence network of soil bacterial and fungal communities and their implications for a new indicator of grassland degradation. Ecol Indic 129:107989 https://doi.org/10.1016/j.ecolind.2021.107989

Xiao X, Liang Y, Zhou S et al (2017) Fungal community reveals less dispersal limitation and potentially more connected network than that of bacteria in bamboo forest soils. Mol Ecol 27:550–563. https://doi.org/10.1111/mec.14428

Xue R, Wang C, Zhao L et al (2022) Agricultural intensification weakens the soil health index and stability of microbial networks. Agric Ecosyst Environ 339:108118. https://doi.org/10.1016/j.agee.2022.108118

Yahdjian L, Sala OE (2002) A rainout shelter design for intercepting different amounts of rainfall. Oecologia 133:95–101. https://doi.org/10.1007/s00442-002-1024-3a

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4. https://doi.org/10.1016/j.tplants.2008.10.004

Yang X, Mariotte P, Guo J et al (2021a) Suppression of arbuscular mycorrhizal fungi decreases the temporal stability of community productivity under elevated temperature and nitrogen addition in a temperate meadow. Sci Total Environ 762:143137. https://doi.org/10.1016/j.scitotenv.2020.143137

Yang X, Yuan M, Guo J et al (2021b) Suppression of arbuscular mycorrhizal fungi aggravates the negative interactive effects of warming and nitrogen addition on soil bacterial and fungal diversity and community composition. Appl Environ Microbiol 87:e01523–e01521. https://doi.org/10.1128/AEM.01523-21

Yang X, Zhu K, Loik ME, Sun W (2021c) Differential responses of soil bacteria and fungi to altered precipitation in a meadow steppe. Geoderma 384:114812. https://doi.org/10.1016/j.geoderma.2020.114812

Yang Y, Luo W, Xu J et al (2022) Fallow land enhances carbon sequestration in glomalin and soil aggregates through regulating diversity and network complexity of arbuscular mycorrhizal fungi under climate change in relatively high-latitude regions. Front Microbiol 13:930622. https://doi.org/10.3389/fmicb.2022.930622

Yang X, He P, Zhang Z et al (2023) Straw return, rather than warming, alleviates microbial phosphorus limitation in a cultivated Mollisol. Appl Soil Ecol 186:104821 https://doi.org/10.1016/j.apsoil.2023.104821

Yang X, Song W, Yang X et al (2024) Microbial network structure, not plant and microbial community diversity, regulates multifunctionality under increased precipitation in a cold steppe. Front Microbiol 14:1349747. https://doi.org/10.3389/fmicb.2023.1349747

Zhu X, Li X, Xing F et al (2020) Interaction between root exudates of the poisonous plant *Stellera chamaejasme* L. and arbuscular mycorrhizal fungi on the growth of *Leymus chinensis* (Trin.) Tzvel. Microorganisms 8:364. https://doi.org/10.3390/microorganisms8030364