

## Responses of soil microorganisms to simulated climate change in desert grassland in northern China

Yi Zhang<sup>a</sup>, Ying-Zhong Xie<sup>a,b</sup>, Hong-Bin Ma<sup>a,b</sup>, Juan Zhang<sup>a</sup>, Le Jing<sup>a</sup>, Yu-Tao Wang<sup>id</sup><sup>a</sup> and Jian-Ping Li<sup>a,b,\*</sup>

<sup>a</sup> College of Agriculture, Ningxia University, Ningxia 750021, China

<sup>b</sup> State Key Laboratory Cultivation Base for Northwest Degraded Ecosystem Recovery and Reconstruction, Ningxia 750021, China

\*Corresponding author. E-mail: lijianpingsas@nxu.edu.cn

 Y-TW, 0000-0002-4100-3078

### ABSTRACT

The study evaluates how simulated climate change affects microorganism communities in the desert grassland of Ningxia Autonomous Region, China. It explores the soil microorganism community and relationships among the soil microorganism community, chemical properties, soil respiration (SR), and plant biomass under climate change. We established a field experiment with five levels of rainfall using rainout shelters and two levels of temperature by the Open-Top Chamber (OTC). The results show that in fungal communities, normal precipitation will promote the number and base number of valid sequences the most, and R66 will significantly promote the mean length of the valid sequence. In the bacterial communities, the interaction of increasing temperature and R133 will promote the number of valid sequences and R166 will promote the length of valid sequences. Neither rainfall nor rising temperature promotes not only the soil community  $\alpha$ -diversity but also the soil microorganism community  $\beta$ -diversity. Soil microorganism communities show resistance to rainfall. SR will limit the soil microorganism diversity. Soil organic carbon (SOC), soil total nitrogen (STN), and soil total phosphorus (STP) will promote soil microorganism abundance (SMA) and soil microorganism diversity (SMS). Aboveground living biomass (ALB) and soil temperature (ST) will promote soil  $\alpha$ -diversity, whereas the effect of root biomass (RB) on the soil  $\alpha$ -diversity is the opposite.

**Key words:** climate change, plant biomass, soil chemical properties, soil microorganism community, soil respiration, desert grassland, plant biomass

### HIGHLIGHTS

- Climate change effects on microorganism communities and relationships in Ningxia Autonomous region are examined.
- Field experiments used five levels of rainfall and two of temperature.
- Results show normal precipitation best promotes the number and base number of valid sequences.
- R66 was found to significantly promote the number of valid sequences.

### INTRODUCTION

Human activities have caused the concentration of atmospheric greenhouse gases, which have increased the global mean temperature by 0.85 °C since 1980 (Shen *et al.* 2020). The Intergovernmental Panel on Climate Change (IPCC)'s Fifth Assessment Report (AR5) suggests that the climate warming system is unquestionable. The effect of geographical patterns of rainfall on global climate change is one of the significant challenges in the supply of water for agricultural and ecology activities (Daba & You 2020). It is speculated that drought-stressed areas occupy nearly 30% of the total global land area. For example, the reduced rainfall, in some regions, is one of the most severe problems facing sustainable agriculture due to the rising temperature. The global annual and monthly rainfall erosivity decreased from 1980 to 2017 (Liu *et al.* 2020). Temperature and rainfall are the compelling factors in determining biodiversity and ecosystem function in terrestrial ecosystems, and rainfall patterns are expected to shift, as climate warming has become a reality.

The feedback of soil microorganism communities to climate change is positive in a relatively arid ecosystem (approximately 350 mm mean annual rainfall). In a moister prairie ecosystem, a negative feedback was proved by researchers (approximately

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900 mm mean annual rainfall). Thus, it is significant to research microorganism feedback, especially in ecosystem types under concurrent changes of temperature and rainfall. The aboveground plant communities are also affected. The semi-arid steppe ecosystem in northern China is a crucial component of the Eurasian grassland biome. The changes in moisture and temperature conditions also affect aboveground plant communities (Chen *et al.* 2020), which manage the type and abundance of many organic substrates provided by soil microorganism heterotrophs. Hence, climate change should also indirectly impact microorganism communities through shifts in plant composition and productivity.

A number of recent studies have investigated the soil microorganism response to climate change from different perspectives and have achieved significant results. However, there are still some blind spots that need further inquiry as follows:

- How can the soil microorganism community in desert grassland ecosystems be evaluated under the interaction of temperature and rainfall? As temperature and rainfall change continues, have sensitive indicators in ecosystem components changed?
- In the context of climate change, what are the correlations among soil respiration (SR), soil microorganism abundance (SMA), soil microorganism diversity (SMS), and soil microorganism coverage (SMC)?
- How do soil properties respond to SMA, SMS, and SMC?
- How does plant biomass affect the soil microorganism community?

To further research the above-mentioned blind spots, our study chooses the desert steppe (south edge of the Mu Us Sandy Land) of Yanchi County, Ningxia as the research object. To simulate temperature increases, we used the OTC (Open-Top Chamber) and shelters and artificial watering to simulate rainfall changes under the interaction of human factors: (i) the soil microorganism community; (ii) the relationship between the soil microorganism community and SR; (iii) the response of soil chemical properties to the soil microorganisms community; and (iv) the main driving factors on the soil microorganisms community. This research provides a reliable theoretical basis for formulating a reasonable response strategy in a desert steppe.

## MATERIALS AND METHODS

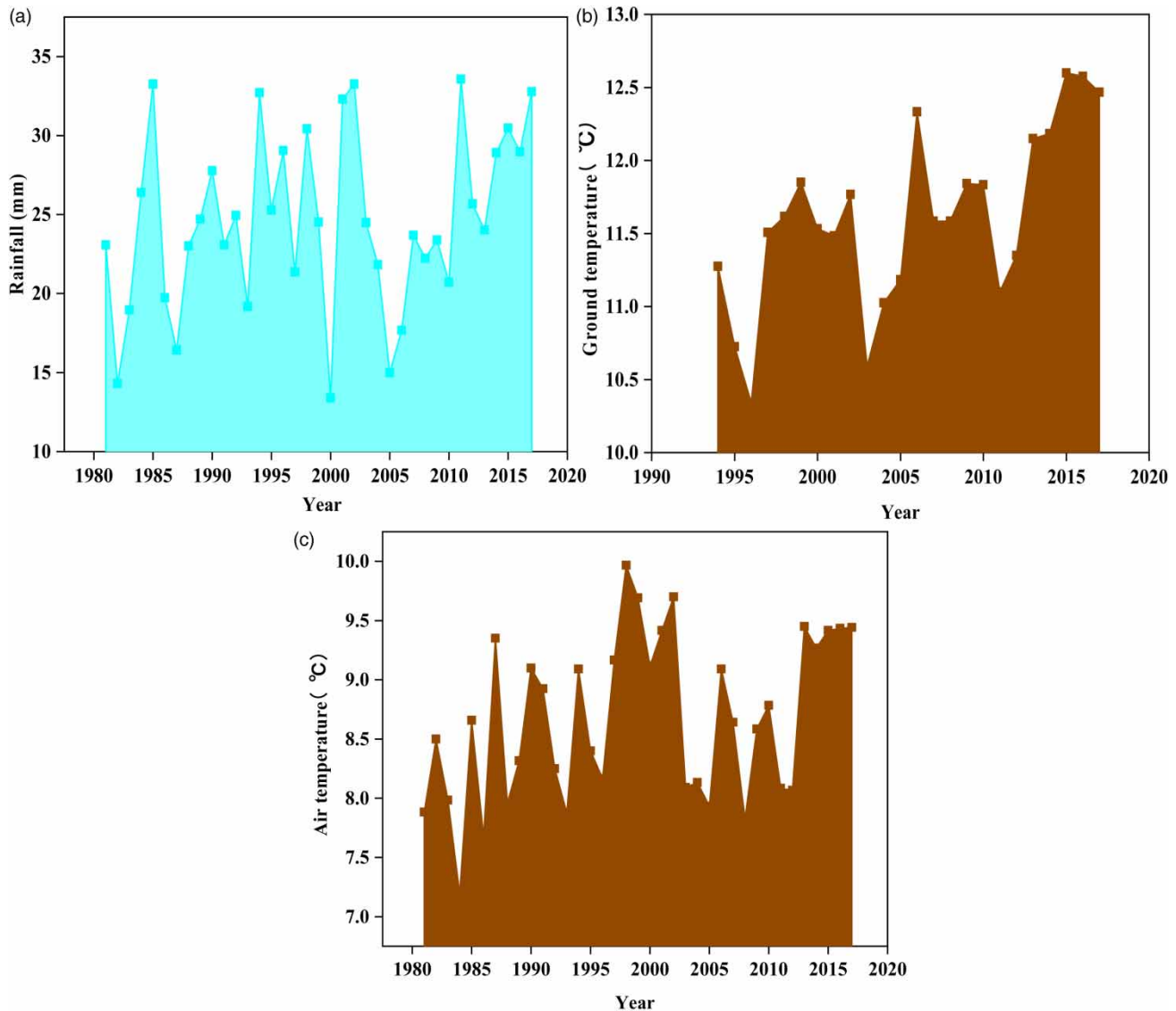
### Study site

The study site is located in the desert steppe of the Ningxia Autonomous Region, China (37°47'N, 107°25'E). The climate is of a typical continental climate with an average annual temperature of 8.1 °C. The annual accumulated temperature is 3,430.3 °C, and the annual rainfall is 295 mm (average in 1981–2017). The rainfall from July to September accounts for approximately 61% of the annual total. The annual evaporation is 2,131.8 mm, and the frost-free period is about 162 days. The soil is light gray calcareous soil, sandy soil, and silt soil. Vegetation is a desert steppe, mainly dominated by xerophytes and mesophytes. The main distribution is perennial plants such as *Stipa breviflora*, *Cleistogenes squarrose*, *Leymus scallions*, and *Lespedeza davurica*, and annual plants such as *Setaria viridis* and *Salsola collina*.

### Experimental design

According to the meteorological monitoring on the study site from 1981 to 2017, the annual average rainfall, ground temperature, and air temperature all showed a rising trend. Based on the 37-year average rainfall and fluctuation extremes, 66 and 133% rainfall gradients were achieved using artificial rain-collecting greenhouses and sprinkler irrigation techniques to ensure that the rainfall treatment is within the range of natural rainfall extremes. Due to the steady increase of ground temperature and atmospheric temperature, two temperature increase gradients are set, and the OTC device is used to achieve a temperature increase of about 2 °C (data from the preliminary experiment) (Figure 1).

The designed rainout shelter was well-ventilated in November 2018 (Figure 2). The rainfall gradient is constructed with artificial shelters and artificial sprinklers. A two-factor completely randomized experimental design is used based on rainfall and temperature factors. Five levels of rainfall are used: 33% (R33), 66% (R66), 100% (CK), 133% (R133), and 166% (R166) of annual average. The first two rainfall conditions are obtained by using the following two rainout shelters with manipulated rainfall doses: 97 mm (R33) and 194 mm (R66). For the other three rainfall conditions, unsheltered plots in addition to rainfall are used: 295 mm (CK), 392 mm (R133), and 490 mm (R166). Rainfall is increased using a watering pot. Temperature is two levels of the actual temperature (CK), and the interaction between the rainfall and temperature increases about 2 °C (T) to achieve a temperature change using the OTC in each plot. The area of each plot is (6 × 6) m, and each treatment ( $n = 5$ ) is repeated three times, for a total of 15 plots. (The diagram at the top left of each plot corresponds to the OTC, and thus, the temperature treatment is applied to each rainfall condition.) On the 15th and 30th of each month, R33 and R66 of the actual



**Figure 1** | Rainfall, ground temperature, and air temperature from 1981 to 2017.

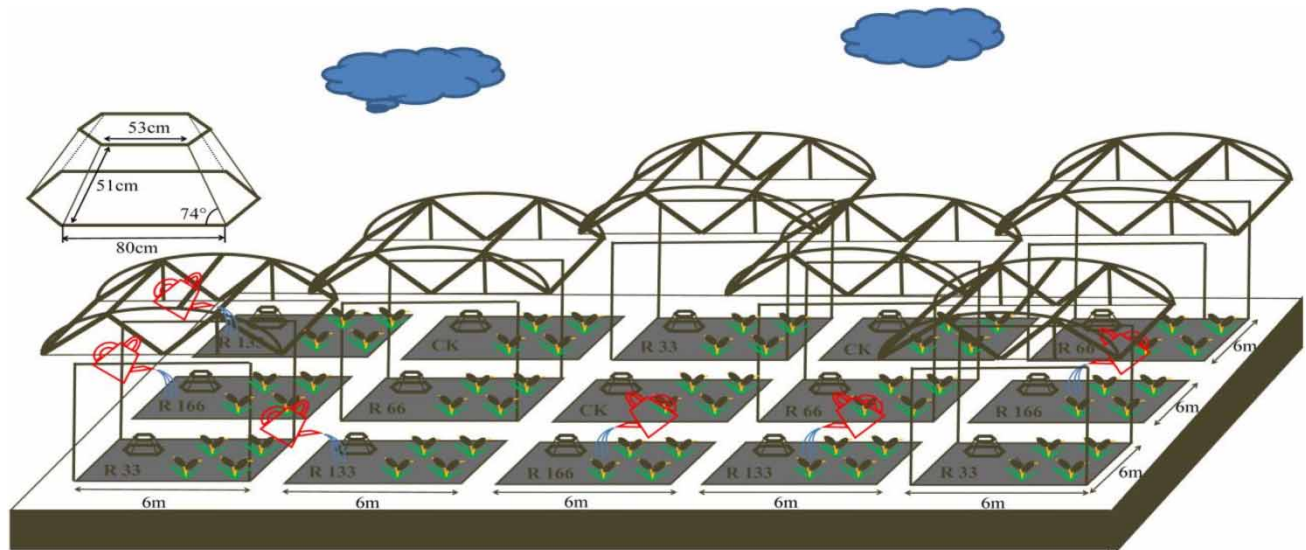
rainfall during 1st–15th and 16th–30th of the month are collected from the actual rainfall, respectively, and then evenly replenished to the plots containing R133 and R166 using a watering pot.

### Collection of soil microorganism samples

In each plot, including the inner OTC, we took 30 cm of soil from each sample plot (inner and outer OTC) and then divided it into three layers, 0–10, 10–20, and 20–30 cm, separately. We removed covering from soil (plants, moss, visible roots, litter, and visible soil animals) and wiped the sample with alcohol cotton. After the alcohol had completely evaporated, a 6 cm diameter drill was used to soak the sample (S1 Canada). The steps were repeated every time the sample was changed. Three samples were taken from the same plot and mixed as one soil sample. Then, we mixed soil into a 10 ml centrifuge tube and transferred it to a  $-80^{\circ}\text{C}$  refrigerator for the determination of soil microbes. [Tables 1](#) and [2](#) show the data analysis of the soil sample.

### Soil property analysis

SR was measured using an open soil  $\text{CO}_2$  flux system (LI-8100 Automated Soil  $\text{CO}_2$  Flux System, Li-COR, Lincoln, NE, USA). For a sample of each plot in the square, a circular PVC pipe with a diameter of 20 cm and a height of 3 cm was embedded in



**Figure 2** | Rain shelter construction and OTC arrangements of the subplots at the study sites. The factors are precipitation and temperature, and five levels of precipitation are 33, 66, 100, 133, and 166% of normal rainfall (recorded as R33, R66, CK, R133, and R166), two rainout shelters with manipulated precipitation doses (i) of 97 mm (33% of annual average) and (ii) 194 mm (66% of annual average), along with three unsheltered plots with manipulated precipitation doses (iii) of 295 mm (normal annual precipitation), (iv) 392 mm (133% of annual average), and (v) 490 mm (166% of annual average), increased precipitation due to watering pot. Temperature is two levels of the actual temperature, and the interaction between the rainfall and temperature increases about 2 °C to achieve a temperature change by the OTC in each plot (recorded as CK and T).

**Table 1** | Soil fungal sequence information in the grassland (mean ± SE)

| Sample | Number of valid sequence | Base number of valid sequence | Mean length of valid sequence |
|--------|--------------------------|-------------------------------|-------------------------------|
| TR33   | 61,229.75 ± 1,435a       | 11,508,850.75 ± 259,397b      | 188.03 ± 2.52a                |
| R33    | 49,461.33 ± 7,859b       | 12,652,044.33 ± 1,907,253a    | 256.48 ± 2.35a                |
| TR66   | 47,107.33 ± 3,532b       | 11,964,428.67 ± 663,741b      | 254.81 ± 5.97a                |
| R66    | 50,879.67 ± 8,124b       | 13,105,869.67 ± 1,966,051a    | 258.49 ± 3.61a                |
| TCK    | 53,078.33 ± 4,334b       | 13,578,317.67 ± 381,018a      | 258.36 ± 6.17a                |
| CK     | 72,175.01 ± 1,352a       | 17,110,279.67 ± 215,969a      | 237.14 ± 2.68b                |
| TR133  | 59,858.01 ± 5,609a       | 15,308,982.67 ± 1,249,745a    | 256.38 ± 3.38a                |
| R133   | 61,703.78 ± 4,505a       | 15,332,526.67 ± 795,513a      | 250.63 ± 3.89b                |
| TR166  | 69,618.03 ± 2,953a       | 16,479,432.33 ± 508,250a      | 236.96 ± 3.52a                |
| R166   | 55,546.67 ± 5,098a       | 13,059,563.67 ± 1,375,884a    | 234.51 ± 3.68b                |

Different lowercase letters indicate significant ( $p < 0.05$ ) differences between the variation percentage of precipitation. The factors are precipitation and temperature, and five levels of R are 33, 66, 100, 133, and 166% of normal precipitation (recorded as R33, R66, CK, R133, and R166), two rainout shelters with manipulated precipitation doses (i) of 97 mm (33% of annual average) and (ii) 194 mm (66% of annual average), along with three unsheltered plots with manipulated precipitation doses (iii) of 295 mm (normal annual precipitation), (iv) 392 mm (133% of annual average), and (v) 490 mm (166% of annual average), increased precipitation due to watering pot. Temperature is two levels of the actual temperature, and the interaction between the rainfall and temperature increases about 2 °C to achieve a temperature change by the OTC in each plot (recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase of about 2 °C (T), R33 is the first site of 33% rainfall and other marks are the same.

the soil to a depth of 12 cm. After that, we removed the plants inside the circular pipe and then measured SR. The measurement frequency was once every 15 days, and the time point was 10 am–2 pm. A 30 cm soil sample (divided evenly into three layers, 10 cm each layer) was drilled in each plot and then taken in separate plastic to test the physical and chemical properties of the soil.

Soil organic carbon (SOC) was measured by the external heating method: potassium dichromate–sulfuric acid digestion, ammonium ferrous sulfate titration (Titrette 50 ml automatic titrate), and soil total nitrogen (STN) were tested using an elemental analyzer (Vario EL/micro cube, Germany). Soil total phosphorus (STP) was studied by sulfuric acid–perchloric

**Table 2** | Soil bacterial sequence information in the grassland (mean  $\pm$  SE)

| Sample | Number of valid sequence | Base number of valid sequence  | Mean length of valid sequence |
|--------|--------------------------|--------------------------------|-------------------------------|
| TR33   | 40,642.67 $\pm$ 2,517b   | 16,956,583.67 $\pm$ 1,036,601b | 417.25 $\pm$ 51.39a           |
| R33    | 56,770.01 $\pm$ 4,088a   | 23,712,154.01 $\pm$ 1,725,704a | 417.64 $\pm$ 44.34a           |
| TR66   | 11,751.47 $\pm$ 6,785b   | 4,910,451.396 $\pm$ 2,835,133b | 417.78 $\pm$ 26.10a           |
| R66    | 39,241.67 $\pm$ 1,329b   | 16,379,041.67 $\pm$ 578,387b   | 417.35 $\pm$ 35.59a           |
| TCK    | 46,201.01 $\pm$ 7,632b   | 19,284,866.01 $\pm$ 3,199,386b | 417.33 $\pm$ 41.10a           |
| CK     | 65,527.67 $\pm$ 6,013a   | 27,335,827.01 $\pm$ 2,514,505a | 417.14 $\pm$ 25.11a           |
| TR133  | 70,880.67 $\pm$ 7,143a   | 29,579,004.33 $\pm$ 3,019,459a | 417.21 $\pm$ 22.83a           |
| R133   | 61,646.01 $\pm$ 5,309a   | 25,745,725.33 $\pm$ 2,543,678a | 417.59 $\pm$ 43.86a           |
| TR166  | 58,378.33 $\pm$ 743a     | 24,355,855.33 $\pm$ 3,348,754a | 417.20 $\pm$ 45.86a           |
| R166   | 45805.01 $\pm$ 7871b     | 19,136,455.01 $\pm$ 3,269,671b | 417.95 $\pm$ 46.69a           |

Different lowercase letters indicate significant ( $p < 0.05$ ) differences between the variance percentage of precipitation. The factors are precipitation and temperature, and five levels of R are 33, 66, 100, 133, and 166% of normal precipitation (recorded as R33, R66, CK, R133, and R166), two rainout shelters with manipulated precipitation doses (i) of 97 mm (33% of annual average) and (ii) 194 mm (66% of annual average), along with three unsheltered plots with manipulated precipitation doses (iii) of 295 mm (normal annual precipitation), (iv) 392 mm (133% of annual average), and (v) 490 mm (166% of annual average), increased precipitation due to watering pot. Temperature is two levels of the actual temperature, and the interaction between the rainfall and temperature increases about 2 °C (N) to achieve a temperature change by the OTC in each plot (recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase of about 2 °C (T). R33 is the first site of 33% rainfall, and other marks are the same.

acid digestion, antimony molybdenum calorimetry, and UV spectrophotometer determination (Hyener I5 Photometer). The soil pH value was measured by an acidifying agent (PHS-3C pH audiometer, China).

### Plant biomass measurement

Plant biomass was measured in a 1 m<sup>2</sup> quadrant, which was randomly selected for outer OTC and 0.25 m<sup>2</sup> inner OTC in each plot at the end of July 2019. All plants in each plot were dug from the soil, and then the aboveground living plant was cut. Moreover, the plant roots were cut and sorted according to the species and placed in their respective envelopes. Finally, these species were taken to the laboratory and dried at 65 °C in the oven for 48 h. Then, the plant aboveground living biomass (ALB) and plant root biomass (RB) were calculated. All plant samples are collected from the desert grassland, and the ownership of the grassland belongs to Ningxia University. Therefore, we were able to conduct experiments on the grassland without a license certificate.

### DNA extraction and polymerase chain reaction amplification

The microorganism community's genomic DNA was extracted from soil samples using the MP Fast DNA Spin Kit for soil according to the manufacturer's instructions (MP Biomedicals, Irvine, CA, USA). The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined by the Nano Drop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The primer region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by an ABI Gene Amp<sup>®</sup> 9700 PCR thermocycler (ABI, CA, USA). The polymerase chain reaction (PCR) amplification of 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, and single extension at 72 °C for 10 min and 10 °C until halted by the user.

The PCR mixtures contain 5× Transport Fast Pfu buffer, 4 µL; 2.5 mM dNTPs, 2 µL; forward primer (5 µM), 0.8 µL; reverse primer (5 µM), 0.8 µL; Trans Start Fast Pfu DNA polymerase, 0.4 µL; template DNA, 10 ng; and finally, H<sub>2</sub>O added up to 20 µL. PCRs were performed in triplicate. The PCR product was extracted from 2% agarose gel and purified using the Axy Prep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using the Quantus<sup>™</sup> Fluorometer (Promega, Madison, WI, USA). We determined the  $\alpha$ -diversity and  $\beta$ -diversity of soil microorganism samples using the metabarcoding approach.

### Statistical analysis

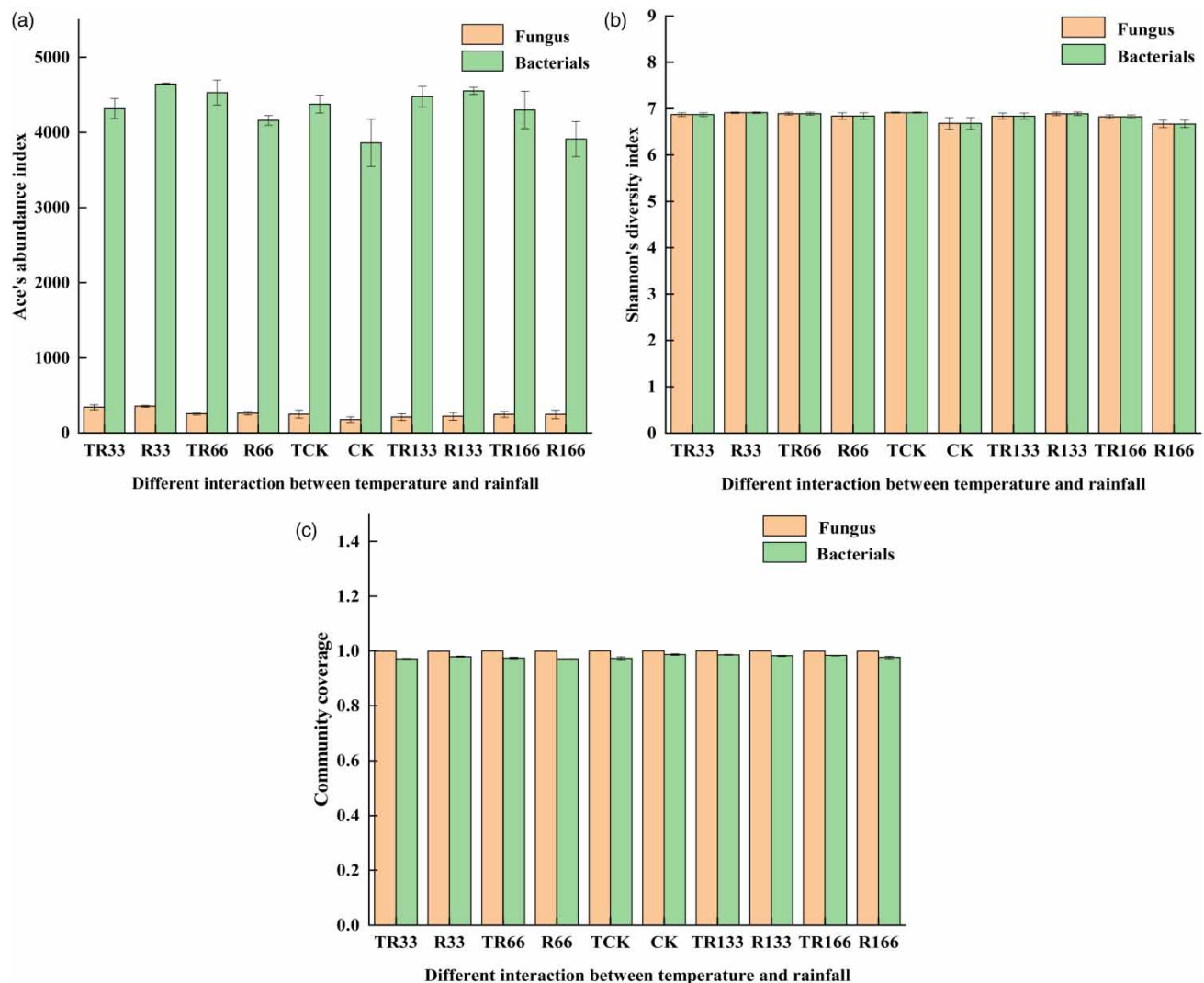
Statistical analyses and figures of rain shelter construction and OTC arrangements of the subplots at the study sites were performed using the Microsoft Excel. The figures of  $\alpha$ -diversity of soil samples, species community composition, and community abundance of  $\beta$ -diversity by principal co-ordinates analysis (PCoA) were performed using Origin 21.0. All data were analyzed

first using a ‘repeated-measures’ statement and the standard errors of individual means were obtained. Meanwhile, correlation analysis and regression equation were performed in SPSS 21.0. Redundancy analysis (RDA) eliminates redundant variables depending on other measured variables, which are based on Canon 5.

## RESULTS AND DISCUSSION

### Soil microorganism community

The  $\alpha$ -diversity of the soil microorganisms in our study includes the following three parts: SMA (the ACE estimator, which explains the soil microorganism abundance), SMS (the Shannon Diversity Index), and SMC (the Good’s coverage). The number of the bacterial community’s richness is obviously more than the fungal community’s richness. However, the SMA, SMS, and SMC of fungal and bacterial communities do not show significant differences under temperature and precipitation increases (Figure 3).



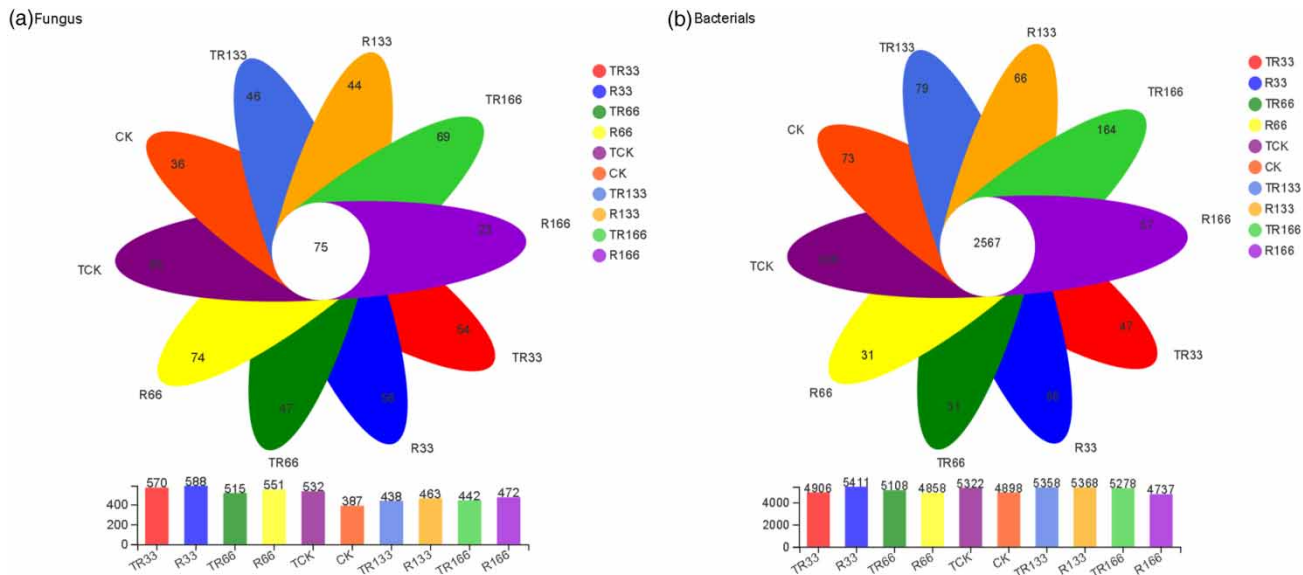
**Figure 3** |  $\alpha$ -diversity of soil samples of (a) community abundance, (b) community diversity, and (c) community coverage in the study sites. The factors are precipitation (R) and temperature (T), and five levels of precipitation are 33, 66, 100, 133, and 166% of normal precipitation (recorded as R33, R66, CK, R133 and R166), two rainout shelters with manipulated precipitation doses (i) of 97 mm (33% of annual average) and (ii) 194 mm (66% of annual average), along with three unsheltered plots with manipulated precipitation doses (iii) of 295 mm (normal annual precipitation), (iv) 392 mm (133% of annual average), and (v) 490 mm (166% of annual average), increased precipitation due to watering pot. Temperature is two levels of the actual temperature, and the interaction between the rainfall and temperature increases about 2°C to achieve a temperature change by the OTC in each plot (recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase about of 2 °C (T), R33 is the first site with 33% rainfall, and other marks are the same.

Under different interactions of temperature and rainfall treatments, the number of bacteria in soil was significantly higher than the number of fungi. In fungal communities, the total kinds of microbes are highest under R33, the number of common species of microbes' operational taxonomic units (OTUs) under different treatments was 75, and the number of unique species of microbes' OTUs under R166 was the highest. In the bacterial communities, the total kinds of microbes are also highest under R33. The number of common species' OTUs of microorganisms under different treatments is 2,576, and the number of unique species' OTUs is highest under the interaction of normal rainfall and temperature (Figure 4).

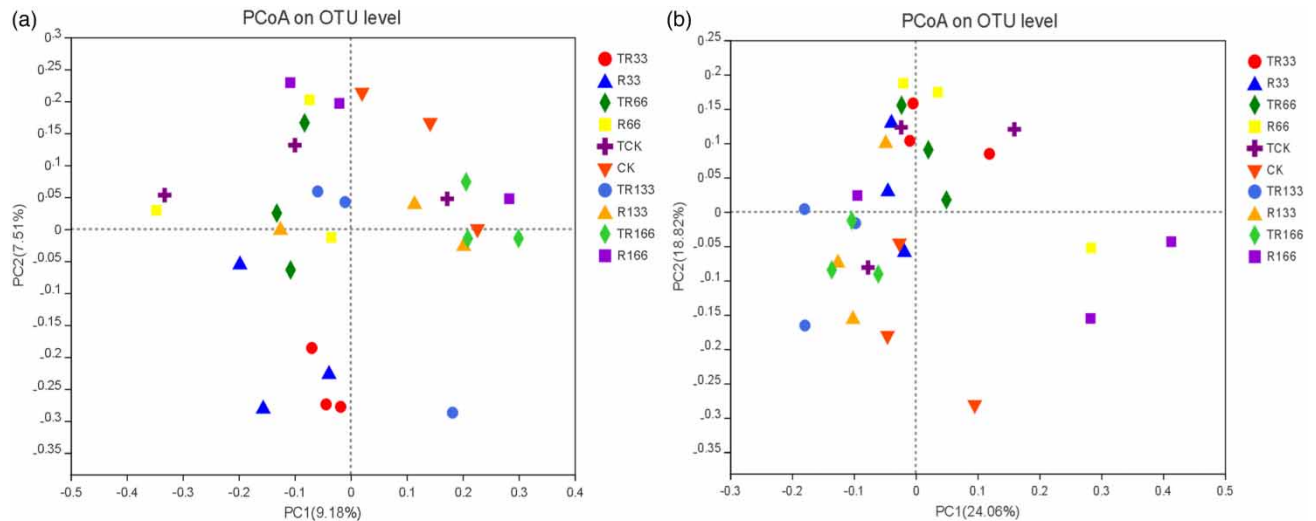
In the fungal communities, the distance between each sample point is the farthest under R166. Thus, the corresponding  $\beta$ -diversity is the highest. However, sample points have the shortest distance under TR33; therefore, the corresponding  $\beta$ -diversity is the lowest. In the bacterial communities, the distance between each sample point is farthest under R166. Thus, the corresponding  $\beta$ -diversity is the highest. However, sample points have the shortest distance under R33; therefore, the corresponding  $\beta$ -diversity is the lowest (Figure 5).

In the fungal communities, the number of valid sequences and the base number of valid sequences are the highest at CK. This may be because normal precipitation will promote the number and base number of valid sequence. The mean length of a valid sequence is highest under R66 (Table 1), which may be because R66 will promote the mean length of the valid sequence. In the bacterial communities, the number of valid sequences is highest under TR133, perhaps because that may be due to the interaction of increasing temperature and R133 will promote the number of valid sequences, the base number of valid sequences is highest under TR66, and the reason may be that the interaction of increasing temperature and the mean length of valid sequences are highest under R166 (Table 2). The reason may be because R166 will promote the mean length of the valid sequence.

The number of SMA of bacterial  $\alpha$ -diversity is obviously more than the fungal communities. The reason may be that bacterial spores have strong drought-resistance ability. However, the temperature and precipitation increases have few effects on SMA, SMS, and SMC of fungal and bacterial communities (Figure 3). This may be because the temperature increase is only 2 °C and the range of precipitation is such a small change that it hardly changes growth and reproduction of the fungal and bacterial communities.



**Figure 4** | Species community composition analysis of (a) fungal and (b) bacterial communities in the study sites. The factors are precipitation and temperature, and five levels of precipitation are 33, 66, 100, 133, and 166% of normal precipitation (recorded as R33, R66, CK, R133, and R166), two rainout shelters with manipulated precipitation doses (i) of 97 mm (33% of annual average) and (ii) 194 mm (66% of annual average), along with three unsheltered plots with manipulated precipitation doses (iii) of 295 mm (normal annual precipitation), (iv) 392 mm (133% of annual average) and the interaction between the rainfall and temperature increases about 2 °C to achieve a temperature change by the OTC in each plot (recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase of about 2 °C (T), R33 is the first site with 33% rainfall, and other marks are the same.



**Figure 5** | Community abundance of  $\beta$ -diversity of (a) fungal and (b) bacterial communities in the study sites by the PCoA. The factors are precipitation and temperature, and five levels of precipitation are 33, 66, 100, 133, and 166% of normal precipitation (recorded as R33, R66, CK, R133, and R166), two rainout shelters with manipulated precipitation doses (i) of 97 mm (33% of annual average) and (ii) 194 mm (66% of annual average), along with three unsheltered plots with manipulated precipitation doses (iii) of 295 mm (normal annual precipitation), (iv) 392 mm (133% of annual average), and (v) 490 mm (166% of annual average), increased precipitation due to watering pot. Temperature is two levels of the actual temperature, and the interaction between the rainfall and temperature increases about 2 °C to achieve a temperature change by the OTC in each plot (recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase of about 2 °C (T), R33 is the first site with 33% rainfall, and other marks are the same.

R166 promotes the number of unique species of microbe OTUs (Figure 4), maybe because in the desert grassland, the microorganisms do not have enough water to absorb to live, so when we supplement the water to our study site, the microbes get proper water to live.

Temperature and rainfall do not have any significant impact on microorganism activities because these indexes adapt to environmental changes caused by experimental treatment through self-regulation. However, the  $\beta$ -diversity of fungal and bacterial communities is highest in R166. Therefore, rainfall increases have a significant impact on microorganism activities (Figure 5), which agrees with the previous studies.

The total kinds of fungal and bacterial communities are high under R33, so reducing rainfall could promote the microorganism species. The reason may be environmental adaptability; and microbes have better tolerance to environmental moisture changes, thus showing resistance to rainfall changes.

The effect of temperature on the fungus and bacteria is not significant, and the abundance of fungal and bacterial communities is lowest under natural rainfall. With the continuous increasing temperatures and decreasing rainfall, the microorganism abundance gradually increases. Hence, the difference between natural rainfall and rainfall changes is significant because the change in rainfall will promote the increase of the microorganism abundance.

### Relationship between the soil microorganism community and SR

The final product of respiration is CO<sub>2</sub> and new cytoplasm. More than 70% of the CO<sub>2</sub> loss caused by SR comes from soil microorganism respiration.

In fungal communities, SMA is significantly negatively related to SR ( $r = -0.775$ ;  $p < 0.05$ ). SMS is significantly negatively correlated with SR ( $r = -0.215$ ;  $p < 0.05$ ) but has no significant correlation with other factors (Table 3(a)). In the bacterial communities, SMA, SMS, and SMC have no significant correlation with other factors (Table 3(b)). The reason may be that the fungal communities are fewer than bacterial communities, so with the specific range, SR will limit the soil fungus respiration but has few effects on the respiration of soil bacteria.

### Response of soil chemical properties to the soil microorganism community

The activity of the soil microorganism community is a key factor affecting the soil environment. This is because soil microbes are a vital factor in promoting the soil nutrient cycling process, and they react rapidly to changes in environmental factors.

**Table 3** | Correlations among SR, SM, ST, soil microbial ace (SMA), soil microbial shannon (SMS), and SMC of (a) fungal and (b) bacterial communities separately in the desert grassland

|              | SR      | SM     | ST     | SMA    | SMS    | SMC |
|--------------|---------|--------|--------|--------|--------|-----|
| (a) Fungus   |         |        |        |        |        |     |
| SR           | 1       |        |        |        |        |     |
| SM           | 0.072   | 1      |        |        |        |     |
| ST           | -0.575* | -0.052 | 1      |        |        |     |
| SMA          | -0.775* | -0.039 | 0.242  | 1      |        |     |
| SMS          | -0.215* | -0.319 | 0.193  | 0.285  | 1      |     |
| SMC          | 0.001   | 0.009  | 0.001  | 0.001  | 0.001  | 1   |
| (b) Bacteria |         |        |        |        |        |     |
| SR           | 1       |        |        |        |        |     |
| SM           | 0.072   | 1      |        |        |        |     |
| ST           | -0.575* | -0.052 | 1      |        |        |     |
| SMA          | -0.186  | -0.081 | -0.352 | 1      |        |     |
| SMS          | -0.215  | -0.308 | 0.192  | 0.348  | 1      |     |
| SMC          | 0.214   | -0.054 | 0.299  | -0.315 | -0.110 | 1   |

\*Significant correlation at the level of  $p < 0.05$ .

This finding (in fungal communities) suggests that SMA and SMC have no significant correlation with other factors. SMS is negatively correlated with STP ( $r = -0.602$ ;  $p < 0.05$ ) (Table 4), which agrees with previous studies showing the soil microorganism community as important in soil biogeochemical cycles, soil formation, and ecosystem resilience to the external environment and a driver of soil properties and processes (Fay *et al.* 2008). Soil microorganism community is an intrinsic

**Table 4** | Correlations among SOC, STN, STP, soil microbial ace (SMA), soil microbial shannon (SMS), and SMC, pH of (a) fungal and (b) bacterial communities separately in the desert grassland

|              | SMA    | SMS      | SMC   | pH       | SOC     | STN     | STP |
|--------------|--------|----------|-------|----------|---------|---------|-----|
| (a) Fungus   |        |          |       |          |         |         |     |
| SMA          | 1      |          |       |          |         |         |     |
| SMS          | 0.285  | 1        |       |          |         |         |     |
| SMC          | 0.001  | 0.002    | 1     |          |         |         |     |
| pH           | -0.184 | 0.088    | 0.001 | 1        |         |         |     |
| SOC          | -0.046 | -0.488   | 0.002 | -0.642** | 1       |         |     |
| STN          | -0.089 | -0.386   | 0.001 | -0.762** | 0.951** | 1       |     |
| STP          | -0.063 | -0.602*  | 0.001 | -0.484   | 0.773** | 0.819** | 1   |
| (b) Bacteria |        |          |       |          |         |         |     |
| SMA          | 1      |          |       |          |         |         |     |
| SMS          | 0.348  | 1        |       |          |         |         |     |
| SMC          | -0.351 | -0.110   | 1     |          |         |         |     |
| pH           | -0.107 | 0.084    | 0.121 | 1        |         |         |     |
| SOC          | -0.138 | -0.483   | 0.403 | -0.642** | 1       |         |     |
| STN          | -0.176 | -0.381   | 0.362 | -0.762** | 0.951** | 1       |     |
| STP          | -0.337 | -0.596** | 0.144 | -0.484   | 0.773** | 0.819** | 1   |

\*Significant correlation at the level of  $p < 0.05$ .\*\*Significant correlation at the level of  $p < 0.01$ .

and sensitive aspect of soil. Although it only accounts for a small part, as an essential source and reservoir of nutrients, it has a vital role in the improvement of nutrient cycling and physical and chemical properties of soil. Furthermore, it can directly reflect soil fertility (Luo *et al.* 2020). The soil microorganism community is regarded as a part of available or labile soil organic matter. A small fraction of the total soil organic matter is readily decomposed and included in nutrient cycling (Xu *et al.* 2020a, 2020b).

Meanwhile, nitrogen and phosphorus are also essential nutrients that limit the primary productivity of terrestrial ecosystems (Xu *et al.* 2020a, 2020b). Soil microbes directly drive the soil carbon, nitrogen, and phosphorus cycle process. Therefore, the study of its distribution characteristics under different rainfall and temperature conditions is of great significance for understanding soil carbon, nitrogen, and phosphorus sales/revenue (Wang *et al.* 2020). Soil nitrogen and phosphorus sales/revenue are serious microorganism-mediated processes, controlled by a range of environmental factors, particularly rainfall and temperature (Su *et al.* 2016).

### Effect of plant biomass on the soil microorganism community

The changing composition of the soil microorganism community is not only influenced by soil physicochemical factors but also by plant properties. These findings demonstrated that plant identity was a more important factor in deciding the soil microorganism community structure than plant species diversity (Zuo *et al.* 2020). The plant ALB and plant RB have been proposed to provide a framework for better understanding of how vegetation composition influences variation in soil microorganism communities (Zhao *et al.* 2020).

In the present study, in fungal and bacterial communities, the SMA, SMS, and SMC were closely positively related to plant ALB but closely negatively related to plant RB (Table 5). A possible explanation is that ALB and soil microorganisms' diversity are interrelated and inseparable. Plants act on the living environment of soil microorganisms by affecting the soil environment, such as water content, pH, carbon, nitrogen, and phosphorus content and ratio, and thus ALB has an effect on microorganism diversity. The soil with high ALB is conducive to the growth and reproduction of microorganisms and is more conducive to the metabolic process of microorganism and the production of diversity. Plant secretions can make microorganisms more abundant resources; vegetation cover makes soil moisture more suitable for community development. The SMA, SMS, and SMC of fungal and bacterial communities were negatively correlated with plant RB. The reason may be that in the desert grassland, some root secretions inhibit the reproduction of microorganisms in the soil (Benizri *et al.* 2007).

### The main driving factors on the soil microorganism community

Soil temperature and moisture changes caused by environmental factors may have an unpredictable effect on the soil microorganism community (Li *et al.* 2018). Although soil microorganisms are involved in most of the physiological and metabolic processes in the soil, there are still few studies on the response laws of the soil microorganism community structure and function under the background of global climate change and how it affects aboveground ecosystem processes. Related field experiments to simulate global climate change show that increasing temperature can significantly affect the content of soil microbes in grassland and forest ecosystems (Zhang *et al.* 2020). For example, some researchers have found that the increase in air temperature has no noticeable effect on the soil microorganism community in the heath forest (Ali *et al.* 2019). Moreover, some scholars believe that increasing temperatures do not have a significant effect on the composition of the grassland soil microorganism community and its biomass (Zhou *et al.* 2019). Increasing the temperature can increase the activity of the

**Table 5** | Regression equation based on soil microbial ace (SMA), soil microbial Shannon (SMS), SMC, ALB, and RB in different temperatures (inner OTC and outer OTC) and variation with precipitation for the prediction of soil microbial ace, soil microbial Shannon and SMC of fungal and bacterial communities separately

| Microbial | Regression equation  |
|-----------|--|
| Fungus    | $\text{SMA} = 2.060 (\pm 2.053) (\text{ALB}) - 1.257 (\pm 0.908)(\text{RB}) + 260.178 [R^2 = 0.068] [p = 0.384]$ $\text{SMS} = 0.013 (\pm 2.053) (\text{ALB}) - 0.013 (\pm 0.005)(\text{RB}) + 4.609 [R^2 = 0.193] [p = 0.021]$ $\text{SMC} = 8.312 (\pm 0.010) (\text{ALB}) - 2.195 (\pm 0.001)(\text{RB}) + 1 [R^2 = 0.057] [p = 0.450]$         |
| Bacteria  | $\text{SMA} = 3.686 (\pm 8.469) (\text{ALB}) - 10.417 (\pm 3.745)(\text{RB}) + 5053.836 [R^2 = 0.266] [p = 0.015]$ $\text{SMS} = 0.003 (\pm 0.003) (\text{ALB}) - 0.004 (\pm 0.001)(\text{RB}) + 7.014 [R^2 = 0.279] [p = 0.012]$ $\text{SMC} = 2.060 (\pm 2.053) (\text{ALB}) - 1.257 (\pm 0.908)(\text{RB}) + 260.178 [R^2 = 0.068] [p = 0.384]$ |

microorganism community and increase the rate of metabolism. The soil microorganism community will tend to adapt to a broader temperature range and a higher metabolic rate.

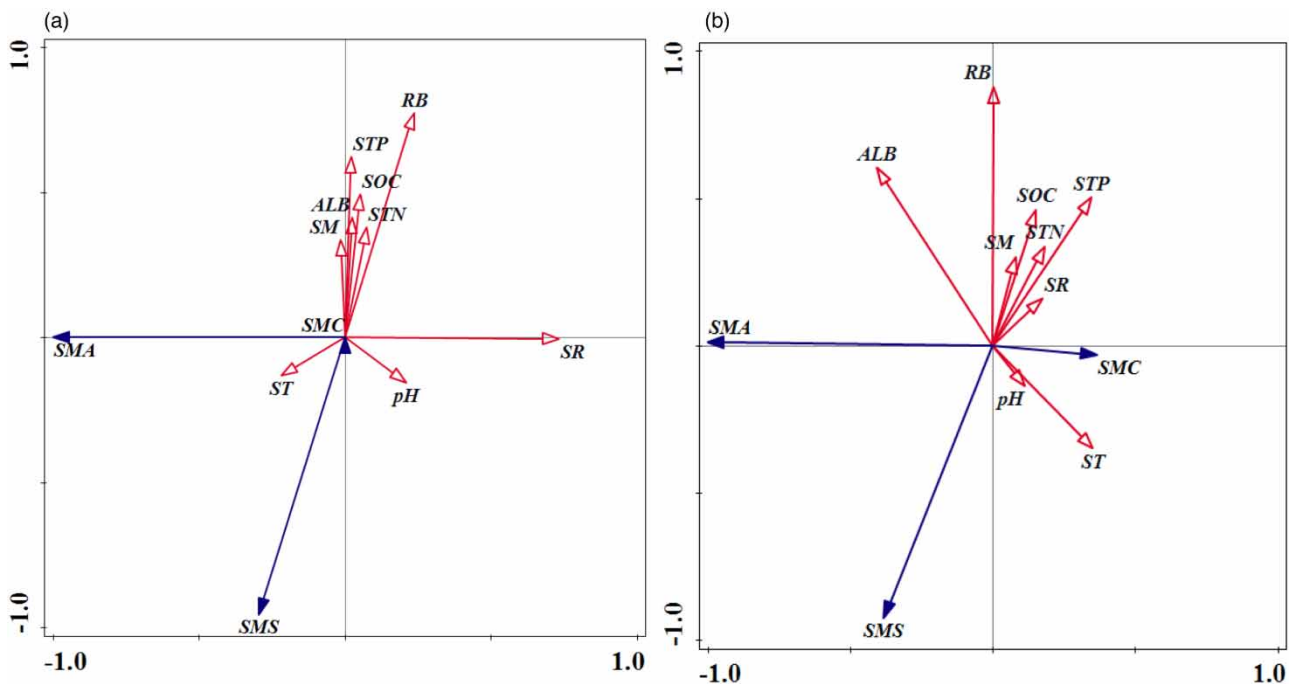
Meanwhile, in addition to the increase in temperature, changes in rainfall value and rainfall frequency will also affect the soil microorganism community (Hu *et al.* 2020). Field rainfall control experiments on grassland ecosystems show that seasonal changes in rainfall have a significant effect on changes in the soil microorganism community content. The experiments conducted by related researchers on the effect of water on soil microorganisms concluded that the water environment can significantly affect forest soil bacterial communities but does not have a significant effect on grassland soil microorganisms (Khan & Khan 2020). However, through field rainfall control experiments, Taylor concluded that neither rainfall decline nor rainfall increase has any significant effect on the biomass of soil microorganism communities. Related research reports point out that changes in rainfall patterns will affect the ratio of fungal and bacterial communities and have a significant effect on soil microorganism communities (Mellado-Vázquez *et al.* 2018).

Most of the above reports pertain to the research and discussion of the soil microorganism community under the effect of single climatic factors. The actual effect of the interaction of multiple climatic factors on the soil microorganism community may be different from the effect of a single climatic factor. For example, the increasing temperature may increase soil microorganism activity, but the impact of declining rainfall may mask this increase. The decrease in rainfall causes a decrease in soil moisture, which reduces the biomass and metabolic rate of litter and then affects the soil microorganism action. However, the interactive effects of such climatic factors have not been reported in terrestrial ecosystems.

In the present study, in fungal communities, SMA is positively correlated with ALB and SM, and the correlation was  $ALB > SM$ . SMA is negatively correlated with other indicators, and the rank of negative correlation is  $RB > SR > STP > SOC > STN > SM > pH > ST$ .

SMS is positively correlated with ST and pH, and the correlation was  $ST > pH$  but is negatively correlated with other indicators, and the rank of the correlation is  $RB > SR > STP > SOC > STN > ST$ . SMC has no significant correlation with various indicators.

In the bacterial communities, SMA positively correlated with ALB and negatively correlated with other indicators. The correlation is  $STP > ST > SOC > STN > SM > SR$ . SMS is positively correlated with pH and ST, and the correlation was  $ST > pH$ . SMS is negatively correlated with other indicators, and the correlation is  $ALB > STP > SOC > STN > SM > SR > pH$ . SMC is positively correlated with SM, SOC, STN, STP, SR, ST, pH, and the correlation is  $STP > ST > SOC > STN > SM > SR$ . SMC is negatively correlated with ALB (Figure 6).



**Figure 6** | RDA plots showing the influence of ST, SM, pH, SR, SOC, STN, STP, soil microbial ace (SMA), soil microbial Shannon (SMS), SMC, ALB, and RB of (a) fungal and (b) bacterial communities.

The findings of this study suggest the lowest ACE indices of fungi and bacteria are both in CK, which indicates that an increase or decrease in both rainfall and temperature could increase the community abundance. In the bacterial and fungal communities, the distance between samples gradually increases with the temperature and rainfall, so rising temperature and rainfall will promote community  $\beta$ -diversity. In the fungal and bacterial communities, abundances are negatively correlated with ST, so increasing temperature will limit the abundance of fungus and bacteria. SMS is negatively correlated with soil CO<sub>2</sub> flux and ST, so soil CO<sub>2</sub> flux and ST will limit soil microorganism diversity. SMC was positively correlated with soil CO<sub>2</sub> flux, so SR will promote the SMC, which is correlated with the previous study.

## CONCLUSIONS

Our finding mainly includes three points. Firstly, in the fungal communities, the normal precipitation will promote the number and the base number of the valid sequence the most, and R66 will significantly promote the mean length of the valid sequence. In the bacterial communities, the interaction of increasing temperature and R133 will promote the number of valid sequences, and R166 will promote the length of valid sequences.

Secondly, in general, bacterial spores have strong drought-resistance ability. Both rainfall and temperature increase could not promote the soil community  $\alpha$ -diversity. However, it can promote the soil microorganism community  $\beta$ -diversity. Fungal and bacterial communities have better tolerance to environmental moisture change, thus showing resistance to change in rainfall. The effect of temperature on fungus and bacteria is not significant, but the change of rainfall will promote the increase of microorganism abundance.

Finally, in soil microorganism communities, SR and ST will limit the soil microorganism diversity; meanwhile, SR will promote the soil microorganism coverage. Plant ALB and ST promote the soil microorganism  $\alpha$ -diversity. The soil microorganism community is important for soil biogeochemical cycles, soil formation as well as ecosystem resilience to the external environment and as a driver of soil properties and processes. These findings may be adopted to other desert steppe ecosystems.

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## DECLARATION OF COMPETING INTERESTS

None of the authors have any conflict of interest.

## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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