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Variations in diversity, composition, and species interactions of soil microbial community in response to increased N deposition and precipitation intensity in a temperate grassland

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Abstract

Background Global climate change has resulted in precipitation regimes exhibiting an increasing trend in rainfall intensity but a reduction in frequency. In addition, nitrogen (N) deposition occurs simultaneously in arid and semi-arid regions. Microbial biomass, diversity, composition, and species interactions are key determinants of ecological functions. We examined the effects of changes in precipitation intensity and N addition on the soil bacterial and fungal communities in a semi-arid grassland in Inner Mongolia, China.

Methods The microbial biomass (bacterial PLFAs and fungal PLFAs) was determined through phospholipid fatty acid (PLFA) analysis, and microbial diversity (Shannon index and evenness index) was determined with high-throughput sequencing (16S and ITS). Species interactions were determined using a molecular ecological network analysis. The relationships between microbial community (bacterial community and fungal community) and environmental variables were examined by Mantel tests.

Results We found that N addition decreased fungal PLFA under moderate, high, and extreme precipitation intensity treatments and increased fungal community complexity under the high precipitation intensity treatment. Furthermore, N addition increased bacterial diversity under moderate and high precipitation intensity treatments. N addition caused greater environmental stress to the fungal community, which was dominated by deterministic processes.

Conclusions The effects of N deposition on soil bacterial and fungal communities were altered by precipitation intensity. The changes in soil bacterial and fungal communities were different, implying that composition and functional traits adapt differently to projected global changes at a regional scale.

Keywords Microbial community composition, Phospholipid fatty acid, High-throughput sequencing, Precipitation intensity, N deposition

Introduction

Soil microbial community plays an important role in ecosystem responses to global climate change through their effects on nutrient cycling. Microbial biomass, diversity, composition, and species interactions are key determinants of ecological functions (Brussaard 1997; Deng 2012; Philippot et al. 2013; Hong et al. 2022; Romdhane

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et al. 2022). These factors are influenced by increasing N deposition and variable precipitation, especially in semi-arid grasslands, where N and water availability are limiting factors (Stursova et al. 2006). Over the past decade, numerous manipulation experiments and global meta-analyses have been conducted to investigate the responses of soil microbial characteristics to N addition (Nelson et al. 2016; Yang et al. 2021). A growing body of evidence suggests that N deposition has profound adverse effects on soil microbes and their functions (Zhang et al. 2018; Hu et al. 2022). For instance, long-term N input decreases microbial biomass due to direct inhibition and reduced investment in microbes (Janssens et al. 2010; Treseder et al. 2008). Microbial taxa within ecological networks can be classified into different ecological units based on their connectivity other taxa (Toju et al. 2018). Recent research reports that N deposition destabilizes the microbial networks (Sun et al. 2023). The response of microbial diversity or community structure to N deposition may depend on microbial physiology and adaptation to local climatic fluctuations (Meena et al. 2022; Palit et al. 2022). Hence, it is essential to better understand the effects of N deposition on soil microbial communities under global precipitation change.

Together with increasing N deposition, global changes are also characterized by changes in precipitation patterns. Rainfall is predicted to shift from small and frequent to stronger and fewer events without change in total amount as a result of climate change (Easterling et al. 2000; IPCC 2013; Pachauri and Meyer 2014). Many studies have concentrated on the influences of precipitation pulses (Yang et al. 2021), rewetting (Yu et al. 2023), droughts (Neilson et al. 2017), and shifts in amounts of precipitation (Zhang et al. 2021) on soil microbial communities. Nevertheless, much less is known about on the effects of changes in precipitation regime on ecosystem structure and functioning (especially in ecosystems such as semi-arid steppes). Changes in precipitation can affect atmospheric wet N deposition, N leaching, soil pH, and plant photosynthesis (Chomel et al. 2019). Therefore, the coupling effects of precipitation change and N deposition on soil microbial communities are more complex than the effects of either factor alone (Shi et al. 2018). For example, increased precipitation exacerbates the negative effects of N deposition on soil microbial communities (Shi et al. 2018) and stimulates the responses of copiotrophic microbes to N deposition (Fierer et al. 2007; Ramirez et al. 2012). During the past few decades, grasslands in arid and semi-arid regions have been subject to variations in precipitation regimes that have shifted toward fewer but more intense rainfall events, without changing the total precipitation amount (Yan et al. 2014). Changes in the precipitation frequency can lead to substantial changes in belowground ecological processes (Chen et al. 2019; Cui et al. 2022). However, the effects of precipitation frequency on the responses of soil microbial communities to N deposition remain unclear.

In this study, we conducted an 8-year field manipulation experiment in a typical semi-arid steppe on the Mongolian Plateau in China to evaluate the coupling effects of N deposition and precipitation frequency shifts on soil microbial communities. The study was part of a long-term and multiple-level precipitation intensity and N addition experiment established in 2012 and located in a typical semi-arid steppe on the Mongolian Plateau. Precipitation in semi-arid northern China has been increasing delivered by highly variable heavy events (Xu et al. 2015). Such alterations in precipitation and N deposition may have particularly significant consequences in vulnerable and water-limited grasslands. Specifically, we evaluated shifts in bacterial and fungal communities based on metrics such as biomass, alpha diversity, and taxa co-occurrence patterns under gradient precipitation frequencies with and without N addition. We hypothesized that: (1) increased N would reduce microbial biomass (including bacterial and fungal biomass) and alpha diversity; (2) bacterial and fungal communities and their associations would respond differently to N addition under varied precipitation intensities; and (3) high and extreme precipitation intensity would exacerbate the adverse effects of N addition.

Materials and methods

Study site

The study was conducted at the Duolun Restoration Ecology Station of the Institute of Botany, Chinese Academy of Sciences (116° 17′ E and 42° 02′ N, elevation 1324 m) (Fig. 1a). The vegetation in this region is a typical steppe community without any disturbances. The experimental site is in a typical temperate zone characterized by a semi-arid continental monsoon climate. The mean annual temperature is 2.1 °C, with mean monthly temperature ranging from − 17.5 °C in January to 18.9 °C in July. The mean annual precipitation is 379.4 mm, with approximately 80% occurring from June to September. The soil of the experimental site was sandy loam with 62.75 ± 0.04% sand, 20.30 ± 0.01% silt, and 16.95 ± 0.01% clay (Xu et al. 2015), classified as Calcisorthic Aridisol (U.S. Soil Taxonomy classification). This temperate steppe is dominated by perennials, including *Stipa krylovii*, *Artemisia frigida, Potentilla acaulis, Cleistogenes squarrosa, Allium bidentatum,* and *Agropyron cristatum.*

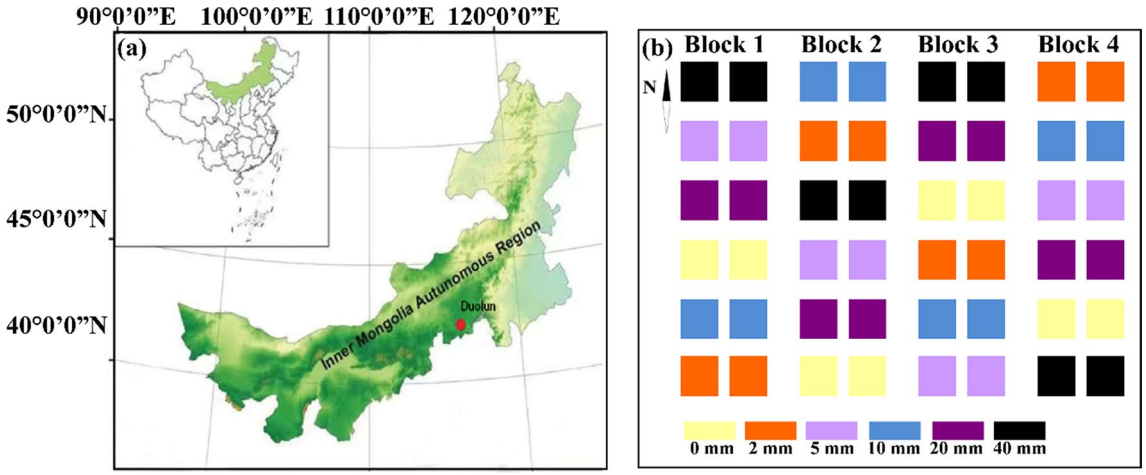

Fig. 1 Collection site (**a**) and experimental design (**b**) in a typical northern grassland

Experimental design

In 2012, a field experiment was conducted to examine the effects of precipitation intensity and N deposition on soil microbes in a semi-arid region. A randomized block design with four replicates was employed at this site, involving two main factors: precipitation intensity, N addition and the interactions between precipitation and N addition (Fig. 1b). Each block consisted of 12 plots (3 m × 4 m), including six precipitation intensity levels (0, 2, 5, 10, 20, and 40 mm) with (N10) and without N addition (N0). We added nitrogen in the form of urea at a rate of 10 g N m^{-2} yr^{-1} (Bai et al. 2010) on rainy days from May to June. If there was no precipitation in the first half of each month, N was added as urea dissolved in a certain amount of water and sprayed into the plot. The amount of added N was comparable to the estimated mean total N deposition rate in northern China (approximately 8.3 g N m^{-2} yr^{-1}; Bai et al. 2010; Xu et al. 2015). In this study, we sprayed the same amount of precipitation (80 mm, 20% of the average annual precipitation) over the growing season but varied the size and frequency of the applied precipitation events (Table 1).

Table 1 Scheme of water addition experiments with different intensities and frequencies (Plot size: 12 m^2)

Rainfall treatment	2 mm	5 mm	10 mm	20 mm	40 mm
Number	40	16	8	4	2
Frequency	1–2 days	3 days	7 days	15 days	1 month
Start date	June 1	June 1	June 1	June 1	Mid-July
Water requirement	24 kg	60 kg	120 kg	240 kg	480 kg

Soil sampling and properties analysis

At the end of the growing season (September, 2020), composite soil samples were collected from each plot by randomly selecting seven cores. Each soil core was collected from the surface (0–10 cm) with a 2.5-cm-diameter soil auger. All the collected samples were immediately transported to the laboratory. Fresh samples were sieved through 2 mm screens and stored at 4 °C prior to chemical analysis and at − 80 °C prior to phospholipid fatty acid (PLFA) and DNA extraction.

The pH of the air-dried soil was measured using a pH electrode (soil:deionized water ratio of 1:2.5). During the experimental period, volumetric soil moisture (SM) content was recorded every 2 days in each plot between May and September using a portable soil moisture probe (Diviner 2000, Sentek Pty, Ltd., Balmain, Australia). Soil microbial biomass C (MBC) and N (MBN) were measured using the chloroform fumigation–extraction method and total organic C was determined. Soil NH_4^+–N and NO_3^-–N levels were determined by extracting 10 g of soil with 100 mL 1 mol L^{-1} KCl). The concentration of the extract was determined using an automatic flow injection machine (Bran+Luebbe, Germany). Soil organic C (SOC) was measured using the potassium dichromate oxidation method. Soil total N (TN) was analyzed using the micro-Kjeldahl method. Soil total P (TP) was determined using the Mo–Sb antispectrophotometric method. All living plants were clipped as aboveground biomass (AGB). After removing the aboveground plants, three root cores (internal diameter, 5 cm; depth, 10 cm) were collected and mixed. The mixed root cores were washed with tap water in a 1 mm sieve, washed with distilled water three times, oven-dried at 65 °C until reaching a constant weight, and weighed as

belowground biomass (BGB). Plant species richness was calculated as the number of plant species in each plot.

Soil microbial community structure

The composition of the soil microbial community was determined using the PLFA pattern, which is an effective method for rapid determination of fungal or bacterial biomass in soil communities (Frostegård et al. 2011). Microbial PLFAs were extracted from 8 g of dry weight equivalent fresh soil and quantified using the method described by Bossio and Scow (1998). Qualitative and quantitative fatty acid analyses were performed with a gas chromatograph (Agilent 7890A, Agilent Technologies, USA) and the MIDI Sherlock Microbial Identification System (MIDI Inc. Newark, DE, USA). We calculated the sum of 15:0, 16:0, 17:0, 18:0, 16:1ω7c, 16:1ω9c, 17:1ω8c, cy17:0, 18:1ω5c, 18:1ω7c, 18:1ω9c, cy19:0, i14:0, i15:0, a15:0, i17:0, and a17:0 to represent the bacterial PLFAs and the fungi were identified by 18:2ω6 (Zelles 1999).

DNA extraction, PCR, and sequencing

Microbial community genomic DNA was extracted from 0.5 g dry weight equivalent fresh soil samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The DNA extract was analyzed on a 1% agarose gel, and DNA concentration and purity were determined using a NanoDrop 2000 UV–Vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable regions V3–V4 of the bacterial 16S rRNA gene were amplified with the primer pairs 338F (5′-ACTCCTACGGGAGGC AGCAG-3′) and 806R (5′-GGACTACHVGGGTWT CTAAT-3′) using an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). Fungal ITS rRNA regions were performed using initial amplification of the ITS (Grades and Bruns 1993) region with the ITS1F and ITS4 (White et al. 1990) primers. The PCR amplification of the 16S rRNA gene was performed as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s, single extension at 72 °C for 10 min, and end at 4 °C. The PCR mixtures contain 5× *TransStart* FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL, forward primer (5 μM) 0.8 μL, reverse primer (5 μM) 0.8 μL, *TransStart* FastPfu DNA Polymerase 0.4 μL, template DNA 10 ng, and ddH_2O up to 20 μL. The PCR reactions were performed in triplicate.

Statistical analyses

Sequence processing, clustering, taxonomic assignments, and biodiversity calculations were performed using the software package QIIME Pipeline-Version 1.9.1 (http://qiime.org/install/index.html). Prior to statistical analysis, we examined whether the data conformed to a normal distribution (Shapiro–Wilk test) and tested for homogeneity of variance (Levene's test). We used non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity matrices to visualize the effects of precipitation intensity and N addition on the compositional variation in bacterial and fungal community structures.

The relative abundances of the bacterial and fungal groups were analyzed by aggregating all taxa at the phylum level. The analysis targeted the most abundant phyla using the lowest relative abundance threshold, which ensured that all taxa in the analysis were present in all samples. The threshold corresponded to a phylum accounting for > 4% of the total number of OTUs sequenced in at least one sample at the time of sampling.

The modified normalized stochasticity ratio (MST) was used to quantify deterministic and stochastic processes (Liang et al. 2020; Ning et al. 2020). Deterministic processes generally refer to non-random ecological processes, whereas stochastic processes indicate diversity patterns indistinguishable from random chance (Chase and Myers 2011; Zhou and Ning 2017). In addition, 0.5 is the boundary point between the deterministic (MST < 0.5) and stochastic (MST > 0.5) assemblies. We analyzed the bacterial and fungal networks for N0 group and N10 group (24 samples each), and all network analyses were performed in R using the igraph package based on Spearman's correlation matrices (Williams et al. 2014). Only OTUs that were present in at least 1/3 of the samples were retained for network analysis (Banerjee et al. 2019; Zhou et al. 2011; Yuan et al. 2021). We generated the sub-networks and calculated the topological features of each sub-network to explore the variations of network complexity under different precipitation intensity treatments (Csardi and Nepusz 2006). The node, edge, average path length, and average clustering coefficient were used to describe the network topology features. The degree of community complexity was quantified using a cohesion metric (Herren and McMahon 2017). The networks were visualized with Gephi (version 0.9.2) software (Shi et al. 2020).

Results

Responses of soil nutrients, soil environment and vegetation properties to changes in precipitation intensity and N addition

N addition significantly increased SOC and TN under low precipitation intensity (10 mm) (Additional file 1: Fig. S1b, d) while significantly increased MBC under extreme precipitation intensity (40 mm) (Additional file 1: Fig. S1n). For soil environment, precipitation intensity significantly increased SM and the highest value occurred

Table 2 Results (*P* values) of two-way ANOVAs on the effects of N addition (N) and precipitation intensity (P) and their interactions on bacterial and fungal PLFAs, diversity, modified normalized stochasticity ratio and network topological characteristics

		Bacterial			Fungal		
		P	N	N×P	P	N	N×P
PLFAs		0.090	0.439	0.390	0.096	**0.000**	**0.003**
Diversity	Shannon index	**0.000**	0.638	**0.001**	0.719	0.323	0.778
	Evenness index	**0.011**	0.318	**0.034**	0.573	0.311	0.909
Modified normalized stochasticity ratio (MST)		0.063	**0.000**	0.395	**0.002**	0.596	0.187
Network	Node numbers	0.928	**0.000**	0.892	**0.000**	**0.017**	**0.029**
	Edge numbers	0.721	**0.000**	0.876	**0.000**	0.153	**0.006**
	Cluster coefficient	0.344	**0.000**	0.559	**0.006**	0.126	**0.001**
	Degree	0.154	0.554	0.769	**0.025**	0.510	**0.027**

Significant differences ($P < 0.05$ and $P < 0.01$) were marked in bold

under heavy precipitation intensity (20 mm) (Additional file 1: Fig. S1q). N addition significantly decreased pH across all the precipitation intensity treatments (Additional file 1: Fig. S1s, t). For vegetation properties, the increase in BGB was larger under strong (10, 20, and 40 mm) than that under low precipitation intensity treatments (2 and 5 mm) (Additional file 1: Fig. S1w, x).

Microbial biomass and alpha diversity

N addition significantly decreased fungal PLFA concentration under the 5 mm, 20 mm, and 40 mm precipitation intensity treatments (N×P interaction; Table 2, Fig. 2b) and fungal PLFA concentration decreased to 0.59 nmol g^{-1}. N addition increased fungal PLFA under 2 mm precipitation intensity treatment, but did not substantially affect bacterial PLFAs (Table 2, Fig. 2a). Interactive effects were observed between precipitation intensity and N addition on soil bacterial diversity (Table 2). Compared with those of the control precipitation treatment (0 mm), N addition increased the bacterial Shannon and evenness indices under the 2 mm, 5 mm, and 20 mm precipitation intensity treatments and decreased under 0 mm and 40 mm (Fig. 2c, e). Neither N addition nor changes in precipitation intensity had any measurable effects on fungal diversity (Table 2, Fig. 2d, f).

Composition and beta diversity of soil bacterial community and fungal community

The bacterial community was dominated by the phyla Actinobacteriota, Acdobacteriota, and Proteobacteria (Fig. 3a). The soil fungal community was dominated by the phyla Ascomycota, Basidiomycota, and Mortierellomycota (Fig. 3c). The results showed that N addition altered the structure of the bacterial community due to a decline in the relative abundance of dominant Acidobacteriota, Actinobacteriota, and Entotheonellaeota and an increase in the relative abundance of Nitrospirota, Planctomycetota, and Bacteroidota (Fig. 3b). Furthermore, N addition altered the structure of the fungal community due to a decline in the relative abundance of dominant Basidiomycota, Mortierellomycota, and others and an increase in the relative abundance of Chytridiomycota and Olpidiomycota (Fig. 3d). The NMDS results showed that N addition considerably altered both bacterial and fungal community composition (Fig. 4a, b). The Mantal test indicated that soil pH, MBC, NH_4^+–N, NO_3^-–N, and plant AGB influenced soil bacterial and fungal community composition. In addition, the soil bacterial community composition was substantially affected by MBN (Table 3, $P = 0.033$). Moreover, the difference in soil fungal community composition between N0 and N10 was significantly higher under the 20 mm precipitation intensity treatment than under the other precipitation intensity treatments (Fig. 4d, $P = 0.028$), whereas there was no significant change in soil bacterial community composition (Fig. 4c, $P = 0.365$).

Assembly processes of soil bacterial and fungal communities

The relative importance of stochastic and deterministic processes in shaping bacterial and fungal communities under stress caused by N addition and precipitation was quantified using an MST (Fig. 5). This framework was developed with 0.5 as the boundary point between the more stochastic (MST > 0.5) and deterministic (MST < 0.5) assembly processes. Our results showed that N addition decreased the MST values of the bacterial community under all precipitation intensity treatments; values lower than 0.5 indicated that determinacy was the predominant force in structuring the community composition. Without N

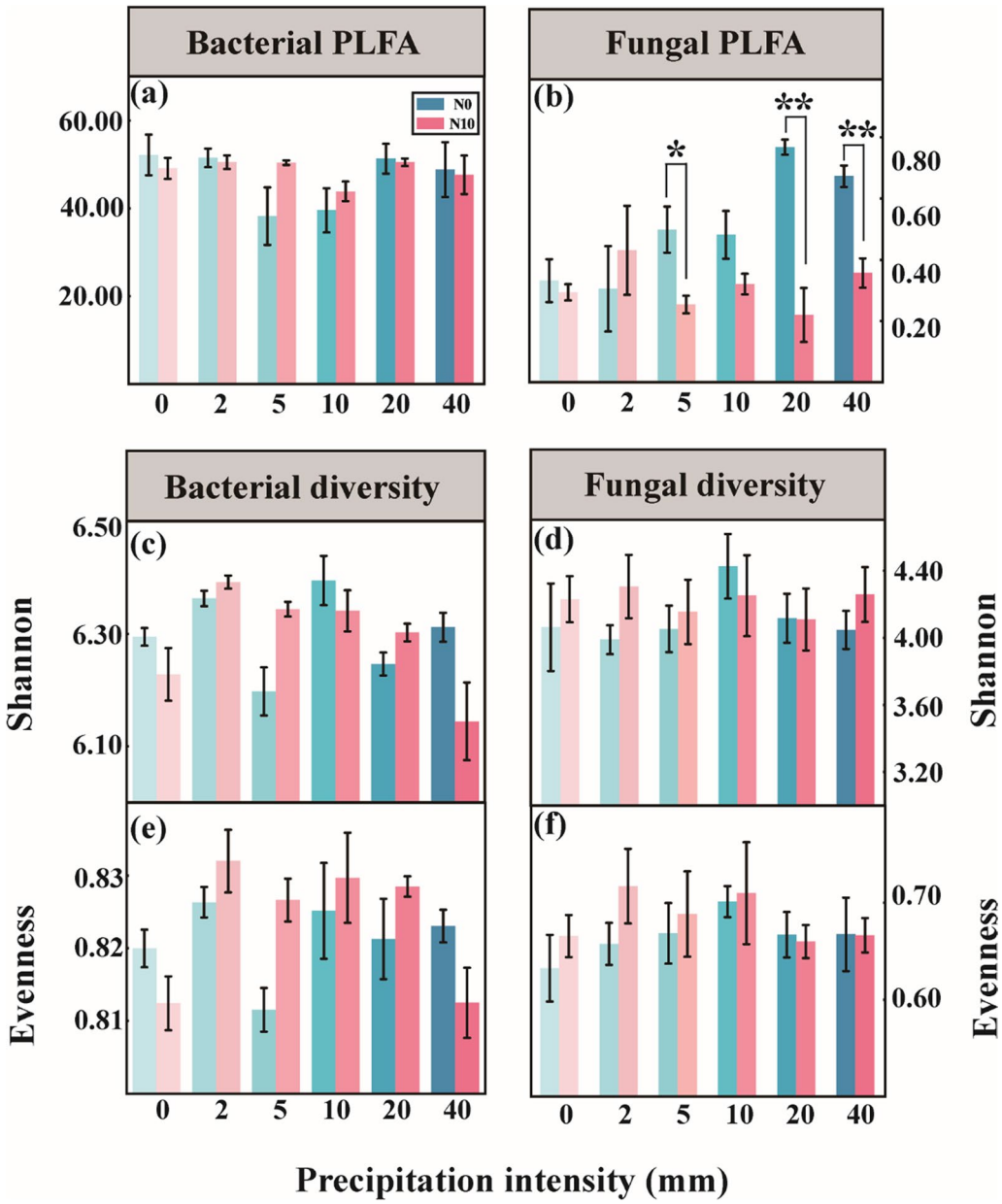

Fig. 2 Responses of soil microbial PLFAs (**a**, **b**) and diversity (**c**–**f**) to precipitation intensity under control (N0) and N addition (N10). Error bar means standard error (S.E.). The number of replicates was 4 ($n = 4$). The asterisk above the horizontal line represents a significant difference between the two groups, with $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$

addition, the mean MST value was approximately 0.6 under 0 mm precipitation intensity, indicating that stochasticity was the predominant force in structuring community composition. Under different precipitation intensity treatments, the median MST values were lower than 0.5, indicating that the dominant factor in community composition changed from stochasticity to determination. The median MST values of the fungal community were below 0.5 with and without N addition, indicating that N addition caused greater environmental stress to the fungal community, which was dominated by deterministic processes (Fig. 5b).

Co-occurrence network

To determine the effects of N addition on soil bacterial and fungal community interactions, two networks were constructed using ecological network analysis (Fig. 6a, b). The effects of N and precipitation intensity on the

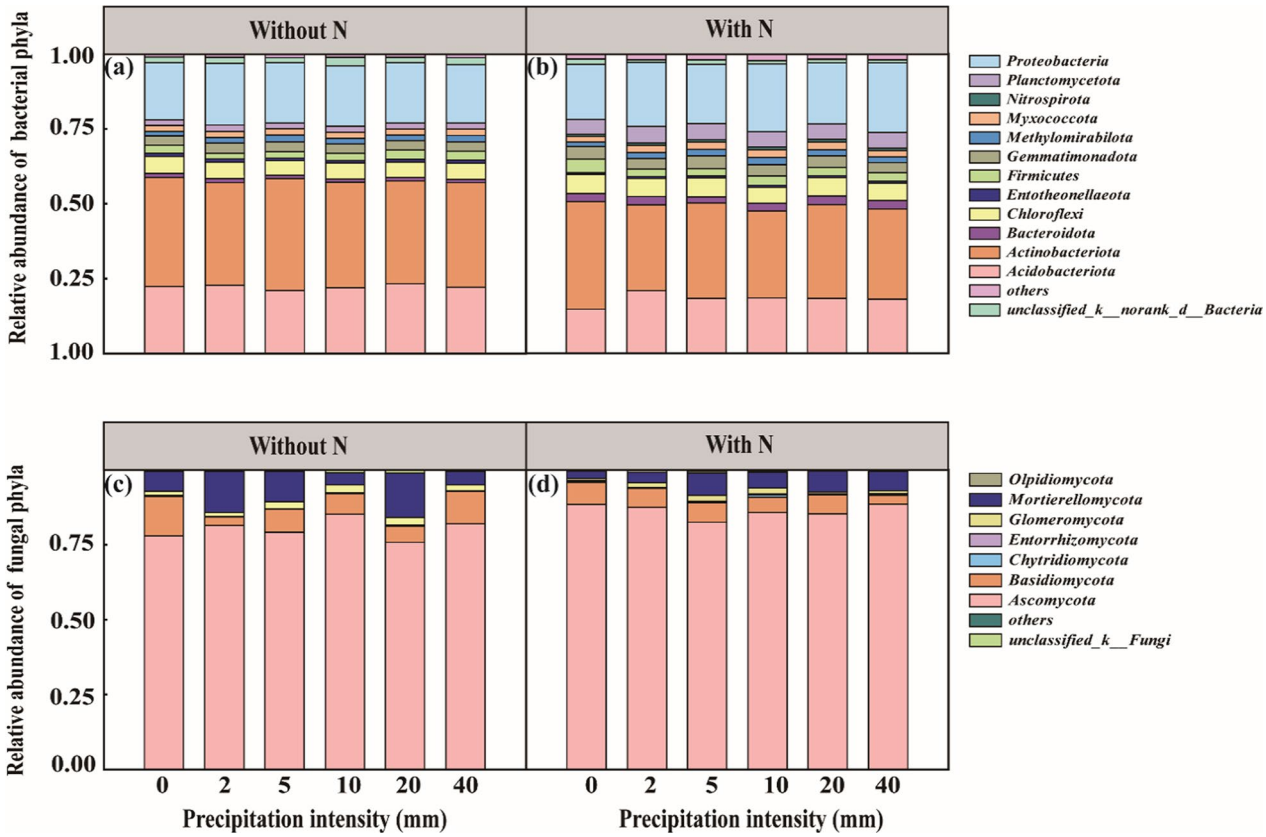

Fig. 3 Responses of relative abundance of dominant phyla of bacteria (**a**, **c**) and fungi (**b**, **d**) to precipitation intensity under control (N0) and N addition (N10)

corresponding topological features are summarized in Table 2 and Fig. 6. For the bacterial network, N addition increased the node numbers, edge numbers, and cluster coefficients under all precipitation intensity treatments (Table 2; Fig. 6c, d, f). Here, we used the network topological parameters of node and edge numbers, degree and cluster coefficient, to assess soil microbial network complexity, with higher node and edge numbers, higher degree and cluster coefficient representing greater network complexity. For the soil fungal network, N addition substantially increased node numbers, edge numbers, degree, and cluster coefficients under the 20 mm precipitation treatment (N × P interaction; Table 2; Fig. 6g–j).

Discussion

Precipitation intensity and N addition were predicted to affect different aspects of soil microbial structure, such as biomass, diversity, and composition. Partially consistent with the first hypothesis, N addition substantially decreased fungal PLFAs, but did not alter bacterial PLFAs in this experiment. Furthermore, N addition tended to increase the NH_4^+–N concentration (Additional file 1: Fig. S1), and the accumulation of NH_4^+ may have a negative impact on soil fungal biomass (Landesman et al. 2010). In particular, the negative effects induced by N addition were stronger following an increase in precipitation intensity, which may be attributed to the solubility of NH_4^+–N in water. Nutrient diffusion and replenishment are rapid in soils with high water availability, which affects the growth of soil microorganisms. Fungal propagation mainly depends on hyphal growth, whereas bacteria proliferate individually (Frey et al. 2004). The acquisition of soil nutrients is more dependent on soil moisture conditions for fungal biomass than for bacterial biomass. The drying–rewetting cycles caused by a longer interval between water additions may inhibit soil fungal growth, as suggested by Hicks et al. (2019).

We found that N addition did not alter the bacterial PLFAs, but did alter the microbial diversity in the experiment. In particular, N addition alone decreased the bacterial Shannon and evenness indices, whereas increased bacterial diversity under moderate and high (10 mm and 20 mm) precipitation intensity treatments. This was not consistent with the third hypothesis that high and extreme precipitation intensity would exacerbate the adverse effects of N addition. Increased

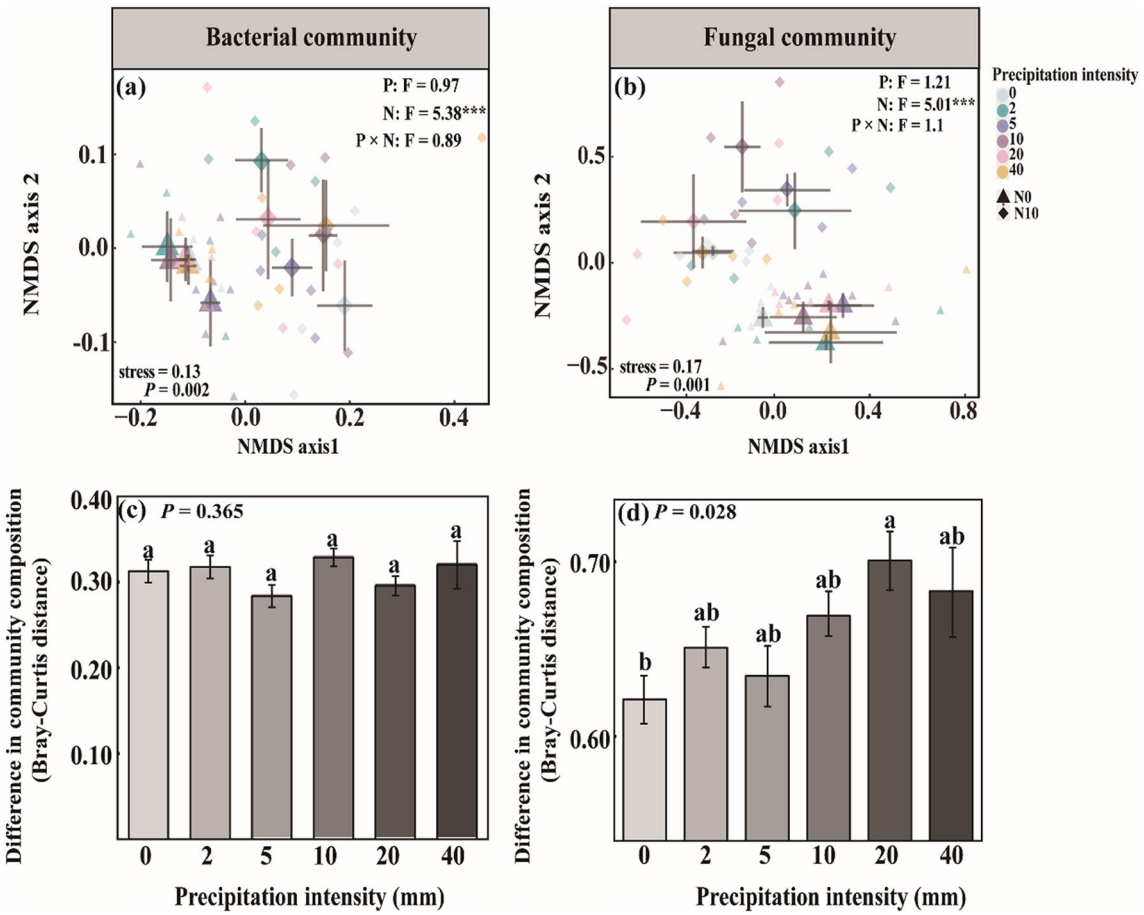

Fig. 4 Multidimensional scaling (NMDS) analysis of bacterial community (**a**) and fungal community (**b**) compositions over precipitation intensity under control (N0) and N addition (N10). The difference between control (N0) and N addition (N10) under precipitation intensity treatments in bacterial (**c**) and fungal community composition (**d**) (Bray–Curtis distance; range 0–1, mean ± s.e.m.)

Table 3 Mantel test (Pearson's correlation) for biotic factors influencing bacterial community and fungal community

	Bacterial community		Fungal community	
	P	*R*	*P*	*R*
SM	0.780	−0.070	0.998	−0.201
pH	**0.000**	0.337	**0.000**	0.350
SOC	0.681	−0.035	0.242	0.045
TN	0.766	−0.056	0.661	−0.035
TP	0.936	−0.112	0.711	−0.047
MBC	**0.001**	0.248	**0.015**	0.166
MBN	**0.033**	0.153	0.240	0.055
AN	0.771	−0.062	0.113	0.101
NH_4^+–N	**0.031**	0.179	**0.045**	0.163
NO_3^-–N	**0.001**	0.321	**0.014**	0.218
AGB	**0.001**	0.220	**0.000**	0.230
BGB	0.505	−0.007	0.478	−0.001
Richness	0.880	−0.086	0.282	0.042

Significant differences ($P < 0.05$ and $P < 0.01$) were marked in bold

precipitation intensity promoted AGB and root growth at the study site (Additional file 1: Fig. S1), suggesting that the higher bacterial diversity was likely explained by higher rhizodeposition (Li et al. 2022). A previous study found that a higher microbial diversity level could favor plant water uptake by increasing soil water availability for the root system (Prudent et al. 2019). Thus, the increased bacterial diversity might be explained by the self-compensation of the plant–soil–microbe system. Moreover, a moderate drought interval increases soil oxygen, decreases soil nutrient leaching, and relieves competition between plants and soil microbes (Gupta et al. 2020), likely resulting in increased bacterial diversity. Notably, N addition decreased bacterial diversity under the extreme precipitation intensity treatment (40 mm) (Heisler-White et al. 2008; Pachauri et al. 2014; Yang et al. 2021). Soil bacterial diversity is frequently reported to be correlated with soil pH, even at large scales, and a narrow pH range for optimal

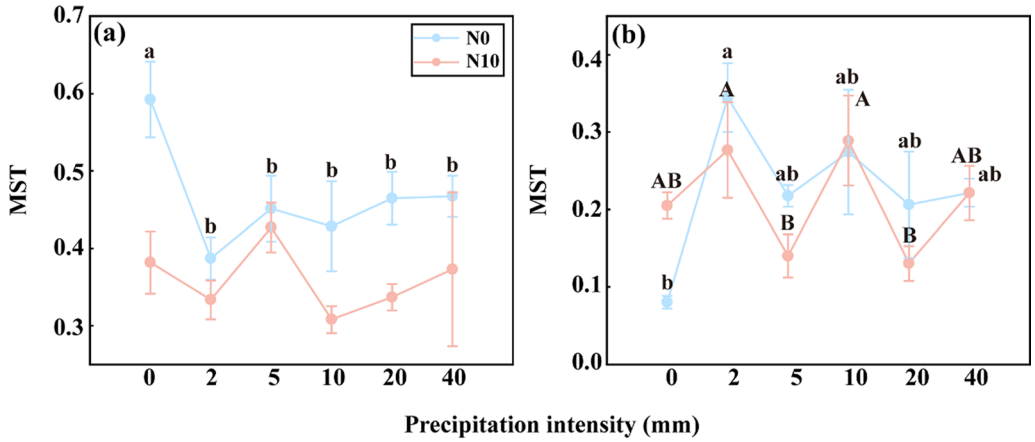

Fig. 5 Modified normalized stochasticity (MST) ratio of bacteria (**a**) and fungi (**b**) under control (N0) and N addition (N10). Different letters indicate significant differences among different precipitation intensities under same N addition treatment ($P < 0.05$). The number of replicates was 4 ($n = 4$)

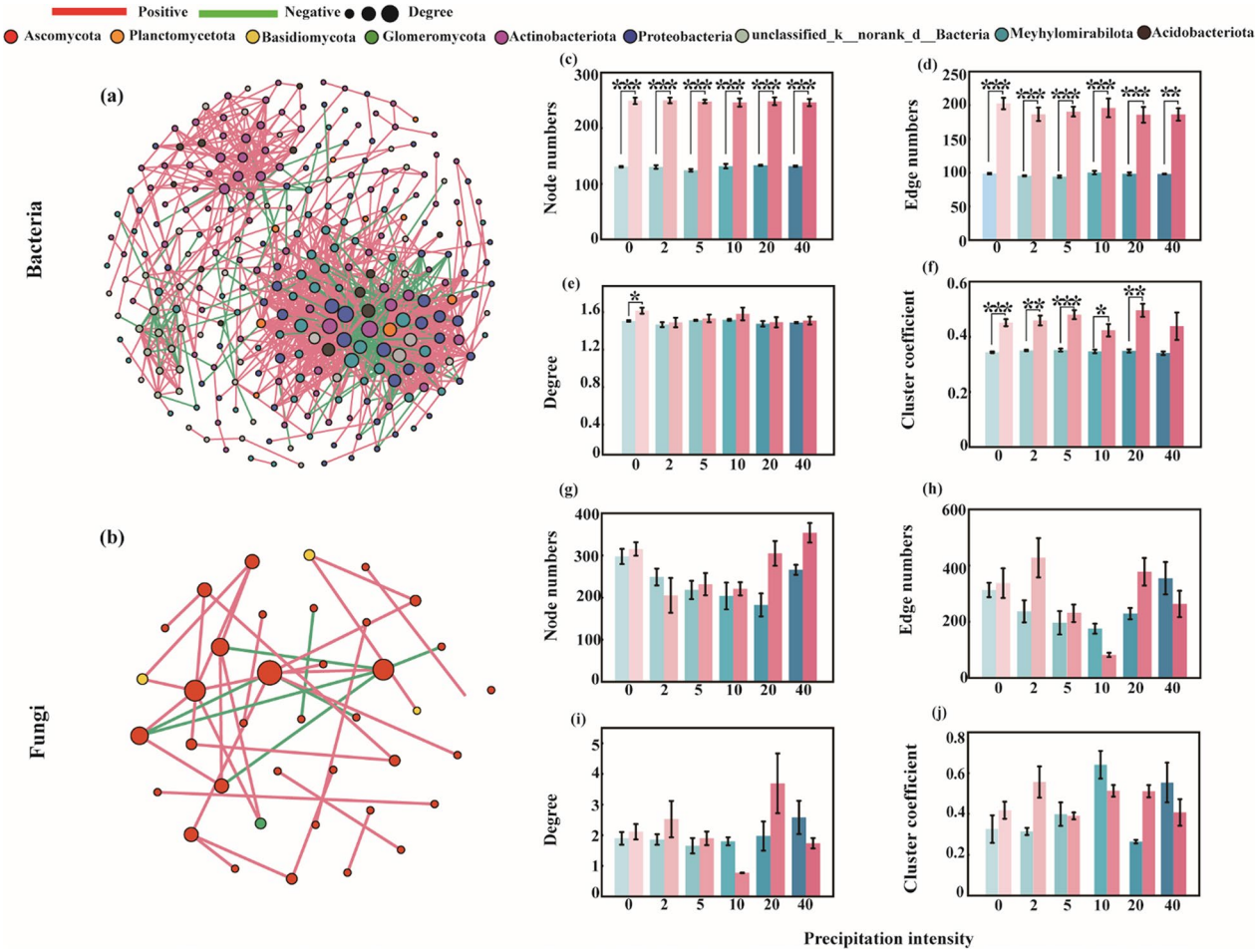

Fig. 6 Ecological networks of soil bacteria (**a**) and fungi (**b**) at OTU level, node numbers (**c**, **g**), edge numbers (**d**, **h**), degree (**e**, **i**) and cluster coefficient (**f**, **j**) were used to estimate the complexity (**c**–**j**). The nodes are colored according to bacterial and fungal models, and node size indicates the degree of connection. The asterisk above the horizontal line represents a significant difference between the two groups, with $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$

bacterial growth may be a primary factor (Lauber et al. 2009; Rousk et al. 2010). We compared N-induced reductions in soil pH among all precipitation intensity treatments and found that the reduction was the largest under the extreme precipitation intensity treatment (40 mm). This inference was supported by the decline in the relative abundances of the dominant Acidobacteriota, Actinobacteriota, and Entotheonellaeota. Consequently, longer drought intervals and greater pH reductions during extreme precipitation events aggravate the negative effects of N addition on bacterial diversity. Our results strongly support the widely held view that N addition alters the microbial structure and has more pronounced effects with increasing precipitation intensity.

Soil microbial communities are characterized by a tremendous diversity of taxa and a myriad of interactions among the members of the microbiome (Wertz et al. 2007; Louca et al. 2018; Bardgett and Caruso 2020). Network analyses provide a way of investigating the ecological understanding of microbial communities by revealing non-random covariation patterns in community organizations (Shi et al. 2016). Consistent with the second hypothesis, the responses of bacterial and fungal community were different. Compared with N0 treatment, the networks under N10 treatment showed the higher node and edge numbers of bacterial networks. That is, N addition substantially increased the complexity of the bacterial community, which was attributed to the N addition increasing plant productivity and associated with more bacterially dominated energy channels (Moore et al. 2003; Wardle et al. 2004). Fungi are less susceptible than bacteria to disturbances induced by N addition (Moore et al. 2021). This is supported by the results of MST in our study. We found that the MST of bacterial community decreased more than that of fungal community under N addition (Fig. 5). It suggests that N addition leads to a type of persistent environmental filtering. This kind of environmental filtering directly resulted in deterministic process gradually beginning to dominate microbial community assembly, and the influence was stronger on bacterial community assembly than on fungal community assembly. A possible reason for this is that bacteria and fungi respond differently to resource availability, likely due to their distinct adaptive strategies in soil environments. Notably, N addition considerably increased the soil fungal network complexity under high precipitation intensity (20 mm) with the highest soil moisture content (Additional file 1: Fig. S1). Fungi are typically more tolerant and nutrient-sensitive than bacteria because of their ability to better access soil water and nutrients through hyphal networks (de Boer et al. 2005; Yuste et al. 2011; Manzoni et al. 2012). The results revealed the effects of N addition on the soil microbial community from different aspects and further demonstrated that the effects can be altered by precipitation intensity.

Conclusion

Understanding the combined effects of rainfall regime and N addition on soil microbes is of specific concern to predict the ecosystem responses to future scenarios of global change in semi-arid grasslands in northern China, especially for different aspects of microbial structure, such as biomass, diversity, and composition. In this 8-year manipulation experiment, the effects of N deposition on soil bacterial and fungal communities were altered by precipitation intensity. When fewer but more intense rainfall events occurred, N addition increased bacterial diversity and the fungal community complexity while reducing fungal PLFAs. The overall changes in soil bacterial and fungal communities were different, implying that composition and functional traits adapt differently to projected global changes at a regional scale.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-023-00445-w.

Additional file 1: Fig. S1. Responses of soil nutrients (a–p), soil environment (q–t) and vegetation properties (u–z) to precipitation intensity under control (N0) and N addition (N10).

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Author contributions
SC: conceptualization, methodology, writing—review and editing. YX: validation, formal analysis, data curation and writing. YZ: review and editing. PW: review and editing. LC: review and editing. GZ: conceptualization, writing—review and editing, project administration, funding acquisition.

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Availability of data and materials
The data sets analyzed in the study can be obtained via the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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