The Responses of Soil Microbial to Changed Rainfall and Increased Temperature in Desert Grassland in Northern China

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Abstract

Background: The study evaluates how rainfall change and temperature increase affect microbial communities in the desert grassland of Ningxia Autonomous Region, China to explore the soil microbial community and the relationships among the soil microbial community, chemical properties, soil respiration (SR) and plant biomass under the climate change. We established the field experiment with five levels of rainfall by rainout shelters and two levels of temperature by Open-Top Chamber (OTC).

Results: The effect of temperature to soil microbial communities is not significant, but with the continuous increase of rainfall, the microbial community gradually increases. Soil microbial diversity negatively correlated with soil CO₂ flux. The α -diversity of microbial communities positively correlated with above-living biomass (ALB) and soil temperature (ST), but negatively correlated with root biomass (RB).

Conclusions: Both rainfall and temperature's rising do not promote the soil community α -diversity, but it can promote soil microbial community β -diversity. Soil microbial communities show resistance to rainfall changing. Soil respiration (SR) will limit soil microbial diversity. Soil organic carbon (SOC), soil total nitrogen (STN), and soil total phosphorus (STP) will promote soil microbial abundance and diversity. ALB and ST will promote the soil α -diversity, but the effect of RB to soil microbial is opposite. These findings maybe provide a reliable theoretical basis for formulating a reasonable response strategy in desert steppe ecosystems.

Background

Human activities have caused the concentration of atmospheric greenhouse gases, which increase 0.85 °C in the global mean temperature since 1880 [1]. The IPCC's Fifth Assessment Report (AR5) [2] suggest that the climate warming system is unquestionable. The effect of geographical patterns of rainfall to global climate change is one of the significant challenges in the supply of water for agricultural activities. It is speculated that drought-stressed areas occupied nearly 30% of the total global land area. The rainfall reduces, such as areas is one of the most severe problems facing sustainable agriculture, due to the temperature rising. The global warming rate has doubled since then, especially in the high latitudes of the northern hemisphere [3].

Meanwhile, the atmospheric temperature continues to increase, a series of changes in the global rainfall distribution pattern has also occurred [4]. Regional rainfall has fluctuated from different years, meanwhile flood and drought events happened frequently. Temperature and rainfall are the forcing factors in determining biodiversity and ecosystem functioning in terrestrial ecosystems, and rainfall patterns are expected to shift with climate warming has become a fact [5].

The finding of feedback of soil microbial communities to climate change is positive in a relatively arid ecosystem (approximately 350 mm mean annual rainfall). In a moister prairie ecosystem, negative feedback was proved by researchers (approximately 900 mm means annual rainfall) [6]. Thus, it is

significant to research microbial feedbacks, especially in ecosystem types under concurrent changes of temperature as well as rainfall changes in moisture and temperature conditions. It also affects aboveground plant communities [7] which manage the type and abundance of many organic substrates provided to soil microbial heterotrophs. So, climate change should also affect microbial communities indirectly by shifts in plant composition and productivity. The semi-arid steppe ecosystem in northern China is a crucial component of the Eurasian grassland biome [8]. The water is an important reason, and soil temperature is low from last autumn to early spring, limiting microbial activity [9]. The changing of moisture and temperature conditions also affect aboveground plant communities [10], which manage the type and abundance of many organic substrates provided to soil microbial heterotrophs. Hence, climate-changing should also impact on microbial communities indirectly through shifts in plant composition and productivity.

The previous studies have researched the soil microbial responding to climate change from different perspectives, and have achieved significant results. However, there is still some blind spot that needs further inquiry as follow:

- i. How to evaluate the soil microbial community in desert grassland ecosystems under the interaction of temperature and rainfall. As temperature and rainfall change continues, has sensitive indicators in components of the ecosystem changed?
- ii. In the context of climate change, what are the correlations among soil respiration, soil microbial abundance, soil microbial diversity and soil microbial coverage?
- iii. How do soil properties response to soil microbial abundance, soil microbial diversity and soil microbial coverage?
- iv. How plant biomass affects the soil microbial community?

To solve the above issues, this study takes the desert steppe (south edge of the Mu Us Sandy Land) of Yanchi County, Ningxia as the research object. We used OTC (Open-Top Chamber) to simulate temperature increase, shelters, and artificial watering to simulate rainfall changes under the interaction of human factors: a) Dynamic changes of soil microbial in desert steppe ecosystems, and b) Ecological responses to desert steppe ecosystem. c) From the response of soil microbes to temperatures rising and rainfall change, finding the ecological sensitivity index among them. This research provides a reliable theoretical basis for formulating a reasonable response strategy in a desert steppe.

Methods

Study site

The study site is located in the desert steppe of Ningxia Autonomous Region, China (37°47'N, 107°25'E). The climate belongs to a typical continental climate with an average annual temperature of 8.1°C higher than 0°C. The annual accumulated temperature is 3430.3°C, and the annual rainfall is 295mm (average in 1981-2017). The rainfall of July to September accounts for approximately 61% of the whole year. The

annual evaporation is 2131.8mm, and the frost-free period is about 162d. The zonal soil is light grey calcareous soil, sandy soil, and silt soil. Zonal vegetation is a desert steppe. It is mainly dominated by xerophyte and mesophyte. The Main distribution is perennial plants such as *Stipa breviflora*, *Cleistogenes squarrosa*, *Leymus scallions*, *Lespedeza davurica* and annual plants such as *Setaria viridis* and *Salsola Collina*.

Experimental design

According to the meteorological monitoring from 1981 to 2017 in the study site, 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 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 Open-Top Chamber (OTC) device is used to achieve a temperature increase about 2°C (data from preliminary experiment).

The designed rainout shelter was well-ventilated in November 2018 (Fig.1). Rainfall gradient was constructed by artificial shelters and artificial sprinklers. A two-factor completely randomised experimental design is used based on rainfall and temperature factor. Five levels of rainfall are 33% (R33), 66% (R66), 100% (CK), 133% (R133), and 166% (R166) of normal rainfall, two rainout shelters with manipulated rainfall doses (i) of 97 mm (33% of annual average) and (ii) 194 mm (66% of annual average), along with three unsheltered plots with manipulated rainfall doses (iii) of 295 mm (normal annual rainfall), (iv) 392 mm (133% of annual average) and (v) 490 mm (166% of annual average), increased rainfall due to watering pot. Temperature is two levels that the actual temperature (CK) and the interaction between the rainfall and temperature increase about 2°C (T) to achieve a temperature change by the OTC (Open-Top Chamber) in each plot. Each plot area is (6 x 6) m, and each treatment repeated three times, 15 plots in total (Temperature treatment included in rainfall treatment). On the 15th and 30th of each month, R33 and R66 of the actual rainfall during 1st-15th and 16st-30st of the month are collected from the actual rainfall respectively, and then evenly replenished to the plots containing R133 and R166 by a watering pot.

Collection of soil microbial samples

In each plot, including inner OTC, we take 30cm of soil from each sample plot and then divided it into three layers, which are 0-10cm, 10-20cm, and 20-30cm separately. We remove covering from the soil (plants, moss, visible roots, litter, and visible soil animals) and wipe the sample with alcohol cotton. After the alcohol has completely evaporated, a 6 cm diameter drill was used to soak the sample (S1 Canada). The steps are repeated every time the sample is changed. Three samples were taken from the same quadrant and mixed as one soil sample. Then, we mixed the soil into a 10ml centrifuge tube and transfer it to a -80°C refrigerator for determination of soil microbes. Table 1 and 2 show the sample's data analysis of soil.

Soil property analysis

Soil respiration (SR) measurement using open soil CO₂ flux system (LI-8100 Automated Soil CO₂ Flux System, Li-COR, Lincoln, NB, USA). 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 the soil to a depth of 12 cm, took off inside plants of circular pipe, and then SR was measured. The measurement frequency is one time /15 days, and the time point is 10am-2pm. A 30 cm soil (divide evenly into three layers, each layer is 10 cm) was drilled in each plot and then put into plastic bags separately to the laboratory to test the soil physical and chemical properties.

Soil organic carbon (SOC) was measured by the external heating method: potassium dichromate-sulfuric acid digestion, ammonium ferrous sulphate titration (Titrette 50 ml Automatic titrate) and soil total nitrogen (STN) were tested using an elemental analyser (Vario EL/micro cube, Germany). Soil total phosphorus (STP) was studied by Sulphuric acid-perchloric acid digestion, antimony molybdenum calorimetry, 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 1m² quadrant which randomly selected in each plot at the end of July 2019. All plants in each plot were dug from the soil and then cut the aboveground living plant. Moreover, the plant roots were cut and sorted according to species and place them in their respective envelope. Finally, these species were taken into the laboratory and drying at 65°C in the oven for 48h. Then the aboveground living biomass (ALB) and root biomass (RB) were calculated. Note: All plant samples collected from the desert grassland and the property right of grassland belongs to Ningxia University, so we can carry out experiments on grassland without license certificate.

DNA extraction and PCR amplification

Microbial community genomic DNA was extracted from soil samples by using the MP Fast DNA Spin Kit for soil according to manufacturer's instructions. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F(5'-ACTCCTACGGGAGGCAGCAG-3') and 806R(5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The 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 30s, 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 FastPfu buffer 4 µL, 2.5 mM dNTPs, 2 µL, forward primer (5µM) 0.8 µL, reverse primer (5µM) 0.8 µL, TransStart FastPfu DNA Polymerase 0.4µL, template DNA 10ng, and finally did H₂O up to 20 µL. PCR reactions were performed in triplicate. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA)

according to the manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA).

Statistical analysis

Statistical analyses were variously performed using the software packages R, Origin 2018 and Microsoft Excel. All data were analysed first using a 'repeated measures' statement as well as obtain the standard errors of individual means and used to perform correlation analyses in SPSS 20.0. PCA eliminates redundant variables depending on other measured variables are based on canoco 5.

Results

Soil microbial community

The α -diversity of soil microbial in our study, including three parts, which are community richness, community diversity and community coverage. The number of bacterial community's richness is obviously more than fungus communities, meanwhile, with the temperature and rainfall increasing, the highest community richness of fungus and bacteria are both in R33, and the lowest is in CK. The highest community diversity of fungus is both in CK, and the lowest are in TCK. The highest community coverage of fungus and bacteria are the same separately Fig.2

Under different interaction of temperature and rainfall treatments, the number of bacteria in the soil was significantly higher than the number of fungus. In the fungus communities, the total kinds of microbes are the highest under the R33, the number of common species of microbes under different treatments was 75, and the number of unique species of microbes under R166 was the highest. In the bacterial communities, the total kinds of microbes are also under the R33. The number of common species of microbes under different treatments is 2576, and the number of unique species is the highest under the interaction of normal rainfall and temperature (Fig.3).

In the fungal and bacterial communities, the distance between each sample point is the farthest under the interaction of R166 and temperature rising. Thus, the corresponding β -diversity is the highest. However, sample point has the shortest distance under R33, therefore, the corresponding β -diversity is the lowest (Fig.4).

In the fungus and bacteria communities, the effect of temperature of them is not significant, their abundance is the lowest under natural rainfall, and with the continuous increase and decrease of rainfall, the microbial abundance gradually increases (Fig.5).

The relationship between soil microbial community and soil respiration

In the fungal communities, soil microbial abundance (ace) is positively related to soil temperature (ST) ($r=0.242$; $p>0.01$) but negatively related to soil moisture (SM) ($r=-0.039$; $p>0.01$) and soil respiration (SR) ($r=-0.775$; $p<0.01$). Soil microbial diversity Shannon (SMS) is positively correlated with ST ($r=0.193$;

$p > 0.01$) but negatively correlated with SR ($r = -0.215$; $p < 0.01$) and SM ($r = -0.319$; $p > 0.01$). Soil microbial coverage has no significant correlation with other factors (Table 3a).

In the bacterial communities, soil microbial abundance is negatively correlated with ST ($r = -0.352$; $p > 0.01$), SR ($r = -0.186$; $p > 0.01$) and SM ($r = -0.081$; $p > 0.01$). Soil microbial diversity is negatively correlated with SR ($r = -0.215$; $p > 0.01$) and SM ($r = -0.308$; $p > 0.01$), but positively correlated with ST ($r = 0.192$; $p > 0.01$). Soil microbial coverage was positively correlated with SR ($r = 0.214$; $p > 0.01$) and ST ($r = 0.299$; $p > 0.01$) but negatively correlated SM ($r = -0.054$; $p > 0.01$) (Table 3b).

The response of soil chemical properties to soil microbial community

In fungal communities, soil microbial abundance is negatively correlated with SOC ($r = -0.046$; $p > 0.01$), STN ($r = -0.089$; $p > 0.01$) and STP ($r = -0.063$; $p > 0.01$). Soil microbial diversity is positively correlated with pH, but negatively correlated with SOC ($r = -0.488$; $p > 0.01$), STN ($r = -0.386$; $p > 0.01$) and STP ($r = -0.602$; $p < 0.01$). Soil microbial coverage has no significant correlation with other factors.

In the bacterial communities, soil microbial abundance is negatively related to other factors and soil microbial diversity is positively related to pH ($r = 0.084$; $p > 0.01$) but negatively related to other factors. Soil microbial coverage is positively correlated with pH ($r = 0.121$; $p > 0.01$), SOC ($r = 0.403$; $p > 0.01$), STN ($r = 0.362$; $p > 0.01$) and STP ($r = 0.144$; $p > 0.01$) (Table 4).

The effect of plant biomass to soil microbial community

In fungal communities, the abundance, diversity, and coverage of microbial communities were positively correlated with ALB ($r = 2.060$, $p > 0.01$; $r = 0.013$, $p > 0.01$; $r = 8.312$, $p > 0.01$), but a negative correlation with RB ($r = -1.257$, $p > 0.01$, $r = -0.013$, $p > 0.01$, $r = -2.195$, $p > 0.01$).

In the bacterial community, the abundance, diversity, and coverage of the microbial community were positively correlated with ALB ($r = 2.060$, $p > 0.01$; $r = 0.013$, $p > 0.01$; $r = 8.312$, $p > 0.01$), but a negative correlation with RB ($r = -1.257$, $p > 0.01$; $r = -0.013$, $p > 0.01$; $r = -2.195$, $p > 0.01$) (Table 5).

Rank of different environmental factors on soil microbial community

In the fungal communities, soil microbial abundance (abundance) positively correlated with ALB and SM, and the correlation was ALB > SM. Soil microbial abundance negatively correlated with other indicators, and the negative correlation is RB > SR > STP > SOC > STN > SM > pH > ST.

Soil microbial diversity positively correlated with ST and pH, and the correlation was ST > pH. Soil microbial diversity negatively correlated with other indicators, and the correlation is RB > SR > STP > SOC > STN > ST. Soil microbial coverage has no significant correlation with various indicators.

In the bacterial communities, soil microbial abundance positively correlated with ALB and soil microbial abundance negatively correlated with other indicators. The correlation is STP > ST > SOC > STN > SM > SR. Soil microbial diversity positively correlated with pH and ST, and the correlation was ST > pH. Soil

microbial diversity negatively correlated with other indicators, and the correlation is $ALB > STP > SOC > STN > SM > SR > pH$. Soil microbial coverage positively correlated with SM, SOC, STN, STP, SR, ST, pH, and the correlation is $STP > ST > SOC > STN > SM > SR$. Soil microbial coverage negatively correlated with ALB (Fig.5).

Discussion

The effect of temperature and rainfall on soil microbial community

Global climate change characterized by climate warming has a significant impact on the global natural environment and social-economic activities, which becomes the research challenge of global sustainable development [11]. The search result of the net change primary productivity (NPP) shows that temperature rise is beneficial to plant growth and rainfall changes. Moreover, the extreme increase in climate events may cause grasslands to face higher levels of risk in future climate change scenarios [12]. The increase of atmospheric CO_2 concentration and temperature will affect the physiological growth process of plants and the structure as well as the function of terrestrial ecosystems through photosynthesis. However, as a feedback to terrestrial ecosystems, soil microorganisms will also change.

Soil microorganisms participate in biochemical cycles, the decomposition of soil organic matter and the formation of soil structure in the ecosystem are sensitive to environmental factors. Domestically and abroad comprehensive researches have found that rainfall changing and temperature increasing in the future are bound to have an impact on plant growth and soil metabolism, which will have a significant impact on soil microbial communities.

The number of bacterial communities abundance (ace) is significantly more than fungus communities. The reason maybe is that bacterial spores have strong ability of drought resistance. The α -diversity of fungus and bacterias' communities do not gradually increase with the rise of temperature and rainfall.

So, temperature and rainfall do not give any significant impact on microbial activities due to these indexes adapt to environmental changes caused by experimental treatment through self-regulation. Yet β -diversity of fungus and bacterial communities are highest in R166. Therefore, rainfall rising promote significant impact on microbial activities, which agreed with the previous studies.

In the fungus and bacteria communities, the distance between each sample point is the farthest under the interaction of R166 and temperature rising. Thus, the corresponding β -diversity is the highest, however, sample point has the shortest distance in R33. Therefore, the corresponding β -diversity is the lowest.

The total kinds of fungus and bacterials are both high under the R33, so rainfall reducing could promote the microbial species. The reason maybe is in environmental adaptability, microbes have better tolerance to environmental moisture changes, thus show resistance to rainfall changing.

The effect of temperature on the fungus and bacteria are not significant, and the abundance of fungus and bacterial communities are both the lowest under natural rainfall. With the continuous increasing and decreasing of rainfall, the microbial abundance gradually increases, hence the difference between natural rainfall and rainfall changes are significant. It is because of the change of rainfall will promote the increase of microbial abundance.

The relationship between soil microbial community and soil respiration

Globally, in the different climate change, a better understanding of the mechanisms that regulate R_s dynamics is essential in predictions of future atmospheric C concentrations and accurate estimations of the C balance.

A primary function of soil microorganisms is the processing and restoration of key nutrients in the input of cuttings and the accumulation of soil organic matter. It is a storage reservoir of soil nutrients and an important source of nutrients available for plant growth. It can reflect the state of soil fertility and nutrient cycling meanwhile play the most prominent role in the cycle. Photoautotrophic microorganisms fix CO_2 as organic matter, which can be consumed or respired by heterotrophic microorganisms. The final product of respiration is CO_2 and new cytoplasm. More than 70% of CO_2 loss caused by soil respiration comes from soil microbial respiration.

In the present study, the fungal and bacterial communities, soil microbial abundance (ace) is positively related to ST but negatively related to soil moisture (SM) and soil CO_2 flux. Soil microbial diversity (shannon) is positively correlated with ST and negatively correlated with soil CO_2 flux and SM. Soil microbial coverage has no significant correlation with other factors in fungal communities, but was positively correlated with soil CO_2 flux and ST meanwhile negatively correlated with SM in bacterial communities.

The response of soil chemical properties to soil microbial community

The soil microbial community's activity is a key factor affecting the soil environment. It is because soil microbes are a vital factor in participating in the process of soil nutrient cycling, and they react rapidly to changes in environmental factors.

The finding of this study (in the fungus' communities) shows that with the temperature and rainfall variation, SOC, STN, and STP will promote soil microbial abundance, diversity and coverage separately. In the bacterial communities, SOC, STN, and STP will promote soil microbial abundance and diversity, but limit the coverage. It was same as previous studies showing that soil microbial community, as an important soil biogeochemical cycles, soil formation as well as ecosystem resilience to the external environment and a driver of soil properties and processes[13]. Soil microbial community is an intrinsic sensitive factor of soil. Although it only accounts for a small part of it, as an essential source and reservoir of nutrients, it acts a vital role in the improvement of nutrient cycling and soil physical and chemical properties. It can directly reflect soil fertility [14]. Soil microbial community regarded as a part of

available or labile soil organic matter. The small fraction of the total soil organic matter is a readily decomposed and concluded in nutrient cycling [15].

Meanwhile, nitrogen and phosphorus are also an essential nutrient which limits primary productivity of terrestrial ecosystems [16]. Soil microbes directly drive the soil carbon, nitrogen and phosphorus cycle process. Therefore, the study of its distribution characteristics under different rainfall and temperature is of great significance for understanding the soil carbon, nitrogen and phosphorus turnover [17]. Soil nitrogen and phosphorus turnover are serious microbial mediated processes, which controlled by a range of environmental factors, particularly in rainfall and temperature [18].

The effect of plant biomass to soil microbial community

Composition changing of soil microbial community is not only influenced by soil physicochemical factor (etc. pH, SOC, nutrient availability, SM and ST) but also by plant properties (etc. plant community type, plant microbial interactions, and plant functional traits). These demonstrated that plant identity was more important factors in deciding the soil microbial community structure than plant species diversity [19]. It has been proposed that plant above-living biomass and plant root biomass economics spectrum could provide a framework for better understand how vegetation composition influences variation in soil microbial communities [20].

In the present study, the abundance, diversity, and coverage of fungal and bacterial communities were positively correlated with ALB but a negative correlation with RB. It was in agreement with previous studies showing that ALB will promote the characters of microbial communities [21].

The main driving factors on soil microbial community

Soil temperature and moisture, changing caused by environmental factors may have an unpredictable effect on the soil microbial community and further affect the decomposition process of organic matter in the soil [22]. Although soil microorganisms are involved in most of the physiological, metabolic processes in the soil, there are still few studies on the response laws of soil microbial community structure and function under the background of global climate change, and how it affects the 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 [23]. For example, some researchers have found that the increase in air temperature has no noticeable effect on the soil microbial community in the heath forest. However, in the soil heating experiment, the biomass of the fungus group decreased, and the bacterial biomass increased [24]. Besides, some scholars believe that increasing temperature does not have a significant effect on the composition of the grassland soil microbial community and its biomass [25]. Increasing the temperature can increase the activity of the microbial community and increase the rate of metabolism. The soil microbial 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 microbial community [26]. Field rainfall control experiments on grassland ecosystems

show that seasonal changes in rainfall have a significant effect on changes in soil microbial community content. The experiments conducted by related researchers affect the water on soil microorganisms concluded that the water environment can significantly affect forest soil bacteria, but has no significant effect on grassland soil microorganisms [27]. However, through field rainfall control experiments, Taylor concluded that neither rainfall decline nor rainfall increase has any significant effect on the biomass of soil microbial communities. Related research reports pointed out that changes in rainfall pattern will affect the ratio of fungus and bacteria, and also have a significant effect on soil microbial communities [28]. Most of the above reports are about the research and discussion of the soil microbial community under the effect of single climatic factors. The actual effect of the interaction of multiple climatic factors on the soil microbial community may be different from the effect of a single climatic factor. For example, the increasing temperature may increase soil microbial activity, but the impact of falling 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 microbial action. However, the interactive effects of such climatic factors have not been reported in terrestrial ecosystems.

In the present study, the lowest ace indices of fungus and bacteria are both in CK, which indicate an increase or decrease both rainfall and temperature could increase community abundance. In the bacterial and fungal community, the distance between samples gradually increases with the temperature and rainfall, so temperature and rainfall rising will promote community β -diversity. In the fungus and bacterial communities, abundances are negatively correlated with ST, so temperature rising will limit the abundance of fungus and bacteria, soil microbial diversity is negatively correlated with soil CO₂ flux and ST, so soil CO₂ flux and ST will limit soil microbial diversity. Soil microbial coverage was positively correlated with soil CO₂ flux, so the SR will promote the soil microbial coverage, which is correlated with the previous study.

Conclusions

This finding suggests that in general bacterial spores have strong ability of drought resistance, both rainfall and temperature's rising could not promote the soil community α -diversity. However, it can promote soil microbial community β -diversity. Fungus and bacteria have better tolerance to environmental moisture changing, thus show resistance to change of rainfall. The effect of temperature to fungus and bacteria are not significant, but the change of rainfall will promote the increase of microbial's abundance.

In the soil microbial communities, SR and ST will limit soil microbial diversity; meanwhile, the SR will promote the soil microbial coverage. ALB and ST promote the soil microbial α -diversity. These findings maybe provide a reliable theoretical basis for formulating a reasonable response strategy in desert steppe ecosystems.

Abbreviations

soil respiration: SR.

Open-Top Chamber: OTC.

above-living biomass: ALB.

root biomass: RB.

soil moisture: SM.

soil temperature: ST.

root biomass: RB.

soil organic carbon: SOC.

soil total nitrogen: STN.

soil total phosphorus: STP.

net change primary productivity: NPP.

soil microbial ace: SMA.

soil microbial shannon: SMS.

soil microbial coverage: SMC.

Declarations

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Author's contributions

- YZ performed the experiments, analysed the data, prepared figures and/or tables, and approved the final draft.
- XYZ and MHB conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- JL, ZJ and WYT performed the experiments, prepared figures and/or tables, and approved the final draft.

- LJP conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

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Availability of data

All data generated or analysed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

None of the authors have no conflict of interest.

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Tables

Table 1 Soil fungus in the grassland (mean±SE). Different lowercase letters indicate significant ($p < 0.05$) differences between 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, 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 are two levels that the actual temperature and the interaction between the rainfall and temperature increase about 2°C, to achieve a temperature change by OTC (Open-Top Chamber) in each plot (Recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase about 2°C (T), R33 is the first site of 33% rainfall, another marks are the same.

Sample\Info	Seq_num	Base_num	Mean_length
TR33	61229.75±1435a	11508850.75±259397b	188.03±2.52a
R33	49461.33±7859b	12652044.33±1907253a	256.48±2.35a
TR66	47107.33±3532b	11964428.67±663741b	254.81±5.97a
R66	50879.67±8124b	13105869.67±1966051a	258.49±3.61a
TCK	53078.33±4334b	13578317.67±381018a	258.36±6.17a
CK	72175.01±1352a	17110279.67±215969a	237.14±2.68b
TR133	59858.01±5609a	15308982.67±1249745a	256.38±3.38a
R133	61703.78±4505a	15332526.67±795513a	250.63±3.89b
TR166	69618.03±2953a	16479432.33±508250a	236.96±3.52a
R166	55546.67±5098a	13059563.67±1375884a	234.51±3.68b

Table 2 Soil bacteria in the grassland (mean±SE). 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, 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 are two levels that the actual temperature and the interaction between the rainfall and temperature increase about 2°C (N), to achieve a temperature change by OTC (Open-Top Chamber) in each plot (Recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase about 2°C (T), R33 is the first site of 33% rainfall, another marks are the same.

Sample\Info	Seq_num	Base_num	Mean_length
TR33	40642.67±2517b	16956583.67±1036601b	417.25±51.39a
R33	56770.01±4088a	23712154.01±1725704a	417.64±44.34a
TR66	11751.47±6785b	4910451.396±2835133b	417.78±26.10a
R66	39241.67±1329b	16379041.67±578387b	417.35±35.59a
TCK	46201.01±7632b	19284866.01±3199386b	417.33±41.10a
CK	65527.67±6013a	27335827.01±2514505a	417.14±25.11a
TR133	70880.67±7143a	29579004.33±3019459a	417.21±22.83a
R133	61646.01±5309a	25745725.33±2543678a	417.59±43.86a
TR166	58378.33±743a	24355855.33±3348754a	417.20±45.86a
R166	45805.01±7871b	19136455.01±3269671b	417.95±46.69a

Table 3 Correlations among soil respiration (SR), soil moisture (SM), soil temperature (ST), soil microbial ace (SMA), soil microbial shannon (SMS) and soil microbial coverage (SMC) of a) fungus and b) bacteria separately in the desert grassland. (Note: * represent significant correlation at the lever of P<0.05, ** represent significant correlation at the lever of P <0.01)

a) Fungus

	SR	SM	ST	SMA	SMS	SMC
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) Bacterials

	SR	SM	ST	SMA	SMS	SMC
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

Table 4 Correlations among soil organic carbon (SOC), soil total nitrogen (STN), soil total phosphorus (STP), soil microbial ace (SMA), soil microbial shannon (SMS) and soil microbial coverage (SMC), pH of a) fungus and b) bacteria separately in the desert grassland. (Note: * represent significant correlation at the lever of $P < 0.05$, ** represent significant correlation at the lever of $P < 0.01$).

a) Fungus

	SMA	SMS	SMC	pH	SOC	STN	STP
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	SMS	SMC	pH	SOC	STN	STP
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

Table 5 Regression equation based on soil microbial ace (SMA), soil microbial Shannon (SMS), soil microbial coverage (SMC), above-ground living biomass (ALB), and root biomass (RB) in different temperature (Inner of OTC and Out of OTC) and variation with precipitation for the prediction of soil microbial ace, soil microbial shannon and soil microbial coverage of fungus and bacteria separately.

Microbial	Regression equation
	SMA = 2.060 (±2.053) (ALB) + 1.257 (±0.908)(RB) + 260.178 [R ² =0.068] [P= 0.384]
Fungus	SMS = 0.013 (±2.053) (ALB) + 0.013 (±0.005)(RB) + 4.609 [R ² =0.193] [P= 0.021]
	SMC = 8.312 (±0.010) (ALB) + 2.195 (±0.001)(RB) + 1 [R ² =0.057] [P= 0.450]
	SMA = 3.686 (±8.469) (ALB) + 10.417 (±3.745)(RB) + 5053.836 [R ² =0.266] [P= 0.015]
Bacterials	SMS = 0.003 (±0.003) (ALB) + 0.004 (±0.001)(RB) + 7.014 [R ² =0.279] [P= 0.012]
	SMC = 2.060 (±2.053) (ALB) + 1.257 (±0.908)(RB) + 260.178 [R ² =0.068] [P= 0.384]

Figures

Figure 1

Rain shelter construction and Open-Top Chamber (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, 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 are two levels that the actual temperature and the interaction between the rainfall and temperature increase about 2°C, to achieve a temperature change by OTC (Open-Top Chamber) in each plot (Recorded as CK and T).

Figure 2

α -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, 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 are two levels that the actual temperature and the interaction between the rainfall and temperature increase about 2°C, to achieve a temperature change by OTC (Open-Top Chamber) in each plot (Recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase about 2°C (T), R33 is the first site of 33% rainfall, another marks are the same.

Figure 3

Species community composition analysis of a) Fungus b) Bacteria 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, 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 are two levels that the actual temperature and the interaction between the rainfall and temperature increase about 2°C, to achieve a temperature change by OTC (Open-Top Chamber) in each plot (Recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase about 2°C (T), R33 is the first site of 33% rainfall, another marks are the same.

Figure 4

Community abundance of β -diversity of a) fungus and b) bacteria in the study sites by principal component analysis (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, 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 are two levels that the actual temperature and the interaction between the rainfall and temperature increase about 2°C, to achieve a temperature change by OTC (Open-Top Chamber) in each plot (Recorded as CK and TR). TR33 is the first site of interaction between 33% precipitation (R33) and the temperature increase about 2°C (T), R33 is the first site of 33% rainfall, another marks are the same.

Figure 5

Principal components analysis (PCA) plots showing the influence of soil temperature (ST), soil moisture (SM), pH, soil respiration (SR), soil organic carbon (SOC), soil total nitrogen (STN), soil total phosphorus (STP), soil microbial ace (SMA), soil microbial shannon (SMS) and soil microbial coverage (SMC), above-ground living biomass (ALB), and root biomass (RB) of a) fungus and b) bacterial communities.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Rawdata.xlsx](#)