

## Changes in soil microbial community response to precipitation events in a semi-arid steppe of the Xilin River Basin, China postprint

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### Abstract

In the context of climate change, precipitation is predicted to become more intense at the global scale. Such change may alter soil microbial communities and the microbially mediated carbon and nitrogen dynamics. In this study, we experimentally repackaged precipitation patterns during the growing season (from June to September) of 2012 in a semi-arid temperate steppe of the Xilin River Basin in Inner Mongolia of China, based on the 60-year growing season precipitation data. Specifically, we manipulated a total amount of 240 mm precipitation to experimental plots by taking the following treatments: (1) P6 (6 extreme precipitation events, near the 1st percentile); (2) P10 (10 extreme precipitation events, near the 5th percentile); (3) P16 (16 moderate precipitation events, near the 50th percentile); and (4) P24 (24 events, 60-year average precipitation, near the 50th percentile). At the end of the growing season, we analyzed soil microbial community structure and biomass, bacterial abundance, fungal abundance and bacterial composition, by using phospholipid fatty acid (PLFA), real-time quantitative polymerase chain reaction (RT-qPCR) and 16S rRNA gene clone library methods. The extreme precipitation events did not change soil microbial community structure (represented by the ratio of PLFA concentration in fungi to PLFA concentration in bacteria, and the ratio of PLFA concentration in gram-positive bacterial biomass to PLFA concentration in gram-negative bacterial biomass). However, the extreme precipitation events significantly increased soil microbial activity (represented by soil microbial biomass nitrogen and soil bacterial 16S rRNA gene copy numbers). Soil fungal community showed no significant response to precipitation events. According to the redundancy analysis, both soil microbial biomass nitrogen and soil ammonium nitrogen (NH<sub>4</sub>-N) were found to be significant in shaping soil microbial community. Acidobacteria, Actinobacteria and Proteobacteria were the dominant phyla in soil bacterial composition, and responded differently to the extreme precipitation events.

Based on the results, we concluded that the extreme precipitation events altered the overall soil microbial activity, but did not impact how the processes would occur, since soil microbial community structure remained unchanged.

## Full Text

### Preamble

#### Changes in Soil Microbial Community Response to Precipitation Events in a Semi-Arid Steppe of the Xilin River Basin, China

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## Abstract

In the context of climate change, precipitation is predicted to become more intense globally, which may alter soil microbial communities and the microbially mediated carbon and nitrogen dynamics. In this study, we experimentally manipulated precipitation patterns during the 2012 growing season (June to September) in a semi-arid temperate steppe of the Xilin River Basin in Inner Mongolia, China, based on 60 years of growing-season precipitation data. Specifically, we applied a total of 240 mm precipitation to experimental plots using four treatments: (1) P6 (6 extreme precipitation events, near the 1st percentile); (2) P10 (10 extreme precipitation events, near the 5th percentile); (3) P16 (16 moderate precipitation events, near the 50th percentile); and (4) P24 (24 events, representing 60-year average precipitation, near the 50th percentile). At the end of the growing season, we analyzed soil microbial community structure and biomass, bacterial abundance, fungal abundance, and bacterial composition using phospholipid fatty acid (PLFA) analysis, real-time quantitative polymerase chain reaction (RT-qPCR), and 16S rRNA gene clone library methods. The extreme precipitation events did not change soil microbial community structure, as represented by the ratio of fungal to bacterial PLFA concentration and the ratio of gram-positive to gram-negative bacterial PLFA concentration. However, extreme precipitation events significantly increased soil microbial activity, as indicated by soil microbial biomass nitrogen and bacterial 16S rRNA

gene copy numbers. The soil fungal community showed no significant response to precipitation events. Redundancy analysis revealed that both soil microbial biomass nitrogen and ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) were significant factors shaping the soil microbial community. Acidobacteria, Actinobacteria, and Proteobacteria were the dominant phyla in soil bacterial composition and responded differently to extreme precipitation events. Based on these results, we conclude that extreme precipitation events altered overall soil microbial activity but did not impact the fundamental processes, since soil microbial community structure remained unchanged.

**Keywords:** extreme precipitation event; phospholipid fatty acid (PLFA); soil microbial community; RT-qPCR; soil bacteria; soil fungi

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## 1 Introduction

General atmospheric circulation models (GCMs) predict shifts in precipitation regimes toward more extreme conditions, characterized by precipitation events with large amounts but low frequency (Easterling et al., 2000; Weltzin et al., 2003; Pachauri et al., 2014). This change represents a remarkable contrast to the historical pattern in semi-arid ecosystems, where precipitation events have typically been small but frequent across relatively short intervals (Heisler-White et al., 2008; Hu et al., 2017). While many studies have focused on the effects of precipitation pulses, rewetting, drought, and changing precipitation amounts on ecosystems (Landesman and Dighton, 2010; Hueso et al., 2012; Bouskill et al., 2013; Sorensen et al., 2013), little research has investigated the effects of shifts in precipitation event patterns on ecosystems—particularly in semi-arid steppes—and their belowground environments, including soil microbial community biomass and structure (Knapp et al., 2008; Sorensen et al., 2013; Knapp et al., 2017).

Persistent shifts in precipitation patterns that fall within the range of statistical extreme climate events can substantially affect soil nutrient cycling and microbial communities in grassland ecosystems (Fierer and Schimel, 2002; Knapp et al., 2002). Soil microbial community structure and biomass are sensitive to changes in precipitation patterns (Steenwerth et al., 2005; Sorensen et al., 2013; Canarini et al., 2016). However, soil bacteria and fungi respond differently to precipitation patterns (Barnard et al., 2013). Soil fungi are generally considered more resistant to water variability than bacteria due to their extensive mycorrhizal networks that facilitate water transfer (Allen, 2011; Bell et al., 2014; Zhang et al., 2016). In contrast, soil bacteria are more sensitive to drought and rewetting at both DNA-based and RNA-based levels in California grasslands, owing to their cell structure and life strategy (Che et al., 2015; Kaisermann et al., 2015). Previous studies have shown that extreme desiccation and rewetting events significantly affect the present and potential active bacterial community but not the fungal community in California grasslands (Barnard et al., 2013).

Alternatively, laboratory experiments have demonstrated that increasing the number of rewetting events not only reduces soil CO<sub>2</sub> loss but also alters soil microbial biomass dynamics (Fierer and Schimel, 2002). Drying-rewetting cycles can decrease soil bacterial biomass while increasing the fungal-to-bacterial growth ratio (Bapiri et al., 2010). Other studies suggest that seasonal precipitation variability alters soil fungal community abundance and consequently influences soil nutrient cycling (Cregger et al., 2012; Bell et al., 2014). Shifts in precipitation event frequency may also affect soil matrix aeration status and soil functional microbes (both directly and indirectly), influencing ecosystem processes more than gradual climate change (Horz et al., 2004, 2005; Jentsch et al., 2007; Zhang et al., 2013). Despite these general patterns, extreme precipitation events in semi-arid ecosystems do not affect soil nutrient availability and microbial communities in the same way as drying-rewetting events or precipitation amount (Belnap et al., 2004; Heisler-White et al., 2008; Chen et al., 2015).

In this study, we established a field experiment simulating different precipitation patterns (frequency and amount) in a semi-arid steppe in the Xilin River Basin, China. Our overall goal was to determine whether shifts in precipitation patterns, as predicted by GCMs, would cause changes in soil microbial community structure and biomass and subsequently affect soil physicochemical properties such as pH, carbon, and nitrogen. We hypothesized that extreme precipitation events (large in amount but low in frequency) would decrease soil bacterial and fungal biomass and alter soil microbial community structure. Additionally, we addressed the following questions: Would extreme precipitation events affect the biomass and abundance of soil bacteria and fungi as well as soil microbial community structure? If so, would soil microbial community structure correlate with soil nutrient pools?

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## 2.1 Study Area

The field experiment was conducted at the Inner Mongolia Grassland Ecosystem Research Station, Chinese Academy of Sciences (43°32' N, 116°40' E; 1200 m a.s.l.), located in a semi-arid steppe in the Xilin River Basin, Inner Mongolia, China. The research site has been fenced since 1979. The region has a temperate continental climate with an annual mean temperature of -0.48°C and mean annual precipitation of 358 mm (Hao et al., 2010). Precipitation during the growing season accounts for 89% of total annual precipitation, with 75% (approximately 240 mm) considered ecologically effective precipitation (EP; daily precipitation >3 mm) (Hao et al., 2011, 2012, 2013). Soil cation exchange capacity is 15.7 cmol/kg, and soil pH is 7.5. The steppe vegetation comprises a mixture of annual grasses, annual and perennial forbs, and perennial shrubs, with xeric rhizomatous grass (*Leymus chinensis*) and needlegrass (*Stipa grandis*) as dominant species (Liu et al., 2017). The soil is loamy sand or dark chestnut.

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## 2.2 Experimental Design

Precipitation treatments were applied during the 2012 growing season (June 1 to September 30) to alter dry interval duration and precipitation event frequency. We maintained a constant total precipitation of 240 mm during the experimental period, approximately equivalent to the 60-year average for the growing season. Four precipitation treatments were established in 16 plots using a randomized block design with four replicates per treatment: (1) P6 (6 extreme precipitation events, near the 1st percentile); (2) P10 (10 extreme precipitation events, near the 5th percentile); (3) P16 (16 moderate precipitation events, near the 50th percentile); and (4) P24 (24 events, representing 60-year average precipitation, near the 50th percentile) (Fig. 1 [Figure 1: see original paper]). Precipitation intervals were 20, 12, 7, and 5 days for P6, P10, P16, and P24 treatments, respectively. The amount of each precipitation event varied according to the 60-year average for the same season, with average event amounts of 10.0 mm (range: 3.8–21.8 mm), 15.0 mm (range: 6.0–26.5 mm), 24.0 mm (range: 11.6–41.5 mm), and 40.0 mm (range: 22.2–72.6 mm) for P24, P16, P10, and P6 treatments, respectively. Precipitation was applied using a sprinkling can. Based on long-term data analysis by Huang et al. (2010), we applied larger events at 2–3-day intervals to ensure that maximum daily precipitation in experimental plots never exceeded 24.0 mm. Volumetric soil water content (SWC) was monitored weekly using a TDR 300 Soil Moisture Meter (Spectrum Technologies, USA) at depths of 5 and 20 cm.

Each plot measured 2 m × 2 m and was surrounded by metal flashing extending approximately 10 cm above and 40 cm below ground to isolate roots and prevent lateral water flux. A rain-exclusion shelter (3 m × 3 m) consisting of a steel frame supporting a clear 0.8-mm-thick fiberglass-reinforced polyester roof (90% light transmission, minimal shading effects) was installed on June 1 in each plot to exclude ambient precipitation. To evaluate microclimate effects, we measured photosynthetically active radiation (PAR) using a LI-190SB quantum sensor (LI-COR, Inc., Lincoln, NE, USA) and air temperature using an HMP45C temperature probe (VAISALA, Woburn, MA, USA) under shelters and in open spaces near plots. No significant differences in PAR or air temperature were found between plots and ambient surroundings.

Historical precipitation data for 1953–2010 were obtained from the Meteorological Administration of Xilin Gol League, but only EP data were used in our analyses.

### 2.3 Soil Sampling and Analysis

At the end of the experimental period (September 30), three soil cores (3 cm diameter, 10 cm depth) were collected from the first three replicate plots of each precipitation treatment and mixed after removing plant litter and roots. Soil samples were sieved through a 2-mm mesh to minimize dilution of surface litter effects on soil microbial community composition. Each sample was divided into two subsamples: one was kept fresh for measurements of microbial community composition, microbial biomass, and soil inorganic nitrogen; the other was air-dried for determination of soil organic carbon (SOC), pH, and total nitrogen (TN).

SOC was determined using the  $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$  oxidation method (Islam and Weil, 1998). TN was analyzed by the Kjeldahl digestion method (Mckenzie and Wallace, 1954). Inorganic nitrogen ( $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$ ) was extracted with 2 M KCl and analyzed using a continuous-flow ion auto-analyzer (AutoAnalyzer 3, Seal Analytical, England). Soil pH was measured in a 1:2.5 soil:water suspension.

Soil microbial biomass carbon and nitrogen were measured using the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987) with modifications. Briefly, 10 g of paired soil samples were weighed and incubated at 4°C for 24 h. One sample was fumigated for 24 h with ethanol-free  $\text{CHCl}_3$  in darkness, while the other remained non-fumigated. Both fumigated and non-fumigated samples were extracted with 0.5 M  $\text{K}_2\text{SO}_4$  on a shaker for 30 min. Extracts were filtered after 30 min of standing and analyzed using a vario TOC analyzer (Elementar, Germany).

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### 2.4 Phospholipid Fatty Acid (PLFA) Analysis

PLFA analysis was used to evaluate coarse-scale changes in soil microbial community structure following Frostegard et al. (1993). Briefly, 8 g of fresh soil sampled at the end of the experiment was extracted with a single-phase mixture of citrate buffer, methanol, and chloroform on a horizontal shaker at room temperature for 2 h. Phospholipids were quantified by gas chromatography-mass spectrometry (GC-MS, Agilent 6850, USA). Fatty acids (14:0, a14:0, a15:0, i15:0, i16:0, 16:1\$5c, 16:17c, i17:0, cy17:0, a17:0, 18:18, 18:17c, cy19:0, and cy20:0) represented bacterial biomass (Frostegard et al., 1993). The fatty acid 18:26,9 indicated fungal biomass (Xue et al., 2005). Fatty acids 10Me18:0, 10Me16:0, and 10Me17:0 determined Actinobacteria. Branched saturated fatty acids (i14:0, a15:0, i15:0, i16:0, i17:0, and a17:0) classified gram-positive bacterial biomass, while cyclopropane-unsaturated and monoenoic fatty acids (16:15c, 16:17c, 16:19c, 17:18c, 18:15c, cy17:0, and cy19:0) classified gram-negative bacterial biomass (Xue et al., 2005). All results were expressed on a dry weight basis.

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## 2.5 Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

DNA was extracted from 0.35 g of fresh soil sampled at the end of the experiment using a soil DNA extraction kit (Mobio Powersoil DNA Isolation Kit, Carlsbad, USA) and quantified with a NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, USA).

RT-qPCR was performed with specific primers on a StepOne Plus Real-time PCR System (Applied Biosystems, USA) in 20- $\mu$ L reaction volumes containing 10  $\mu$ L SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> (Takara Bio Inc., Japan), 10 nM each primer, and 2  $\mu$ L template DNA. Forward primer 338F (5'-ACT CCT ACG GGA GGC AG-3') and reverse primer 518R (5'-ATT ACC GCG GCT GCT GG-3') targeted total bacteria (Edwards et al., 1989). Forward primer gITS7 (5'-GTG ART CAT CGA RTC TTT G-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') amplified the fungal ITS region. Bacterial 16S rRNA gene analysis used the following conditions: denaturation at 94°C for 5 min, followed by 42 cycles of 94°C (60 s), 56°C (45 s), and 72°C (45 s), with final extension at 72°C for 10 min. ITS gene analysis conditions were: denaturation at 95°C for 5 min, followed by 40 cycles of 95°C (20 s), 54°C (25 s), and 72°C (45 s), with final extension at 72°C for 10 min (Ihrmark et al., 2012). Melting curve analyses ensured PCR amplicon specificity (Bustin et al., 2009; Zhang et al., 2017).

Standard curves were generated from serial dilutions of sequences extracted from plasmids (pGEM-T easy vector, Promega) containing 16S rRNA gene and ITS gene fragments amplified from *Escherichia coli* DH5 $\alpha$  competent cells (Takara Bio Inc., Japan). Curves showed linear correlation over six orders of magnitude ( $R^2 > 0.98$ ).

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## 2.6 16S rRNA Gene Clone Library

To analyze soil bacterial community composition in the surface layer (0-10 cm depth), we constructed a bacterial 16S rRNA gene clone library for the four treatments. Approximately 500-bp 16S rDNA fragments were amplified in a MyCycler thermocycler (Bio-Rad, Hercules, CA, USA) using forward primer 27F (5'-GAGTTTGATCMTGGCTCAG-3') and reverse primer 519R (5'-TATTACCGCCGCKGCTG-3') (Hill et al., 2003). PCR conditions were: initial denaturation at 94°C for 5 min, 35 cycles of 94°C (45 s), 56°C (45 s), and 72°C (50 s), and final extension at 72°C for 10 min. PCR products were analyzed by gel electrophoresis in 1% (wt/vol) agarose gel and purified using a gel purification kit (Axygen, USA). Products were ligated into pUCm-T vector (Promega, USA) and transformed into

*Escherichia coli* DH5 $\alpha$  competent cells. Positive clones were screened on indicator plates by color-based selection and confirmed by PCR using pUCm-T vector-specific primers T7 (5'-TAATACGACTCACTATAGGG-3') and M13 (5'-CAGGAAACAGCTATGACC-3') under the same conditions as 16S rRNA gene amplification.

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## 2.7 Data Analysis

PLFA, microbial abundance, and soil property data were analyzed using one-way ANOVA with treatment as a fixed effect in SAS 8.0 (Institute Inc., Cary, USA). Significance levels were set at  $P < 0.05$ . Before testing, normality of error terms was assessed using the Kolmogorov-Smirnov test, and homoscedasticity was evaluated using Levene's test. Non-metric multidimensional scaling (NMDS) of soil microbial PLFA data was performed to determine relationships between community structure and precipitation events, with stress  $< 0.05$  indicating good representation of overall community structure. Redundancy analysis (RDA) tested relationships between PLFA samples and environmental variables. NMDS and RDA were conducted in R 3.2.5 (R Development Core Team, 2016) using the vegan package.

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## 3.1 Variations of Volumetric Soil Water Content (SWC)

Variations in volumetric SWC under different precipitation treatments during the 2012 growing season are shown in Figure 2 [Figure 2: see original paper]. Precipitation treatments significantly altered SWC at 20 cm depth ( $F = 3.32$ ,  $P = 0.02$ ) but not at 5 cm depth ( $F = 2.27$ ,  $P = 0.08$ ). SWC under the four treatments showed significant differences on the same sampling day (Figs. 2a and c). Mean SWC was higher under P24 than under the other three treatments throughout the experimental period at both depths (Figs. 2b and d). Mean SWC ranged from 11.5% under P6 to 14.4% under P24 at 5 cm depth (Fig. 2b), and from 14.4% under P6 to 16.1% under P24 at 20 cm depth (Fig. 2d).

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## 3.2 Soil Properties and Microbial Biomass

During the 2012 growing season, precipitation treatments did not significantly influence SOC ( $F = 2.80$ ,  $P = 0.13$ ), TN ( $F = 0.00$ ,  $P = 0.96$ ), or soil pH ( $F = 0.29$ ,  $P = 0.60$ ) (Table 1). However, treatments significantly affected instantaneous soil properties: NH<sub>4</sub>-N ( $F = 7.77$ ,  $P = 0.03$ ) and NO<sub>3</sub>-N ( $F = 6.94$ ,  $P = 0.03$ ). NH<sub>4</sub>-N values ranged widely (4.36-10.43 mg/kg) among treatments, with higher values under extreme precipitation events (P6 and P10) than under moderate events (P16 and P24; Table 1). NO<sub>3</sub>-N values ranged

from 0.88 to 1.54 mg/kg across treatments, with the lowest value under P6, significantly lower than under P24 (Table 1).

Extreme precipitation events in the P10 treatment significantly influenced soil microbial biomass nitrogen ( $F = 18.89$ ,  $P < 0.01$ ; Fig. 3a [Figure 3: see original paper]). Soil microbial biomass nitrogen under P10 ( $167.51 \pm 6.61$  mg/kg) was twice that under P24 ( $71.69 \pm 0.51$  mg/kg). However, extreme precipitation events (P6 and P10) did not significantly alter soil microbial biomass carbon ( $F = 0.66$ ,  $P = 0.44$ ; Fig. 3b) or the ratio of microbial biomass carbon to microbial biomass nitrogen ( $F = 0.39$ ,  $P = 0.55$ ; Fig. 3c).

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### 3.3 Response of Soil Microbial Community Structure to Precipitation Events

Soil microbial community structure responses to different precipitation treatments are shown in Figure 4 [Figure 4: see original paper]. Precipitation treatments did not significantly influence PLFA concentrations of aerobic bacteria ( $F = 0.37$ ,  $P = 0.56$ ; Fig. 4a), anaerobic bacteria ( $F = 0.03$ ,  $P = 0.86$ ; Fig. 4b), Actinobacteria ( $F = 0.00$ ,  $P = 0.96$ ; Fig. 4c), fungi ( $F = 1.71$ ,  $P = 0.23$ ; Fig. 4d), or total bacteria ( $F = 0.53$ ,  $P = 0.49$ ; Fig. 4e). However, all PLFA concentrations were lower under P6 than under P24, except for Actinobacteria. The lowest PLFA concentrations in aerobic and anaerobic bacteria occurred under P6 and P10 treatments.

Extreme precipitation events did not significantly affect the ratio of fungal to bacterial PLFA concentration ( $F = 1.70$ ,  $P = 0.24$ ; Fig. 4f), with values ranging from 0.11 to 0.13 and a maximum under P24 (0.12). Similarly, the ratio of gram-negative to gram-positive bacterial PLFA concentration was not significantly affected ( $F = 0.24$ ,  $P = 0.63$ ; Fig. 4g). However, the NMDS plot (Fig. 5 [Figure 5: see original paper]) revealed that soil microbial community structure, characterized by overall PLFA concentration, differed between extreme and mean precipitation events (stress = 0.015).

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### 3.4 Relationship Between Soil Nutrient and Microbial Community Structure

RDA showed that soil  $\text{NH}_4\text{-N}$  ( $F = 3.8$ ,  $P = 0.05$ ) and microbial biomass nitrogen ( $F = 5.4$ ,  $P = 0.03$ ) were significant factors shaping soil microbial community structure. The first and second RDA axes explained 53.3% and 1.4% of total variation in soil microbial community structure, respectively (Fig. 6 [Figure 6: see original paper]).

### 3.5 Response of Soil Bacterial and Fungal Abundance to Precipitation Events

Altered precipitation patterns significantly influenced soil bacterial 16S rRNA gene copy numbers ( $F = 9.05$ ,  $P = 0.02$ ; Fig. 7a [Figure 7: see original paper]). Extreme precipitation events tended to increase bacterial abundance, with 16S rRNA gene copy numbers ranging from  $9.38 \times 10^9$  to  $13.20 \times 10^9$  copies/g dry soil across treatments, peaking under P10 and reaching a minimum under P24 (Fig. 7a). However, precipitation treatments did not significantly impact fungal abundance ( $F = 0.60$ ,  $P = 0.63$ ; Fig. 7b). Fungal ITS gene copy numbers ranged from  $4.19 \times 10^9$  to  $4.96 \times 10^9$  copies/g dry soil, with maximum values under P6 and minimum under P10. Fungal ITS gene copy numbers were substantially lower than bacterial 16S rRNA gene copy numbers (Figs. 7a and b).

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### 3.6 Response of Soil Bacterial Composition to Precipitation Events

Identified 16S rDNA sequences contained abundant major bacterial phyla under all four precipitation treatments. The dominant phyla were Acidobacteria, Actinobacteria, and Proteobacteria (Fig. 7c). Compared with P24, mean relative abundance of Actinobacteria increased by 21.0% and 43.8% under P10 and P6, respectively, while Acidobacteria decreased by 49.4% and 67.4% under P10 and P6, respectively. Other common phyla included Gemmatimonadetes, Planctomycetes, Verrucomicrobia, and additional Planctomycetes across all treatments. Notably, Firmicutes was detected only under P6.

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## 4 Discussion

Extreme precipitation events decreased precipitation frequency but increased intensity (amount), thereby extending drought intervals (Gong et al., 2004; Heisler-White et al., 2008; Pachauri et al., 2014). These events differ from drought, dry-rewetting, and precipitation amount patterns (Berard et al., 2011; Barnard et al., 2013; Zhang et al., 2013) by integrating dynamics of all three experimental conditions. We found that extreme precipitation events significantly decreased SWC, particularly at 5 cm depth, which may alter soil microbial communities and microbially mediated carbon and nitrogen dynamics.

Soil microbial communities in the Xilin River Basin temperate steppe exhibited high variability in response to extreme precipitation events during the growing season. We hypothesized that integrated effects of extended dry intervals, precipitation magnitude, and increased dry-rewetting cycles would shift soil microbial community structure toward higher fungi-to-bacteria ratios and lower bacterial abundance, correlating with soil nutrient availability and SWC (Placella

et al., 2012; Bell et al., 2014; Gschwendtner et al., 2014). However, extreme precipitation events did not alter fungal abundance, the ratio of microbial biomass carbon to microbial biomass nitrogen, or the fungal-to-bacterial PLFA ratio, but instead increased bacterial abundance. Higher  $\text{NH}_4\text{-N}$  content likely contributed to increased bacterial abundance under extreme precipitation events. These findings indicate that extreme precipitation events (P6 and P10) did not modify soil microbial properties during the growing season, while fungal communities showed high resistance. In contrast, extreme precipitation events affected bacterial abundance and significantly influenced soil nutrient availability, particularly inorganic nitrogen. Similar results were reported by Bell et al. (2014) in Chihuahuan Desert grasslands, where 25% changes in seasonal precipitation frequency significantly affected soil nutrients and microbial properties.

Changes in precipitation amount, frequency, and timing may impact soil carbon and nutrient pools (Nielsen and Ball, 2015; Wilcox et al., 2015). Extreme precipitation events did not alter SOC or TN but changed soil nutrient availability, including  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  contents, thereby increasing microbial activity (as indicated by microbial biomass nitrogen). Moreover, altered  $\text{NH}_4\text{-N}$  and microbial biomass nitrogen were significantly correlated with soil microbial community structure under extreme precipitation events, likely linked to changes in microbial heterotrophic respiration and rhizosphere activity (Chen et al., 2009; Li et al., 2012), which feedback to underground ecosystem nutrient balance and cycling (Broeckling et al., 2008). Changes in soil inorganic nitrogen reflected more instantaneous responses of the soil nutrient bank to extreme precipitation events. Previous studies have shown that precipitation pulses stimulate nitrogen mineralization and nitrification (Dijkstra et al., 2012), while drought influences nitrogen losses through denitrification (Evans and Burke, 2013).

Fungi are generally considered more resistant to extreme climate than bacteria, especially under drought conditions (Gordon et al., 2008; de Vries et al., 2012; Li et al., 2012). Similarly, we found that fungal abundance and biomass remained stable at both gene and community levels. Changes in the ratios of microbial biomass carbon to nitrogen and fungal-to-bacterial PLFA concentration often indicate shifts in microbial community structure (Zeglin et al., 2013). However, we found the fungal-to-bacterial PLFA ratio was not sensitive to extreme precipitation events. While bacterial abundance and composition were sensitive, the gram-negative-to-gram-positive bacterial PLFA ratio—a stress indicator, as gram-positive bacteria have thicker cell walls and greater drought resistance (Klamer and Bååth, 1998)—was not influenced by extreme precipitation events. Compared with moderate precipitation events, extreme events did not significantly decrease SWC, although SWC has been shown to influence microbial community structure (Tiemann and Billings, 2011; Zeglin et al., 2013).

At the phylum level, Actinobacteria dominated changes in bacterial composition under extreme and moderate precipitation events (Fig. 7c). Mean relative abundance of Actinobacteria was 43.8% higher under P6 than under P24. Subdominant groups included Proteobacteria and Acidobacteria. Previous studies

have shown that under simulated extreme drought, the relative abundance of Actinobacteria (an oligotrophic phylum) increases while Acidobacteria (a copiotrophic phylum) decreases (Fierer et al., 2007; Barnard et al., 2013). These differential responses reflect changes in soil nutrient pools (Barnard et al., 2013). Gram-positive bacteria such as Actinobacteria, with high guanine and cytosine content, can acquire nutrients under adverse conditions (Zvyagintsev et al., 2007). Actinobacteria have been found more drought-resistant than Acidobacteria (Goodfellow and Williams, 1983; Zvyagintsev et al., 2007; Barnard et al., 2013). Similarly, Acidobacteria abundance decreased with increasing precipitation amount (Zhang et al., 2013). In phylum Firmicutes, organisms with gram-positive cell walls and low guanine-cytosine content produce desiccation-resistant endospores, enabling survival under extreme conditions, which partly explains why Firmicutes was detected only under P6.

Overall, extreme precipitation events increased bacterial abundance by up to 40% compared with moderate events, yet we found no significant changes in microbial community structure or biomass among precipitation treatments. This may reflect microbial adaptation strategies to extreme climate events. Different microbial phyla employ different life strategies (Fierer et al., 2007; Barnard et al., 2013). Additionally, soil moisture availability has long been a major constraint on ecosystem productivity in Inner Mongolia grasslands (Bai et al., 2004; Hao et al., 2010), suggesting that soil microbial communities may have adapted to precipitation pattern changes and developed resistance to extreme events (Gong et al., 2004; Bouskill et al., 2013).

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## 5 Conclusions

This study provides important implications for understanding and predicting impacts of changing precipitation events (large amount, low frequency) on soil microbial communities. Our results demonstrate the importance of extreme precipitation frequency—not just amount—on soil microbial community structure and total biomass. We found that extreme precipitation events increased bacterial abundance compared with moderate events, while microbial community structure, total microbial biomass, and fungal abundance remained stable. Bacteria were sensitive to extreme precipitation events in terms of both abundance and composition. These results indicate that soil microbial communities in the Xilin River Basin semi-arid steppe respond differentially to precipitation pattern changes, with bacteria and fungi employing different strategies. We recommend future long-term experiments on extreme precipitation event effects across different ecosystems.

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