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Differential responses of soil bacteria and fungi to altered precipitation in a meadow steppe

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ABSTRACT

Soil microorganisms are essential participants in ecosystem processes, yet their composition, diversity, and function are affected by altered precipitation. The patterns and key processes driving the effects of changes in precipitation on soil bacterial and fungal communities remain unclear. To better understand how changes in precipitation may affect soil microorganisms, we conducted a three-year field precipitation manipulation experiment, with treatments ranging from 50% reduction to 50% increases in precipitation, in a meadow steppe located in northeast China. Our results demonstrated that the bacterial community was more sensitive to changes in precipitation than the fungal community. The fungal community was sensitive to inter-annual differences in precipitation, but not to the treatment-induced changes in precipitation. Increased annual precipitation shifted the dominance of the microbial community from bacteria to fungi. Over the precipitation range (200–280 mm) soil microbial biomass and diversity are maximal, below the long-term mean annual precipitation (430 mm) for this site. Soil water content, pH, and total phosphorus were the main factors related to the variance in soil microbial community diversity. Results show non-linear, time-dependent, and interacting responses of bacterial and fungal biomass and diversity to soil properties under gradients of altered precipitation magnitude in this semi-arid grassland.

1. Introduction

As an important component of the global water cycle, precipitation patterns are likely to be altered in location-specific ways due to global warming (IPCC, 2007; Knapp et al., 2017). Changes in precipitation will have significant ecological consequences including shifts in community composition and variation in ecosystem functions, especially in water-limited ecosystems such as grasslands (Nielsen and Ball, 2015; Zhang et al., 2019). In semi-arid grasslands, soil microorganisms are the most sensitive community component to the resultant changes in soil water availability (Barnard et al., 2015; Chen et al., 2019) and indirect variation in physicochemical conditions (Ochoa-Hueso et al., 2018; Na et al., 2019). Despite numerous studies that have been conducted on the effects of soil water availability on community composition and activities of soil microbes (Huang et al., 2015; Koyama et al., 2018; Qin et al., 2020), we are still far from fully understanding the responses of belowground microbial community structure and functions to the predicted changes in precipitation. Moreover, few studies have tested the responsivity of soil microbial communities and soil properties to a range of precipitation changes *i.e.,* multiple levels of both increases and decreases, under otherwise identical environmental conditions.

Soil microorganisms are key participants in nutrient mineralization and organic matter decomposition, and an increase or decrease in precipitation directly influences the soil microbial community by changing soil water availability (Ren et al., 2017). Soil microorganisms, including bacteria and fungi, differ in their response to variation in soil water availability. Due to different physiological properties and survival strategies, bacteria typically respond faster than fungi to changes in soil water availability (Engelhardt et al., 2018). Bacterial biomass and diversity responds to increased precipitation and soil water content (SWC) because they are highly dependent on water for movement and substrate diffusion (Harris, 1981). However, it has been reported that osmotic stress accompanying increased SWC can lead to a decrease in bacterial biomass and diversity (Kieft et al., 1987). Previous studies have shown that precipitation can drive changes in the bacterial community via physicochemical variability in SWC and pH (Felsmann et al., 2015;

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Ochoa-Hueso et al., 2018). On the other hand, Marschner et al. (2003) and Sessitsch et al. (2001) found that soil organic matter content, carbon/nitrogen ratio, and soil texture significantly affected the bacterial community. Altered precipitation (especially drought) may drive changes in the composition of the soil carbon (C) pool, with consequences for soil C loss (Hueso et al., 2012), leading to limited capability of bacteria for rapid growth. Despite these prior results, the soil abiotic factors that determine bacterial community composition are far from clear.

In contrast to bacterial communities, multiple studies have reported that precipitation changes could affect soil fungal communities by altering both abiotic and biotic factors (Hawkes et al., 2011; Liu et al., 2018). For instance, fungal richness can be shaped by temperature, SWC, pH, and nitrogen (N) availability (Allison et al., 2007; Bi et al., 2012; Tedersoo et al., 2014; Wang et al., 2015), which all tend to co-vary with changes in precipitation. Alternatively, variation in precipitation may indirectly affect the fungal community by changes in plant community composition, diversity, and productivity (Knapp et al., 2002; Suttle et al., 2007; Prober et al., 2015). For example, plants can influence mycorrhizal fungi via root exudates and shift soil pH within the rhizosphere (Richardson et al., 2009). To date, there is no consensus for how altered precipitation leads to fungal community changes. This is because fungal communities are more resistant than bacteria to water limitation, likely due to their hyphal connections that allow them to be better adapted to water-poor pore domains in soil when searching for nutrient resources (De Boer et al., 2005). These prior responses of bacteria and fungi to soil moisture highlight the need to simultaneously quantify the effects of changes in precipitation on bacteria, fungi, and the soil microenvironment.

Soil microorganisms affect land-atmosphere C exchange, modulating ecosystem C fluxes through decomposition and heterotrophic respiration (Hopkins and Gregorich, 2005; Standing et al., 2005; Zhang et al., 2013). At the same time, grasslands contribute to soil C sinks at a global scale (Poulter et al., 2014; Ochoa-Hueso et al., 2018). In northeast China, grasslands are represented by meadow steppe, which is a part of a widely distributed grassland ecosystem across the Eurasian Steppe region. Global circulation models predict that extreme precipitation events and the intensity of future precipitation will be on the rise in this region (IPCC, 2007; Sun and Ding, 2010). Moreover, Liu et al. (2005) have reported that increased annual precipitation is mainly driven by the increase in extreme precipitation events during the growing season. Therefore, understanding the responses of microbial community biomass, diversity, structure, and activity to variation in precipitation will improve our ability to predict the impacts of precipitation regime change on ecosystem C cycling and feedbacks between ecosystem processes and global climate change.

In this study, we examined the effects of altered precipitation on microbial diversity and community structure using a three-year field experiment with five levels of increasing and decreasing precipitation magnitude. Research was conducted in the Songnen meadow steppe, where ecosystem processes are strongly influenced by large inter-annual precipitation variability (Yang et al., 2020). To identify the patterns and key processes controlling soil microbial community diversity in response to precipitation, we measured plant productivity (*e.g.*, aboveground biomass, belowground biomass), soil abiotic properties (*e.g.*, SWC, soil bulk density, soil water filled pore space, soil total porosity, pH, soil organic carbon, total nitrogen, and total phosphorus), microbial community composition, and diversity and structure (using Illumina sequencing). We hypothesized that: (1) effects of the altered precipitation on soil microbial diversity and activity depend on the annual, ambient precipitation; (2) soil microorganisms (both bacteria and fungi) have an optimal precipitation range for biomass and diversity; and (3) SWC and soil nutrient status are the main factors modulating the microbial diversity in response to precipitation.

2. Materials and methods

2.1. Study site

This study was conducted at the Songnen Grassland Ecological Research Station of Northeast Normal University, Jilin Province, northeastern China (44°40′-44°44′N, 123°44′-123°47′E; 160 m in elevation). The study area has a temperate semi-arid monsoon climate. The mean annual air temperature (1950-2014) is 6.3 °C (Zhong et al., 2019) and the mean annual precipitation (1963-2012) is approximately 430 mm. The study site is dominated by *Leymus chinensis* (Trin.) Tzvel., a C_3 perennial rhizomatous grass (Zhong et al., 2017; Zhu et al., 2019; Yang et al., 2020). The grassland study site had a sodic saline meadow soil with a pH value of 9.6, soil organic carbon (SOC) content of 0.9% and soil total nitrogen (TN) content of 0.08% before the experiment commenced in 2015.

2.2. Experimental design

In 2015, we fenced 1 ha of grassland and divided it into four blocks. Within each block, we established five plots (3.5 m × 3.5 m) that were similar in vegetation composition (*L. chinensis* accounted for 85% of plant cover in each plot). Each of the five plots within a block was randomly assigned to one of the five precipitation treatments: ambient (0%), 50% decrease (−50%), 30% decrease (−30%), 30% increase (+30%), and 50% increase (+50%), which resulted in four replicates and a total of twenty plots. Each plot was trenched to a depth of 0.5 m along the border and lined with an impermeable barrier, then refilled prior to the initiation of the experiment in 2015.

To create the precipitation gradient, we set up passive rainfall shelters for each plot. Each rainfall shelter consisted of a metal frame and V-shaped UV-transparent Perspex® (>90% light permeability) that was 3.7 m long, 0.33 m wide, 3 mm thick, and arranged in a longitudinal plait of 120° (Li et al., 2019). The rainfall shelters had a 10° inclination from the lower side (1.3 m tall) to the higher side (1.7 m tall). The intercepted rainfall was collected in a container at each shelter. For the 0%, +30%, and + 50% plots, the collected rainfall was manually irrigated back to the corresponding plots. Meanwhile, the collected rainfall in the −50% and −30% plots was manually added to the + 50% and + 30% plots, respectively. We used a control treatment without rainfall shelters (0%) to identify that our rainfall shelters have a negligible non-drought impact on plant productivity and soil properties (Loik et al., 2019).

2.3. Environmental conditions

Precipitation and air temperature were recorded with a HOBO U30-NRC meteorological station (Onset Computer Corporation, Bourne, MA, USA). The measurements were carried out continuously from mid-April in 2016 to mid-October in 2017.

2.4. Vegetation survey

For each plot, vegetation was surveyed for species composition in a 50 cm × 100 cm quadrat in August 2016 and 2017. We harvested aboveground plant materials for the measurements of aboveground plant biomass (AGB) following the vegetation survey. Belowground plant biomass (BGB) was determined by washing the roots out of two composited soil cores (diameter of 6.5 cm and depth of 30 cm) for each plot. Plant biomass samples were dried at 65 °C until a constant weight was achieved.

2.5. Soil sample collection

In August 2016 and 2017, ten soil cores (2.54 cm diameter, 10 cm deep) were collected from randomly-assigned positions within each plot

and combined to form one composite soil sample. After gentle mixing and removal of roots, the moist soil was passed through a 2 mm mesh sieve and separated into three parts. One part was maintained fresh (4 °C) for measurements of microbial biomass carbon (MBC) and microbial extracellular enzyme activity (EEA). One part was stored at −80 °C for extraction of microorganisms for community composition analysis. The last part was air-dried to determine soil pH, SOC, TN and total phosphorus (TP). For the measurements of SWC, soil bulk density (BD), soil water filled pore space (WFPS), and soil total porosity, we used the ring knife method to collect the soil samples (100 cm^{-3}).

2.6. Soil properties

SWC samples were placed in aluminum boxes, and measured for total fresh mass (Mt). The dry mass (Ms) was measured after oven drying at 105 °C until a constant weight was achieved. SWC, BD, WFPS, and total porosity were calculated as follows:

$$\mathrm{SWC}(\%) = \frac{Mt - Ms}{Ms - M} \times 100$$

$$\mathrm{BD}\left(\mathrm{g}\ cm^{-3}\right) = \frac{(\mathrm{Mt} - \mathrm{M}) \times 100}{V(100 + \mathrm{SWC})}$$

$$\mathrm{WFPS}(\%) = \frac{\mathrm{SWC} \times \mathrm{BD}}{1 - \frac{BD}{\rho}}$$

$$\mathrm{Totalporosity}(\%) = \frac{\rho - BD}{\rho} \times 100$$

where M (g) is the mass of the aluminum boxes; V is the volume of the ring knife (100 cm^{-3}); ρ is the soil particle density of 2.65 g cm^{-3}.

Soil pH was measured using a 1:5 ratio of air-dried soil to deionized water. SOC and TN were analyzed with an elemental analyzer (Vario EL cube, Elementar, Langenselbold, Germany). TP content was determined using the $HClO_4$-H_2SO_4 heating digestion method followed by quantification using an inductively coupled plasma emission spectrometer. MBC was determined following the chloroform fumigation-extraction method (Vance et al., 1987). Fumigated and unfumigated soil samples were extracted in 0.05 M K_2SO_4 (1:4 [wt/vol]) by shaking at 160 rpm for 2 h. Extracts were filtered using Whatman No. 1 filter paper, and 10 ml was immediately used to measure extractable organic carbon using the dichromate oxidation method (Vance et al., 1987).

2.7. Microbial extracellular enzyme activity

We measured the microbial activity of seven hydrolytic soil enzymes that degrade a range of substrates that are common constituents of organic matter (Bell et al., 2014). These enzymes were selected to represent the degradation of N-rich substrates: L-leucine aminopeptidase (LAP) and β-1,4-N-acetylglucosaminidase (NAG); C-rich substrates: α-1,4-glucosidase (AG), β-1,4-glucosidase (BG), β-xylosidase (BX) and β-D-cellobiohydrolase (CBH); and alkaline phosphatase (AP) for the phosphorus (P) cycle (Sinsabaugh et al., 2009). The corresponding substrates and functions of these microbial extracellular enzymes are as described in Matulich et al. (2015) (STable 1).

Soil slurries were prepared by adding 1 g of soil to 100 ml of 50 mM buffer with the pH adjusted to soil samples (pH ± 0.5). The soil slurries and corresponding substrates were then added to a 96-deep-well microplate with an eight-channel repeat pipettor. Slurry control wells contained 50 μl of buffer and 200 μl of sample slurry. Substrate control wells contained 50 μl of substrate solution and 200 μl of the buffer. Reference standard wells contained 50 μl of standard and 200 μl of the buffer. Quench wells contained 50 μl of standard and 200 μl of sample slurry (Shi et al., 2018). There were eight replicates for each sample slurry, slurry control, substrate control, reference standard, and quench wells. Soil slurry with fluorometric substrates was incubated in the dark for 3 h at 25 °C. After the incubation, the reactions were terminated by adding 10 μl of 1 M NaOH to each well. Fluorescence was measured using a fluorescence plate reader (TECAN infinite F200, Tecan Group, Switzerland) with an excitation wavelength of 365 nm and an emission/detection wavelength of 450 nm.

2.8. DNA extractions, PCR amplification, and Illumina sequencing

DNA was extracted from soil using the MO BIO PowerSoil™ DNA Isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The V4 region of the bacterial 16S rRNA genes was generated using the barcoded Illumina sequencing primer 515F/806R. Fungal ITS1 genes of distinct regions were amplified using the specific primer ITS5-1737F/ITS2-2043R with the barcode. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). We mixed the same volume of 1 × loading buffer (containing SYB green) with PCR products and separated them by electrophoresis on 2% agarose gel for detection. We chose samples with bright main strips between 400 and 450 bp for further experiments. PCR products were mixed in equidensity ratios and then were purified with Qiagen Gel Extraction Kits (Qiagen GmbH, Hilden, Germany). Finally, the resultant PCR products were analyzed by an Illumina HiSeq2500 (Illumina Inc., San Diego, CA, USA).

2.9. Bioinformatics analyses

Paired-end reads were assigned to samples according to their unique barcode and truncated by cutting off the barcode and primer sequence. FLASH (Version 1.2.7) was used to merge paired-end reads, and the splicing sequences were called raw tags. Quality filtering was done on the raw tags to acquire high-quality clean tags based on QIIME (Caporaso et al., 2010). The tags were compared with the reference database (Gold database) using the UCHIME algorithm (Edgar et al., 2011) to detect chimera sequences and then were removed (Haas et al., 2011) to obtain effective tags.

UPARSE software (Edgar, 2013) was used to analyze sequences for all the effective tags. Edgar (2013) classified operational taxonomic unit (OTU) sequences as 'Perfect' (identical to the biological sequence), 'Good' (≤1% errors), 'Noisy' (>1% to ≤ 3% errors), 'Chimeric' (>3% errors and chimeric with high confidence), 'Contaminant' (high-identity match to a species not in the targeted community) or 'Other' (>3% errors or a biological sequence missing from the reference databases). Therefore, sequences with > 97% similarity were assigned to the same OTUs. Representative sequences for each OTU were screened for further annotation. Each representative sequence was performed by MOTHUR software, which reads < 150 bp, and ambiguous characters were removed. 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 (Threshold: 0.8 ~ 1) (kingdom, phylum, class, order, family, genus, species); the non-bacterial and non-fungal reads were further removed (Pruesse et al., 2007). To get the phylogenetic relationship of all OTUs, representative sequences were submitted to analysis with MUSCLE (Version 3.8.31, http://www.drive5.com/muscle/). OTU abundance information was normalized via a standard of the sample with the least sequences number. We used the output normalized data for subsequent analyses of alpha diversity and the structure of soil microorganisms.

2.10. Statistical analyses

We used one-way ANOVAs with Duncan's multiple-range tests to detect the significance of all response variables among different precipitation gradients in 2016 and 2017. Two-way ANOVAs were performed on the effects of precipitation (P) and year (Y) on the bacterial and fungal OTU richness. These statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

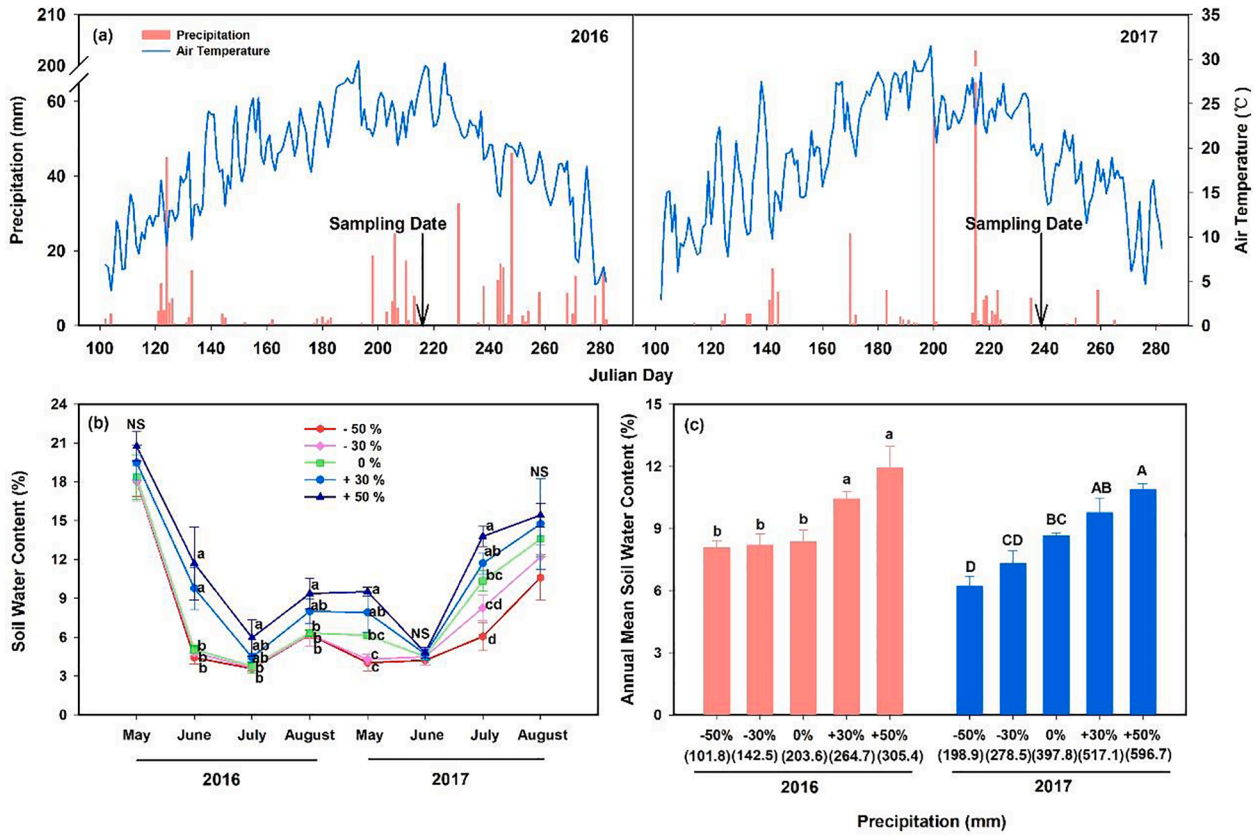

Fig. 1. Precipitation and air temperature (from mid-April to early-October) (a), soil water content (from May to August) (b) and annual mean soil water content (c) in different precipitation levels in 2016 and 2017. Different letters indicate significant differences among the levels of precipitation ($P < 0.05$). Values are means ± 1 SE ($n = 4$).

Alpha diversity of soil microorganisms was applied in analyzing the complexity of species diversity with five indices per sample, including OTU richness, Shannon index, Simpson index, Chao1 index, and ACE. All of these indices in our samples were calculated with QIIME (Version 1.7.0) and displayed with R software (R Development Core Team 2012) (Version 2.15.3).

The relative abundance of bacterial and fungal groups was analyzed by aggregating all taxa at the phylum level. The analysis targeted the most abundant phyla by using the lowest relative abundance threshold that ensured that all taxa in the analysis were present in all the samples. The threshold corresponded to the phylum accounting for > 1% of the total number of OTUs sequenced in at least one sample at one sampling time. The relative abundance of the most common bacterial and fungal taxa were analyzed by ANOVA, after log-transformation of the data to meet assumptions about normal distribution and heteroscedasticity. The explanatory variables were precipitation gradient, taxonomic identity (phylum), and their interactions. In our statistical model, the taxa identity level was nested within the plot level.

We used non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity matrices to visualize the effects of precipitation gradient on the compositional variation of the entire bacterial and fungal community structure. We used variation partition analysis (VPA) to assess the relative contributions of plant factors and soil factors to soil bacterial and fungal communities. The relationships between soil microbial community richness and physicochemical properties were determined using Pearson correlations.

2.11. Accession number

The sequence reads for all samples have been deposited in the National Center for Biotechnology Information Sequence Reads Archive with the accession number SRP200146.

3. Results

3.1. Precipitation patterns

For both experimental years, there was substantial variation in both rainfall event size and seasonal distribution. Despite significant inter-annual differences in the rainfall amount, the precipitation patterns for the two study years were both described as a dry spring with a rainy summer. The amount of rainfall (from April to August sampling date) in 2017 (397.8 mm) was greater than in 2016 (203.6 mm) (Fig. 1a). Considering long-term mean annual precipitation, we defined 2016 as the dry year and 2017 as the wet year.

Air temperature varied substantially for both years, with the highest values occurring during July and August. Mean growing season air temperature was 18.73 ± 0.45 °C in the dry year and 19.19 ± 0.48 °C in the wet year (Fig. 1a).

SWC varied considerably during the growing season for both experimental years. Significant precipitation gradient effects on SWC were detected for the sampling dates of June, July, and August in 2016 and May and July in 2017 (Fig. 1b). There were significant precipitation gradient effects on the mean growing season SWC for 2016 and 2017 (Fig. 1c).

3.2. Plant community characteristics

AGB was significantly influenced by the precipitation gradient: it was higher in the increased precipitation plots than in the decreased precipitation plots for both experimental years. However, the precipitation gradient had no significant effects on BGB, which tended to

Table 1
Vegetation characteristics in decreased or increased precipitation in 2016 and 2017. Significant differences between different precipitation levels are indicated by lowercase letters. Values are means ± 1 SE ($n = 4$).

Years	Vegetation characteristics	Precipitation gradient					*F* Value	*P* Value
		−50%	−30%	0%	+30%	+50%		
2016		**101.8 mm**	**142.5 mm**	**203.6 mm**	**264.7 mm**	**305.4 mm**		
	AGB (g m^{-2})	226.60 ± 21.93^{a}	260.00 ± 24.33^{ab}	346.05 ± 18.49^{bc}	431.35 ± 21.26^{c}	475.05 ± 72.99^{c}	**7.918**	**0.001**
	BGB (g m^{-2})	840.55 ± 107.07	922.52 ± 219.04	950.83 ± 85.21	1087.30 ± 231.84	1136.76 ± 156.39	0.661	0.661
	Plant species richness	2.75 ± 0.85	1.50 ± 0.50	3.00 ± 0.41	2.00 ± 0.41	2.00 ± 0.71	1.034	0.422
	Evenness	0.05 ± 0.02	0.07 ± 0.07	0.14 ± 0.04	0.07 ± 0.04	0.09 ± 0.06	0.536	0.711
	Shannon-Wiener index	0.07 ± 0.04	0.07 ± 0.07	0.17 ± 0.06	0.05 ± 0.03	0.11 ± 0.08	0.6	0.668
2017		**198.9 mm**	**278.5 mm**	**397.8 mm**	**517.1 mm**	**596.7 mm**		
	AGB (g m^{-2})	147.00 ± 26.37^{a}	164.25 ± 32.78^{a}	258.85 ± 27.93^{b}	344.15 ± 12.78^{c}	375.70 ± 26.88^{c}	**15.419**	**0.000**
	BGB (g m^{-2})	677.66 ± 215.53	706.07 ± 187.46	790.25 ± 60.15	828.13 ± 139.51	951.25 ± 66.77	0.540	0.709
	Plant species richness	3.75 ± 1.11	1.75 ± 0.96	2.50 ± 0.65	2.25 ± 0.95	1.75 ± 0.48	1.125	0.382
	Evenness	0.12 ± 0.04	0.08 ± 0.06	0.10 ± 0.05	0.08 ± 0.05	0.06 ± 0.05	0.205	0.932
	Shannon-Wiener index	0.19 ± 0.08	0.08 ± 0.07	0.13 ± 0.08	0.07 ± 0.04	0.06 ± 0.05	0.622	0.654

AGB: aboveground biomass; BGB: belowground biomass.

Table 2
Soil properties in increased or decreased precipitation in 2016 and 2017. Significant differences between different precipitation levels are indicated by lowercase letters. Values are means ± 1 SE ($n = 4$).

Years	Soil properties	Precipitation gradient					*F* Value	*P* Value
		−50%	−30%	0%	+30%	+50%		
2016		**101.8 mm**	**142.5 mm**	**203.6 mm**	**264.7 mm**	**305.4 mm**		
	BD (g cm^{-3})	1.67 ± 0.03	1.63 ± 0.04	1.56 ± 0.07	1.64 ± 0.04	1.45 ± 0.17	1.074	0.404
	WFPS (%)	28.31 ± 2.65	27.12 ± 5.71	24.52 ± 3.03	33.90 ± 2.92	33.40 ± 8.66	0.628	0.650
	Total porosity (%)	36.94 ± 0.96	38.52 ± 1.49	41.31 ± 2.82	38.28 ± 1.45	45.38 ± 6.27	1.074	0.404
	pH	9.60 ± 0.08	9.63 ± 0.04	9.63 ± 0.07	9.70 ± 0.08	9.50 ± 0.12	0.823	0.531
	SOC (mg g^{-1})	5.00 ± 0.40	5.55 ± 0.45	5.52 ± 0.52	6.50 ± 0.68	6.63 ± 0.47	1.848	0.172
	TN (mg g^{-1})	0.81 ± 0.03^{b}	0.85 ± 0.04^{b}	0.83 ± 0.05^{b}	0.99 ± 0.07^{ab}	1.04 ± 0.08^{a}	**3.451**	**0.034**
	TP (mg g^{-1})	0.07 ± 0.02^{b}	0.08 ± 0.02^{b}	0.05 ± 0.01^{b}	0.11 ± 0.02^{ab}	0.15 ± 0.03^{a}	**3.399**	**0.036**
	MBC (mg kg^{-1})	262.23 ± 64.16	303.92 ± 168.59	424.10 ± 144.18	317.49 ± 29.66	189.83 ± 51.60	0.644	0.640
2017		**198.9 mm**	**278.5 mm**	**397.8 mm**	**517.1 mm**	**596.7 mm**		
	BD (g cm^{-3})	1.47 ± 0.07	1.49 ± 0.13	1.42 ± 0.06	1.52 ± 0.06	1.42 ± 0.03	0.316	0.863
	WFPS (%)	34.16 ± 2.87	42.52 ± 6.00	41.11 ± 2.10	52.19 ± 11.26	46.94 ± 1.48	1.275	0.323
	Total porosity (%)	44.36 ± 2.77	43.87 ± 4.88	46.37 ± 2.09	42.70 ± 2.15	46.47 ± 1.36	0.316	0.863
	pH	9.88 ± 0.05	9.84 ± 0.05	9.93 ± 0.04	9.82 ± 0.02	9.88 ± 0.08	0.607	0.664
	SOC (mg g^{-1})	5.79 ± 0.67	6.34 ± 0.14	6.54 ± 0.62	6.95 ± 0.26	7.74 ± 0.39	2.456	0.091
	TN (mg g^{-1})	0.82 ± 0.09	0.88 ± 0.07	0.90 ± 0.06	1.02 ± 0.04	1.04 ± 0.13	1.306	0.312
	TP (mg g^{-1})	0.21 ± 0.00	0.22 ± 0.00	0.21 ± 0.01	0.22 ± 0.01	0.24 ± 0.02	1.648	0.214
	MBC (mg kg^{-1})	885.30 ± 205.81	820.52 ± 205.81	743.74 ± 188.06	698.40 ± 219.67	366.01 ± 44.51	1.108	0.389

BD: soil bulk density; WFPS: soil water filled pore space; SOC: soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; MBC: microbial biomass carbon.

increase with increasing precipitation. There were no significant differences in plant species richness, evenness, or Shannon diversity among the five different precipitation levels (Table 1).

3.3. Soil abiotic properties

The precipitation gradient did not affect soil BD, WFPS, or total porosity in either year. BD in the dry year (~1.59 g cm^{-3}) was higher than in the wet year (~1.46 g cm^{-3}). However, WFPS and total porosity in the wet year were higher than in the dry year. The highest total porosity was detected in the + 50% plots (45.38 ± 6.27% in 2016 and 46.47 ± 1.36% in 2017). The precipitation gradient had no significant effects on soil pH, but soil pH in the wet year (~9.86) was higher than in the dry year (~9.61). Along the increasing precipitation gradient, the content of SOC increased from 5 ± 0.4 to 6.63 ± 0.47 mg g^{-1} in 2016 and from 5.79 ± 0.67 to 7.74 ± 0.39 mg g^{-1} in 2017. For the dry year, TN and TP both significantly increased along the increasing precipitation gradient, from 0.81 ± 0.03 to 1.04 ± 0.08 mg g^{-1} and from 0.07 ± 0.02 to 0.15 ± 0.03 mg g^{-1}, respectively. By contrast, both TN and TP increased with precipitation, but the treatment effects were not significant in the wet year (Table 2).

3.4. Microbial biomass carbon and extracellular enzyme activities

The effects of the precipitation gradient on MBC were not significant in either experimental year. The highest MBC value was detected for the ambient precipitation (424.10 ± 144.18 mg kg^{-1}) plots in the dry year and for the −50% precipitation plots (885.30 ± 205.81 mg kg^{-1}) in the wet year. On average, MBC in the wet year was approximately two times greater than in the dry year (Table 2).

The precipitation gradient had significant effects on the activities of all assessed enzymes in 2016, but only for LAP in 2017. In 2016, activities of C cycling enzymes (AG, BG, BX, and CBH) and N cycling enzymes (LAP and NAG) gradually increased, whereas AP gradually declined along the increasing precipitation gradient. Activities of LAP decreased, comparing the −50% precipitation plots to the + 50% precipitation plots in 2017 (SFig. 1).

3.5. Microbial community dynamics

The precipitation gradient had significant impacts on bacterial OTU richness but not on fungal OTU richness and the ratio of bacterial OTU richness to fungal OTU richness. The ambient precipitation plots had the highest bacterial OTU richness (Fig. 2a). Bacterial and fungal OTU richness in the wet year was significantly greater than in the dry year (Fig. 2a & b). Significant interactive effects between the precipitation

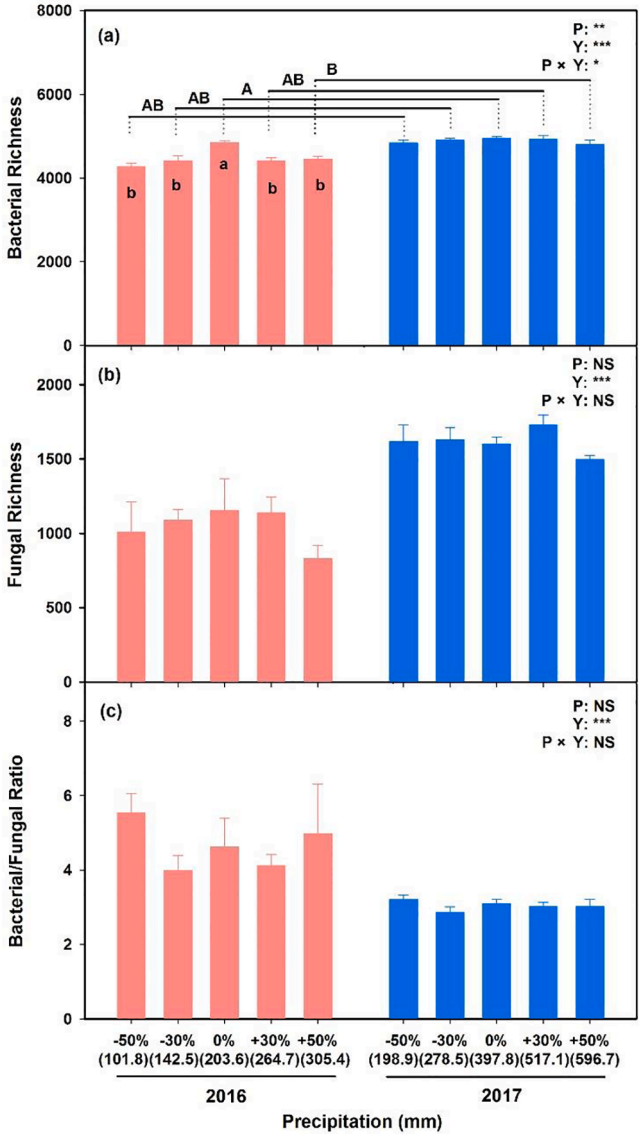

Fig. 2. Bacterial richness (a), fungal richness (b) and bacterial/fungal ratio (c) in different precipitation levels in 2016 and 2017. P: precipitation treatment; Y: year. Significant differences are reported as: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. Values are means ± 1 SE ($n = 4$).

gradient and experimental year were detected only for bacterial OTU richness (Fig. 2a). Inter-annual variation in annual precipitation had greater impacts on fungal OTU richness than bacterial OTU richness, which caused a reduction in the bacterial/fungal ratio in the wet year (Fig. 2c).

We detected strong effects of the precipitation gradient on the bacterial alpha diversity but not on fungal alpha diversity in the dry year. Compared to the ambient precipitation, both decreased and increased precipitation significantly reduced bacterial alpha diversity. The Shannon-Wiener index for bacteria was significantly higher for the ambient precipitation plots; Chao 1 and ACE were highest in the ambient precipitation, followed by the −30% precipitation, and with the lowest value recorded in the −50% precipitation in the dry year. For the wet year, no precipitation gradient effects were detected for bacterial or fungal alpha diversity (STable 2).

The precipitation gradient significantly affected the relative abundances of dominant bacterial and fungal groups in different ways (Fig. 3). The bacterial community was dominated by the phyla *Actinobacteria*, *Proteobacteria*, and *Acidobacteria* (Fig. 3a & b). *Actinobacteria* and *Thaumarchaeota* showed greater abundance in the dry year, while *Proteobacteria*, *Acidobacteria*, and *Gemmatimonadetes* showed greater abundance in the wet year (SFig. 2). The precipitation gradient significantly changed the relative abundances of two fungal phyla (*Zygomycota* and *Glomeromycota*) with the higher value detected for the ambient precipitation plots and the + 30% precipitation plots in the dry year. The most dominant phylum *Ascomycota* (over 60% of the total fungal sequences) showed greater abundance in the wet year than the dry year (SFig. 3).

The precipitation gradient significantly altered the structure of the bacterial community in both years (Fig. 4a & b), but not the structure of the fungal community (Fig. 4c & d). ANOSIM analysis indicated that the bacterial communities in the −50% precipitation ($R = 0.56$, $P = 0.03$) and + 50% precipitation ($R = 0.6$, $P = 0.03$) plots differed significantly from those in the ambient precipitation plots in the dry year. For the fungal community, differences were detected between the −30% precipitation and + 30% precipitation plots ($R = 0.42$, $P = 0.03$) in the dry year. For the wet year differences were detected between 0% and −50% precipitation plots ($R = 0.37$, $P = 0.04$), and between 0% and −30% precipitation plots ($R = 0.49$, $P = 0.03$).

3.6. *Relationships between soil physicochemical properties, plant biomass, and microbial community*

VPA was conducted to assess the relative contributions of plant factors and soil factors to changes in soil microorganisms. Soil factors explained the largest fraction of the variation on bacterial community composition with 44.84% in the dry year (Fig. 5a), but plant factors explained the largest fraction of the variation on bacterial community composition with 20.73% in the wet year (Fig. 5b). For fungal community composition, soil factors and plant factors jointly explained 39.38% (2016) and 41.32% (2017) of the variation, respectively (Fig. 5c & d).

Pearson correlation analysis revealed that among all the soil factors, bacterial diversity had the strongest correlation with TP, followed by soil pH and SWC. For fungal diversity, we found significant positive correlations among SWC, pH, TP, total porosity, WFPS and SOC (Fig. 6).

4. Discussion

4.1. *Soil microbial activity and biomass, and their response to precipitation magnitude*

The precipitation gradient effects were more pronounced in the dry year than in the wet year (SFig. 1). In the dry year, reduced precipitation resulted in soil water scarcity, which constrained plant growth and microbial activity (Delgado-Baquerizo et al., 2013). Lower SWC levels most likely reduced plant transpiration rate, and therefore element uptake (Farooq et al., 2012). The reduced input by plants was likely accompanied by weakened element adsorption capacity of microbes. It is possible that lower microbial activity with drought also reduced their requirements for nutrients (Dijkstra et al., 2015). Despite the precipitation gradient significantly affecting EEAs in the dry year, activities of most studied enzymes were similar across the precipitation gradient in the wet year. This may have been caused by a large extreme precipitation event in 2017 (Fig. 1a) that lead to a period of time with no significant difference in SWC during the sampling period, thereby temporarily reducing the precipitation gradient effects.

Precipitation is a key factor regulating soil microbial biomass (Zeglin et al., 2013; Nielsen and Ball, 2015; Ren et al., 2017). Contrary to our expectations, there were no significant effects detected of the experimental precipitation gradient on MBC, which could be attributed to a large variation in MBC values among the replicated plots (Table 2). Interestingly, the ambient precipitation of 204 mm in the dry year 2016 led to the highest MBC, whereas the −50% precipitation (199 mm) and −30% precipitation (276 mm) treatments yielded higher MBC values in

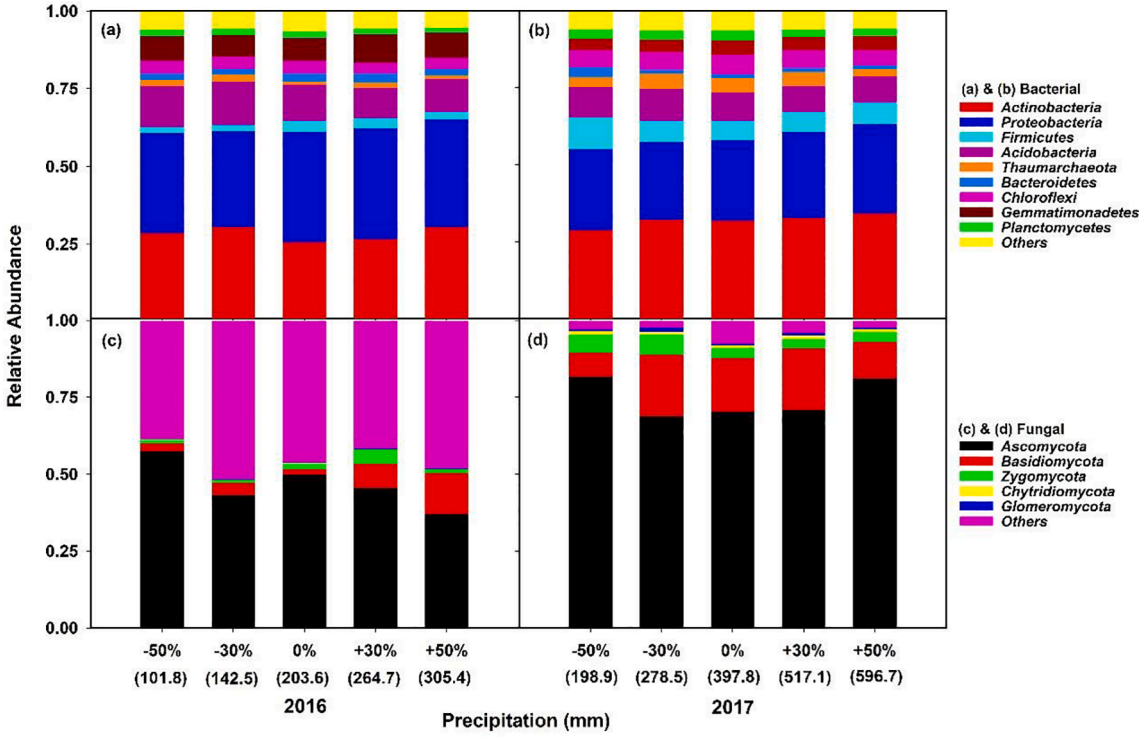

Fig. 3. Relative abundance of bacterial (a & b) and fungal (c & d) phyla as determined by Illumina sequencing in different precipitation levels in 2016 (a & c) and 2017 (b & d).

the wet year. These results support the hypothesis that a moderate range of growing season precipitation (200–280 mm) resulted in higher soil MBC in the studied meadow steppe. Historical precipitation regimes generate regional adaptation in microbial responses to soil moisture, which are likely to constrain the response of microbes to any changes in precipitation patterns (Averill et al., 2016). Long-term annual mean precipitation is 430 mm, but growing season precipitation mean is 241 mm (from early-May to early-August) at our grassland study site, corresponding to the mid-range in the precipitation amount of 200–280 mm that yielded highest MBC values.

4.2. Response of the soil bacterial community to precipitation magnitude

Changes in precipitation and soil water availability led to differential responses of bacterial diversity and structure (Zeglin et al., 2013; Ren et al., 2017). Alpha diversity of 16S bacterial phyla were more sensitive to the altered precipitation in the dry year than the wet year (Richness, Shannon, Chao1, and ACE) (Fig. 2a and STable 2). In the dry year, the ambient precipitation plots had the highest diversity value, which supported our second hypothesis, namely that the optimal precipitation range for bacterial alpha diversity was in the range 200–280 mm. The precipitation reduction treatments significantly decreased soil bacterial alpha diversity, likely because decreased precipitation can greatly constrain microbial growth (Zeglin et al., 2013; Nielsen and Ball, 2015). The increased precipitation also caused a decline in soil bacterial alpha diversity. Some species of bacteria will die from water stress when soil wetting is rapid and cells rupture (Schimel et al., 2007), and isolated heavy rainfall events may cause wetting of multiple soil pore domains and bacterial niches.

Effects of the precipitation gradient on soil bacterial alpha diversity displayed similar patterns, but a smaller effect size, in the wet year compared to the dry year (Fig. 2a and STable 2). Smaller differences among the precipitation gradient plots in 2017 may be associated with the occurrence of the aforementioned extreme rainfall event before the collection of soil samples. The extreme rainfall event enhanced SWC, even in the −50% precipitation subplots, which likely shifted the constraining factors of soil bacterial community diversity from soil water availability to other factors, such as nutrient availability or temperature. Antecedent soil moisture from prior precipitation events can affect many ecological processes (Ogle et al., 2015) and may help to explain some of the differences in the dry *vs.* wet year responses.

NMDS results indicated that the precipitation gradient significantly influenced the bacterial community structure in both years (Fig. 4a & b). Considering that phylogenetic shifts in the composition of soil bacterial community are correlated with community function (Fierer et al., 2012), altered precipitation mainly disturbs the ecological function of the soil bacterial community (Na et al., 2019). This could result in large-scale effects on soil biogeochemical processes, including nutrient cycling (Engelhardt et al., 2018).

4.3. Response of the soil fungal community to precipitation magnitude

Fungal alpha diversity was sensitive to inter-annual differences in precipitation, but not the treatment-induced changes in precipitation amount or current SWC (Fig. 2b and STable 2). The precipitation gradient had little effect on the structure of the fungal community in either year (Fig. 4c & d). This was consistent with the results of Hawkes et al. (2011) and Ren et al. (2017) who found that fungal community responses to altered precipitation were correlated to ambient precipitation and dependent on inter-annual variation in precipitation. We speculate that the lack of significant treatment effects on fungal diversity and structure were mainly because of a threshold effect on fungal hyphal growth under decreased precipitation (Hawkes et al., 2011). Increased precipitation may reduce oxygen (O_2) levels within the soil gas phase, thus affecting root exudate production, coexistence, diversity, and structure of fungal populations and communities (Descamps-Julien and Gonzalez, 2005; Pett-Ridge and Firestone, 2005). Despite these prior results, fungal community diversity was resistant to within-year differences in SWC created by our experimental precipitation gradient.

Our VPA results also showed that other factors were associated with

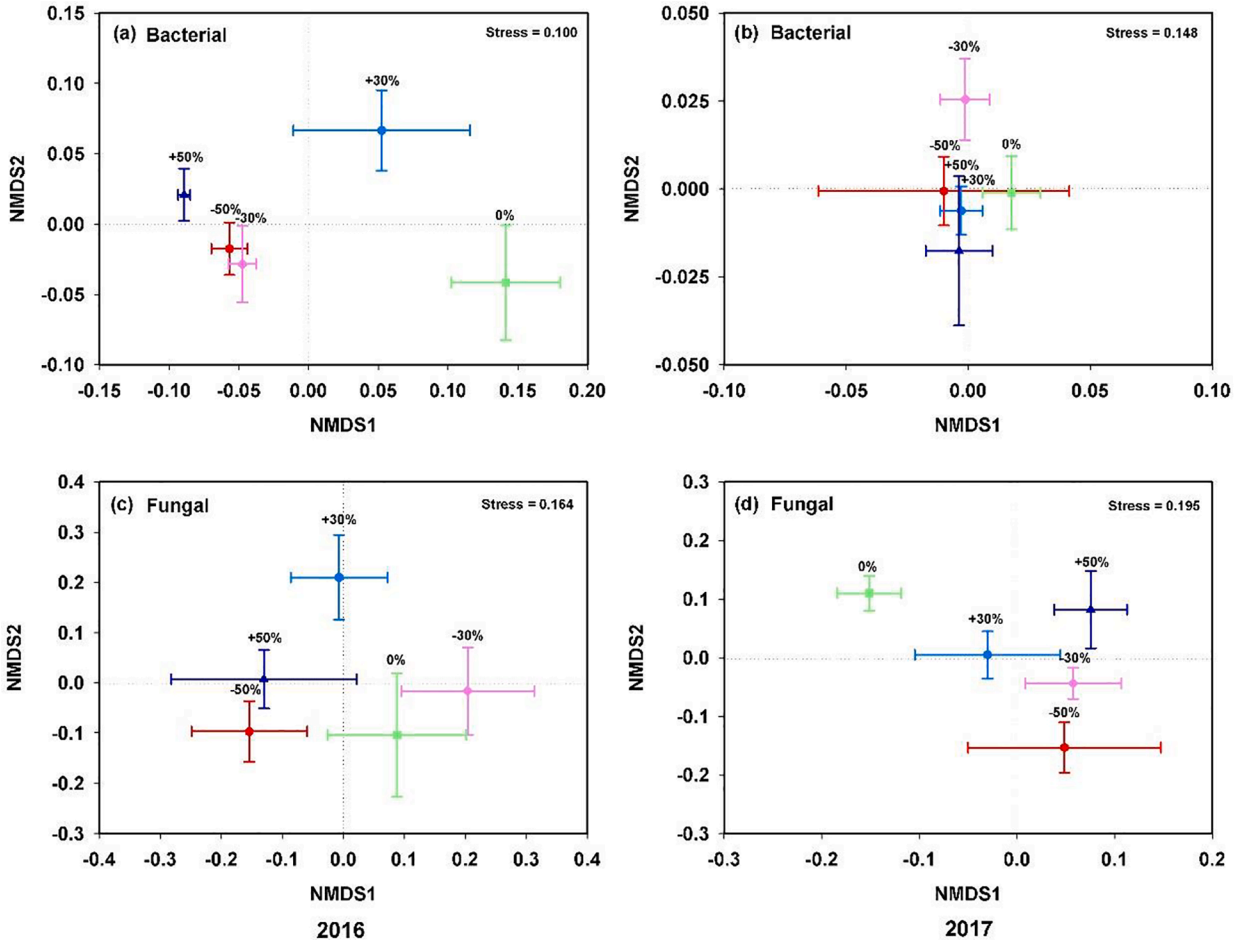

Fig. 4. Non-metric multidimensional scaling (NMDS) of the 16S bacterial (a & b) and ITS fungal (c & d) phyla in different precipitation levels in 2016 (a & c) and 2017 (b & d). The compositional variation is represented with Bray-Curtis distance based on the abundance of OTUs.

fungal community composition and explained 60.62% (2016) and 58.68% (2017) of the variation (Fig. 5c & d). Environmental fluctuations can affect coexistence and stability in fungal communities (Tolkkinen et al., 2015). Certain climatic factors (*e.g.*, mean annual temperature) are usually associated with fungal richness and community composition (Tedersoo et al., 2014). Our results also confirmed that the higher soil fungal richness was positively linked to higher SOC concentration (Fig. 2b and Table 2). Six et al. (2006) reported that fungi on average produce more biomass C per unit of C metabolized than do bacteria, which also leads to a greater proportion of C stored in fungal-dominated systems (Strickland and Rousk, 2010). The bacteria-to-fungi ratio exhibited significant inter-annual differences in our study, and increasing annual precipitation tended to decrease the bacterial/fungal ratio (Fig. 2c). Microbial communities shifted from being dominated by bacteria to fungi, indicating that fungi became prevalent in the year with higher annual precipitation.

Among the dominant fungal groups, the abundance of *Glomeromycota* and *Zygomycota* were significantly increased, especially when precipitation was in the range 200–280 mm (SFig. 3b & c). Likewise, the diversity of dominant phyla of the fungal communities was highest for precipitation in this range. This suggests that certain soil microorganisms have adapted to this range of precipitation that is consistent with long-term growing season precipitation, but not annual precipitation.

4.4. Factors influencing microbial community composition and diversity

Several soil physicochemical properties were measured to identify factors responsible for the changes in soil microbial communities. Pearson correlations indicated that SWC, pH and TP were the best predictors for describing microbial community diversity in our study (Fig. 6). SWC is essential for nutrient diffusion and O_2 content in soils, which could further constrain microbial community diversity (Na et al., 2019). Our results showed that the soil bacterial community tracked soil water availability conditions more closely than the soil fungal community did (Fig. 2a & b), which is consistent with previous findings that fungi are more resistant to variability of soil moisture than bacteria (De Vries et al., 2012; Barnard et al., 2015; Engelhardt et al., 2018).

Soil pH has an important influence on OTU richness of the microbial community, which is in agreement with the results of Siciliano et al. (2014). Soil pH is a master variable in many biogeochemical processes, such as acid-base reactions in soil pore water, ATP membrane transport, and organic matter hydrolysis (Waldrop et al., 2017).

Our results suggest that soil total phosphorus (TP) is a key driver in regulating soil microbial diversity. This result can probably be explained by the strong P limitation in the studied meadow steppe. Arbuscular mycorrhizal fungi can alleviate P limitation and result in the decoupling of N and P (Mei et al., 2019). In addition, in semi-arid ecosystems where N and C are already available for plants and microorganisms, P becomes available through the activity of extracellular phosphatase enzymes, coupling P availability to biological processes (Delgado-Baquerizo et al., 2013). Thus, increased precipitation enhances the availability of P, promoting plant and microbial activity and diversity. Our results indicate that a moderate proportion of soil water availability and P content can maintain soil microbial diversity, further maintaining the productivity of the ecosystem.

We also found that the fungal diversity was positively correlated

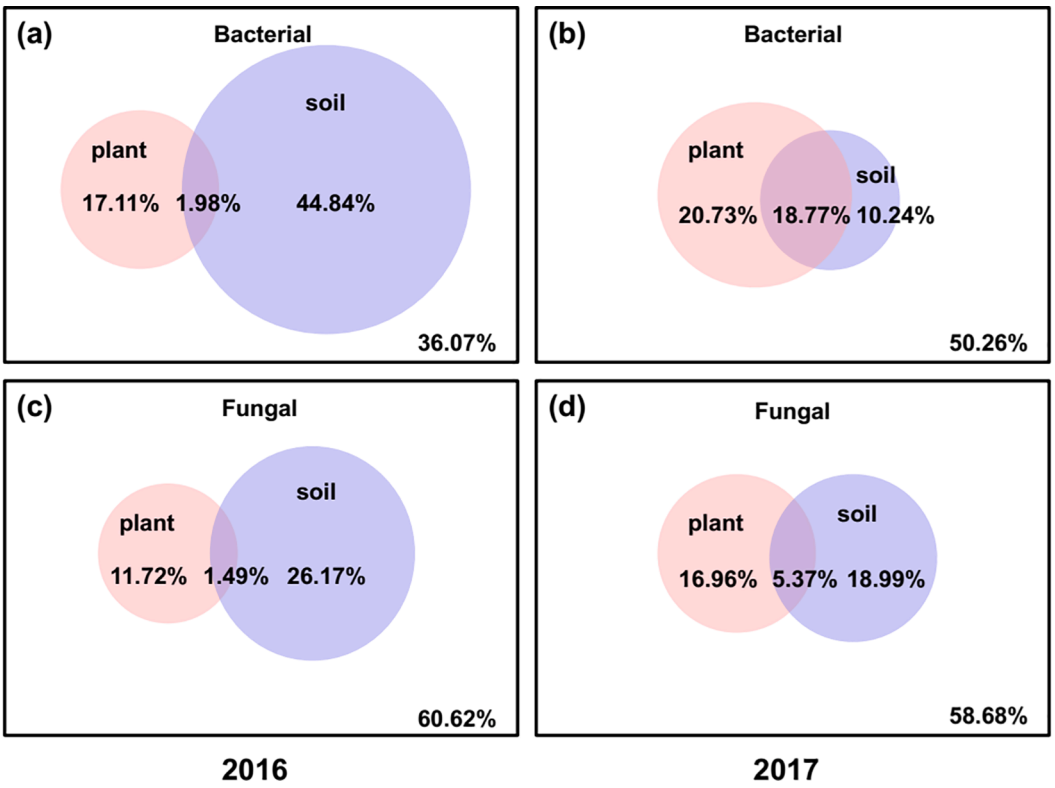

Fig. 5. Variation partition analysis (VPA) for the effects of plant factors and soil factors on the bacterial (a & b) and fungal (c & d) community composition in 2016 (a & c) and 2017 (b & d). The data are percentage (%) of variation explained by the factors.

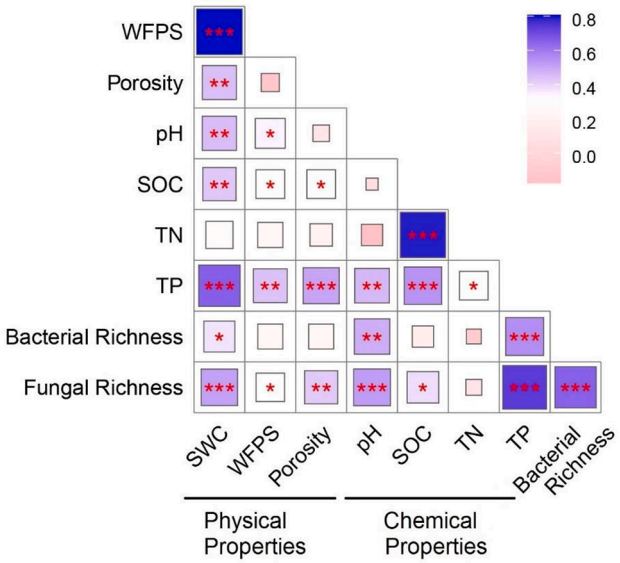

Fig. 6. Pearson correlations between the soil microbial diversity and soil properties. SWC: soil water content; WFPS: soil water filled pore space; Porosity: soil total porosity; SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus. Significant correlations are reported as: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

with the soil total porosity and SOC (Fig. 6). Soil total porosity can strongly interact with SWC to affect fungal communities. Higher porosity increases soil air space at fixed volumetric water content and thus reduces O_2 limitations (Moyano et al., 2013). Fungi may cross air-filled soil pores to access nutrient sources, such as SOC (Barnard et al., 2013; De Vries et al., 2012). It is well known that the microbial growth and activity are highly dependent on SOC (Huang et al., 2008).

Numerous studies have shown that increasing precipitation and soil water availability indirectly increases nutrients in the soil by regulating plant production and C allocation in semi-arid grasslands (Clark et al., 2009; Manzoni et al., 2014). These conditions favor microbial growth and lead to a more complex community composition (Nielsen and Ball, 2015; Hu et al., 2020). Soil hyphal growth can also be affected by rainfall-driven changes in plant production and allocation of photosynthates (Hawkes et al., 2011). However, our results showed that plant biomass was lower in the wet year than in the dry year (Table 1), which might be explained by the following reasons. First, in early-August 2017, an extreme one-time rainfall event increased SWC, which caused large numbers of plants to die because of waterlogging. Second, the sampling date is different between the two study years. The peak plant biomass season in our study area is usually in late-July to early-August. Due to the extreme precipitation event, the sampling date in the wet year was delayed. Thus plant factors can explain only a small part of the variation in microbial community composition (Fig. 5). The specific mechanisms linking plant biomass to microbial community diversity and structure remains unclear, but it may be related to litter quantity and quality.

5. Conclusions

Bacterial diversity and composition were sensitive to the altered precipitation treatments, whereas fungal diversity and composition were highly responsive to inter-annual variation in precipitation. Higher annual precipitation shifted microbial composition from dominance by bacteria to fungi. When shifts in soil microbial communities occurred in response to precipitation, soil water content, total phosphorus, and pH were the most critical drivers, yet the indirect effects of plants also cannot be ignored. According to the results of this field experiment, we predict that shifting precipitation regimes in future climate change scenarios would substantially alter the soil microbial community stability and function, whether precipitation increases due to greater

storminess, or decreases due to more frequent drought. Either way, predicted changes in precipitation patterns may affect the microbial populations, which could result in large-scale effects on soil biogeochemical processes including nutrient cycling.

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.geoderma.2020.114812.

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