Increased precipitation enhances the rate of the soil respiration in semi-arid area of the Loess Plateau in China

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Background. Precipitation influences the vulnerability of grassland ecosystems, especially the upland grasslands, and soil respiration is critical for carbon cycling in the arid grassland ecosystem which typically experiences more drought conditions. **Methods.** We used three precipitation treatments to understand the effect of precipitation on soil respiration of a typical steppe in the Loess Plateau in China. Precipitation was captured and relocated to simulate precipitation rates of 50%, 100%, and 150% of ambient precipitation. **Results and Discussion.** Soil moisture was influenced across all precipitation treatments. Shoot increased insignificantly as precipitation increased, however, the increase or decrease of precipitation can significantly reduce the biomass of the root. There was a positive linear relationship between soil moisture and soil respiration in the study area during the summer (July and August), when most precipitation fell. Soil moisture, soil root biomass, pH, and fungal diversity were predictors of soil respiration based on SPLS (partial least squares regression), and soil moisture was the best predictor. **Conclusion .** Our study highlights the importance of increased precipitation on soil respiration in the drylands. Precipitation changes can cause significant alterations in soil properties, microbial fungi, and the root biomass, and the moisture is fed back into the climate, thereby affecting the rate of soil respiration in the future.

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Abstract

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Methods. We used three precipitation treatments to understand the effect of precipitation on soil respiration of a typical steppe in the Loess Plateau in China. Precipitation was captured and relocated to simulate precipitation rates of 50%, 100%, and 150% of ambient precipitation.

Results and Discussion. Soil moisture was influenced across all precipitation treatments. Shoot increased insignificantly as precipitation increased, however, the increase or decrease of precipitation can significantly reduce the biomass of the root. There was a positive linear relationship between soil moisture and soil respiration in the study area during the summer (July and August), when most precipitation fell. Soil moisture, soil root biomass, pH, and fungal diversity were predictors of soil respiration based on SPLS (partial least squares regression), and soil moisture was the best predictor.

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Introduction

Recent changes in global temperature and precipitation rates have occurred due to the emission of greenhouse gases (Gao et al. 2016). Temperatures are expected to gradually increase in most parts of the world and extremes are anticipated to become more frequent (Garrett et al. 2006). Global warming is expected to cause the atmospheric water vapor to increase significantly and affect the hydrological cycle (O'Gormana & Schneider 2009), impacting global precipitation patterns and causing regional precipitation changes (Pall et al. 2006). The intensity of precipitation events is expected to increase, and extreme precipitation events will occur more frequently according to the IPCC (Intergovernmental Panel on Climate Change) forecast (IPCC

2001).

Previous studies have indicated that changes in precipitation affect the dynamics of the terrestrial carbon cycle and terrestrial carbon pools (Ahlström et al. 2015;Felton et al. 2019;Frank et al. 2015;Wu et al. 2011). Water is a driving factor for chemical and biological processes in ecosystems, including plant survival, photosynthesis, heterotrophic respiration (Gerten et al. 2008;Zhang et al. 2019a), soil nutrient dynamics (Yuan et al. 2017), terrestrial ecosystem functioning (Wu et al. 2009), and microbial activity, diversity, and respiration (Classen et al. 2015;Felton et al. 2019;Frank et al. 2015). Moreover, changes in global precipitation patterns exert profound effects on the vegetation (Gao et al. 2016), especially in arid and semiarid regions where water is the main limiting factor for plant growth (Jing et al. 2010;Knapp et al. 2002).

Global emissions of CO_2 from soil are considered to be one of the largest causes of flux in the global carbon cycle and small changes in soil respiration may have large impacts on atmospheric CO_2 concentrations (Schlesinger & A.Andrew 2000). Several studies have suggested that soil CO_2 flux is more sensitive to higher temperatures, since roots exudates, root mycorrhizae, plant detritus, and other part of the plant have different temperature sensitivities to fluctuations in soil CO_2 levels (Richard D. Boone et al. 1998). Fluctuations in soil CO_2 strongly impact the terrestrial carbon cycle (Fischlin et al. 2007;Frank et al. 2015). However, we still have a poor understanding of the response of soil respiration as related to climate change (Fernandez et al. 2006).

Carbon fluxes have recently been studied in alpine meadows and show that only underground biomass and soil moisture have a direct effect on soil respiration (Geng et al. 2012). Studies on tropical forests have shown that changes in soil respiration due to elevation-related changes of roots and litter resulted in the increases of soil respiration (Zimmermann 2010). In arid temperate grasslands, soil respiration has a positive effect on the extreme precipitation events and the ecosystem carbon cycle (Thomey et al. 2011). However, there are few studies on semi-arid ecosystems which are especially sensitive to precipitation change (Niu et al. 2019). Semi-arid areas account for about 15% of the terrestrial area of the globe (Huang et al. 2015). Grasslands play a key role in the carbon cycle (Li et al. 2017;Poulter et al. 2014;Zhang et al. 2019b), while being very sensitive to large-scale climate change (Feng et al. 2006).

The upland Loess Plateau in northwestern China is a critical transition zone for ecosystems in China (Zhao et al. 2014). It is predicted that temperature and precipitation will increase significantly in this area (Zhao et al. 2014). We conducted field work to simulate increased and decreased precipitation to explore the factors affecting soil CO_2 flux in semi-arid areas in China. Our study has great scientific and practical implications on the effects of precipitation on soil respiration and plant productivity and their effects on regional and global terrestrial carbon cycles. We sought to evaluate the differences in soil properties, plant biomass, and microbial diversity using different precipitation treatments and different soil depths and to determine the main factors affecting soil respiration in the study area.

Materials & Methods

Study sites

The study was conducted at the Experimental Station of the Agriculture of Ningxia University (106°21′E-106°27′ E，36°10′ N-36°17′ N), Yinchuan Province of China, in the Guyuan Yunwu Mountain of Ningxia near the Loess Plateau, at an altitude of 1700- 2148 m. The climate of the area is temperate, continental monsoon. The annual average precipitation is about 439 mm and has varied from 282 mm in 1982 to 706 mm in 2013. More than 50% of the annual precipitation occurs in the summer months (June to August). The average annual temperature is 7.2 °C, and has varied from 5.3 °C in 1984 to 8.7 °C in 2013. The average monthly minimum temperature for the coldest month (January) was -7.2 °C, and the average monthly maximum temperature for the warmest month (July) was 19.6 °C. The annual evaporation is 1300-1640 mm, and the annual duration for sunshine can reach 2500 h, with a frost-free period of 112-140 days. The annual potential evapotranspiration is 1625 mm (meteorological data from the National Meteorological Administration of China from 1981 to 2017). The soils are grey-cinnamon and dark loessial, as classified by the Chinese soil classification system (Office 1998). The vegetation is typical steppe and the main plant species are *Stipa bungeana*, *Artemisia gmelinii*, *Stipa grandis*, *Artemisia frigida*, *Potentilla acaulis* and *Agropyron michnoi.*

Experimental design

Our research site was located near the Loess Plateau, in a semi-arid natural grassland that was left ungrazed for 19 years. The study area was at 2077 m, with a 7-10° slope, and a south-facing, sunny aspect. The annual precipitation in 2019 was 592 mm, which was 20% higher than average. According to local multi-year meteorological data of the study area, the maximum and minimum precipitation were about 50% and 150% of the average annual precipitation. We set three blocks with three 6×6 m plots in each block, to make a total of 9 plots. Rain shelters were set up on three plots to change interfere to intercept with half of the natural precipitation to form a reduced precipitation area (R50), and the actual precipitation of the plots was 50% of the normal precipitation. The intercepted water was piped to the adjacent plot to form an increased precipitation area (R150). The actual precipitation of the plot was 150% of the normal precipitation. The remaining three plots were the controls (R100) and the actual precipitation of the plot was 100% of the normal precipitation. Each rain-shelter was fixed to the ground by steel pillars, and transparent polyethylene plates were fixed in "V" shapes to intercept precipitation and channel it off the plot using the natural slope of the mountain, while forming a stable and well-ventilated structure (Talat & Hema 2019). The intercepted water was piped to the adjacent plot to form the R150 (Fig. 1). Snow was collected from the rain shelters after each snowfall (R50) and was sprinkled evenly into the R150. A 1.2 m deep plastic barrier was used to prevent surface runoff or leakage of soil moisture between plots. The barrier was buried at a depth of 1.1 m and projected 10 cm above ground. Our study ran from May 2017 to May 2019.

Environmental factors

We collected field data in July 2019 under three precipitation treatments that corresponded to the annual period of peak biomass. Three soil sample replicates were collected at depths of 0-9.9 cm, 10-19.9 cm, and 20-30 cm in each plot, after the litter was discarded. The soil samples were separated into two parts: one part was kept moist to determine the microbial diversity of the soil, and the other was air-dried for measurement of soil properties.

Soil organic carbon (SOC) was measured by potassium dichromate-sulfuric acid digestion, with ammonium ferrous sulfate titration. Total nitrogen (TN) was determined using an Elementar analyzer (Elementar, Vario EL III, Germany). Total phosphorus (TP) was measured using Olsen's method (Olsen et al. 1982). The soil pH was measured using a PHS-3C pH audiometer in a 1:5 of fresh soil: water slurry (Huakeyi, Beijing, China).

A 1 m^2 quadrat was randomly selected in each subplot to determine the plant biomass. The litter was raked and bagged and the shoots of the plants were cut. The root biomass (RB) was sampled to a 30 cm depth in three intervals of equal depth and the soil was brushed off the roots. All plant samples were dried at 65℃ in an oven for 75 hours and then weighed.

Soil microbial diversity was determined based on the Illumina HiSeq sequencing platform of the Majorbio Cloud Platform (www.majorbio.com). The bacterial primer was 338F_806R and the fungal primer was ITS1F_ITS2R. Sobs' and Shannon's indices were used to indicate the alpha diversity of bacteria and fungi. Coverage index, as defined by Good (Good 1953) measures how well an environment is sampled, and indicates the percentage of operational taxonomic units (OTUs) sampled in a microbial community (i.e. recovered per sample as a percent of all OTUs found on the site) (Chao 1984;Lemos et al. 2011).

Measurements of soil respiration

Soil respiration (release of CO_2) was measured every 14-16 days by the LI-8100A portable gas exchange system from April 2019 to October 2019 (LiCor, Lincoln NE, USA; chamber 8100-103, diameter of 20 cm). Polyvinyl chloride (PVC) collars were set in place one week before the first measurement to minimize soil disturbance. The height of each collar was 12 cm and the above-ground height was 3 cm, so the soil surface area within the collar was 317.8 cm^2. Five collars were placed randomly in each plot, giving a total of 45 collars. The above-ground parts of the plants inside the collar were removed before taking each flux reading and the roots were left in place (Talat & Hema 2019). Soil fluxes were measured about every 16 days, between 9 am and 1 pm, based on weather conditions. The flux from each collar was measured for 100 s. Soil moisture was measured at a depth of 5 cm with a GS-1 Licor sensor, and the temperature was measured concurrently at a depth of 10 cm using the Licor sensor 6000-09 TC. To avoid pseudo-replication, the five values per plot were averaged for each variable to get a single datum for each measurement timepoint.

Statistical analysis

Statistical analysis was conducted using IBM SPSS (IBM, Chicago, USA) with ANOVA. One-way ANOVA was used to process the aboveground and underground biomass of plants under different precipitation treatments. A two-way ANOVA was used to process root biomass, soil nutrient content, soil pH, and the microbial diversity index under different precipitation treatments and different soil depths. The microbial diversity was calculated by Mothur (Version v.1.30). Origin (Origin Lab 2017, Microcal, Massachusetts, USA) was used for figures. The different dates formed temporal pseudoreplication, so the significance was explored using nlme (https://svn.r-project.org/R-packages/trunk/nlme) in R (R Core Team 2013; Version 3.0.2) for soil temperature, soil moisture, and soil CO_2 flux (using the format lme: flux ~ Precipitations * Date, random = ~1 | plot, weights = varIdent (form = ~1 | Date). The varIdent function in package Predictmeans v1.0.2 (https://www.rdocumentation.org/packages/predictmeans) was used to allow each timepoint to have a different variance. Package ggplot2 (https://www.rdocumentation.org/packages/ggplot2) was used for analyzing the correlating relationships of soil CO_2 flux and all other factors to filter some variables. SPLS (http://mixomics.org/methods/spls/) analysis was used to select normal variables to provide the main influencing factors of the soil CO_2 flux prediction model. Stepwise regression was used to establish a model of the main factors affecting soil respiration.

Results

Precipitation and temperature during the trial

The annual precipitation was 420 mm, 550 mm, and 592 mm in 2017-2019. In 2018 and 2019 precipitation was 30% higher than the average level of precipitation for the last 40 years.

Precipitation in 2019 from April to October (the growing season), was 562 mm, which was about 95% of the annual precipitation (Fig. 2). The average annual air temperature was 7.9 ℃ in 2019, which was 9% higher than the average temperatures from 1980 to 2019. The highest temperature was 19.7 ℃ in July, and the lowest was -6.6 ℃ in January in 2019 (Fig. 2).

Soil properties

Two-way ANOVA results showed that, except for TP, other soil properties had significant differences under precipitation treatments and different soil depths, respectively (P <0.05). The precipitation treatment*soil depth only caused significant differences in soil SOC. The rest of the soil properties had their highest value under R100 in soil 0-30 cm deep (Table 1), with the exception of the soil TP. SOC decreased the most under R50 (23%) as the depth of the soil layer increased, followed by R100 (18%) and R150 (14%). SOC at R50 increased just 1% in the topsoil (0-9.9 cm) and decreased about 6% in soil depths of 20-30 cm, compared with R100, while R150 had same value with R100 in 20-30 cm soil depths but decreased about 3% in 0-9.9 cm soil depths. As the depth of the soil layer increased, the soil pH gradually increased. Soil TN and SOC were the highest in the topsoil (0-9.9 cm), and decreased with soil depth.

Biomass of shoot, litter and root

There was no significant difference in shoot, litter, and total biomass (TB) under different precipitation treatments according to one-way ANOVA results ($P > 0.05$). Shoot biomass was largest in R150, and decreased with the decrease of precipitation. Litter biomass was greatest in R50. Root biomass (RB) 0-30 cm showed significant differences between precipitation treatments according to one-way ANOVA ($P < 0.05$), with R100 having the most root biomass, and R150 and R50 being significantly lower by 52% and 65%, respectively (Fig. 3A). The total biomass (TB = sum of shoots, litter, and RB 0-30) under R50 was lower than R100 and R150. The shoot/root ratio (AGB/RB), was largest in R150, and was significantly lower by 64% in R100 ($P < 0.05$) (Fig. 3B).

Different precipitation treatments and soil depths caused significant differences in root biomasses according to two-way ANOVA ($P < 0.05$). The interaction between the two factors (precipitation * soil depth) also caused the root biomass to differ significantly (P <0.05). All values decreased as the soil depth deepens (Fig. 4). The highest value of root biomass appeared at 0-9.9 cm in R100; R50 and R150 at 0-9.9 cm were significantly lower by 68% and 57%, respectively. Increased precipitation and reduced precipitation significantly reduced the root biomass in the topsoil (0-9.9 cm) (Fig. 4).

Microbial richness and diversity

More than 95% of operational taxonomic units (OTUs) found in the microbial community at the study site were present in each soil sample examined. There was no significant difference in soil microbial richness via Sobs' index and diversity via Shannon's index under different precipitation treatments and precipitation*soil depth (P >0.05). However, there was a significant difference in soil microbial richness and diversity with soil depth (P <0.05), and the difference was greatest at 0-9.9 cm (Table 2). The Sobs' index and Shannon index of bacteria and fungi gradually decreased as soil depth increased.

Soil moisture, soil temperature and soil CO_2 flux

Soil moisture, soil temperature and soil CO_2 flux showed significant differences between precipitation treatments over the entire experimental period (April - October) (Table 3). Measurements over the growing season also differed significantly for all varieties and were significantly affected by precipitation levels (P <0.05) (Table 3).

Soil moisture and CO_2 flux were greater at higher levels of precipitation while soil temperature was lower (Table 4). The response of soil moisture to decreased precipitation (-37.2 % of R100) was greater than to increased precipitation (3.9 % of R100). Mean soil CO_2 flux under R100 was higher by 38.9 % than that for R50, while for R150 the increase relative to R100 was only 8.3 % (Table 4).

All variables (soil moisture, soil temperature and soil CO_2 flux) were strongly influenced by seasonality (Table 3). Soil moisture was highest in April and May, then dropped and rose again in October. They had moderate levels in July and a “W”-shaped relationship with time. Soil temperatures peaked in summer (June-August). The soil CO_2 flux showed an upward trend from

April to July, and peaked before decreasing to its lowest levels in October (Table 5).

Soil moisture was typically greater in R150, with the exception of April and September (Fig. 5A). The highest soil temperature values were typically seen in R50 (Table 4), but it reversed in June and July (Fig. 5B), when precipitation was high in the summer (Fig. 2). Normal levels of precipitation were re-established in August, the period of maximum precipitation (Fig. 5B). Overall, the soil temperature first rose and then decreased with the date of record.

Soil CO_2 flux showed the same trends as soil moisture for most months. The soil CO_2 flux was lower at the lower end of the precipitation gradient and was highest at R150 and lowest at R50. However, across the summer months, from early May to early July, the soil CO_2 flux with enhanced precipitation was lower in R150 than R50 and R100 (Fig 5C). Precipitation had little effect on soil CO_2 flux in the autumn month of October.

Modeling soil CO_2 flux

Based on the results of the correlation analysis (Fig. S1), one of the normally distributed and strongly correlated factors was selected for SPLS analysis. SPLS analysis (Fig. S2) showed that the factors most closely correlated with soil respiration were soil moisture, RB 0-9.9, RB 10-19.9, soil pH, and fungal diversity. We used stepwise regression to filter out the best model of the main factors affecting soil respiration. Our results showed that soil moisture was the only input factor and significantly affected soil CO_2 flux ($P < 0.001$, $R = 0.94$, adjust R^2=0.870), all other variables were excluded (Table 6).

Discussion

Soil organic carbon (SOC), total nitrogen (TN) and pH were significantly different in three precipitation treatments. Soil SOC, TN, and pH had the highest value in R100, and increased and decreased precipitation reduced these soil parameters. Nutrients in the soil can accumulate through the degradation of plants, litter, and root secretions (Qiu et al. 2009;Zhang et al. 2016). Our study showed that different precipitation treatments had no significant impact on shoots and litter, but there was a significant effect on the root biomass. Increases and decreases in precipitation also significantly reduced the root biomass in the study area, and were significantly higher in R100, especially in the topsoil (0-9.9cm). Changes in precipitation may influence a plant's growth as related to the balanced growth hypothesis. Plant will preferentially distribute the obtained water to the root and in low-moisture areas, the proportion of plant roots that obtain moisture is higher than other parts of the plant (Talat & Hema 2019). Therefore, soil properties under different precipitation treatments may be more affected by the root biomass. Root decomposition and the secretion of roots will cause differences in various precipitation treatments, and will affect the SOC, TN and pH. We found that as precipitation decreased in the study area, the water requirements for grass growth were not met. Therefore, the root biomass was significantly lower than in instances of normal precipitation. As precipitation increased, the root biomass was higher in R150 than R50 and R100, but was not significantly different and the root biomass was significantly lower in R150 than in R100. This may be due to the vegetation's own growth strategy when dealing with changes in precipitation. The soil microbial diversity and richness index were unresponsive to the three precipitation treatments, but were responsive to different soil depths. Previous studies have suggested that when available water is manifested, there is a positive correlation in the richness and diversity of the community in the ecological process (Hawkins et al. 2003). However, this rule does not necessarily apply to soil microorganisms since available water is not directly correlated with the diversity of all organisms (Bachar et al. 2010), and it is determinant by isolation of microbial populations in soils (Treves et al. 2003). In addition, short-term precipitation changes have little effect on microbial diversity. Study also showed that one-year precipitation changes and the two-year precipitation changes did not interfere with microbial diversity (Wang et al. 2020). Soil moisture, soil temperature, and soil respiration showed significant seasonal differences under different precipitations throughout the experiment. Soil moisture and soil respiration increased with the precipitation gradient, but soil temperature showed the opposite downward trend. Soil temperature may be affected by the surface vegetation coverage (S. Kang 2000), which increases with increasing precipitation. Areas with higher vegetation coverage have more vegetation canopy that intercepts solar radiation and reduces evaporation, thus lower soil temperature and the temperature of the local microclimate. The plant biomass is lowest in R50, which had the largest area of bare land and experienced more evaporation from the bare soil. Water also has a higher specific heat volume and the treatments with more soil water would likely have a lower soil temperature.

The change of soil temperature reflected the atmospheric temperature in the study area but the highest soil temperature did not correspond when the atmospheric temperature was the highest and more precipitation had a weakening effect on the soil temperature. Temperature regulates soil respiration by changing the rate at which organisms process carbon and nutrients (Crowther & Bradford 2013). Studies on the effect of temperature changes on soil respiration indicate that the temperature sensitivity of soil respiration will decrease under continuous global warming (Peng et al. 2009). Our study showed there was no significant correlation between soil respiration and soil temperature during vigorous plant growth, which may be related to the length of our study. Therefore, changes in soil temperature have limited effects on soil respiration. In addition, an increasing number of studies showed that soil respiration responds more strongly to precipitation pulses rather than soil temperature in arid regions limited by water (Almagro et al. 2009).

Changes in soil moisture content and precipitation were not closely correlated, but showed high values in spring and autumn and trended in a "W" shape. Melting snow in the spring replenishes the soil moisture, and lower evapotranspiration keeps the soil moist. High evapotranspiration in the study area produced no peak of soil moisture and limits growth, even though precipitation is higher in summer. Precipitation events in water-limited areas usually only affect the topsoil and are easily lost by direct evaporation (Schwinning & Sala 2004), which negates soil moisture gains. Soil moisture accumulated as air temperature and evapotranspiration losses were decreased in autumn (September to November) (Felton et al. 2019;Maes & Steppe 2012;Wang et al. 2013). We found that soil moisture under increased precipitation was significantly higher and then decreased, which may be caused by the soil permeability and evaporation. Literature has indicated that the non-negligible factors affecting soil moisture include soil permeability, surface runoff, evaporation, and evapotranspiration (Wang et al. 2012). We found that surface run-off could be ignored with the use of a plastic barrier with an underground depth of 110 cm and a ground height of 10 cm. A better root system can improve soil permeability, aeration, and porosity (Ozalp et al. 2016). The greater root biomass in R100 resulted in improved soil permeability, moisture storage, and increased humidity. The aboveground biomass under increased precipitation and high plant coverage can have an increased blocking effect on soil moisture evaporation before it decreases. More roots and above-ground biomass may lead to more water absorption and evapotranspiration by plants. However, we found that the above-ground biomass had no significant effect related to precipitation, and more roots appeared in R100. Therefore, the vegetation of R150's evaporative utilization of water was lesser due to several factors and R150 held the most soil moisture.

Soil moisture is one of the main environmental factors affecting soil CO_2 flux. Soil moisture affects the physiological performance of microorganisms and nutrient diffusion (Curiel Yuste et al. 2007), especially in arid areas with limited water conditions (Emmett et al. 2004;Lellei-Kovács et al. 2011;Zhang et al. 2010). Soil respiration is affected by soil moisture (Balogh et al. 2011) and has a clear threshold of soil moisture content for soil respiration. Our study suggested that the total soil CO_2 flux in R150 was higher than in R50 throughout the test period, but in April, June, and July when the soil moisture content was higher than in other months, the soil CO_2 flux of R150 was almost equal to or slightly below R50. In arid areas that lack atmospheric precipitation, the soil moisture content has a greater impact on soil respiration. An increase in atmospheric precipitation and soil moisture replenishment from ice and snow melt caused the soil moisture in R50 to reach the maximum threshold of soil moisture for soil respiration in the study area. Increased precipitation produced little or no effect on soil respiration.

Multiple regression analysis showed that soil moisture was the best predictor of soil respiration. Other factors included root biomass, pH, and fungal Shannon diversity based on SPLS. There was a significant positive linear correlation between soil moisture and soil respiration throughout the plant growth period as shown by stepwise regression, with the strongest correlation at the end of July ($R^2 = 0.87$). In the arid research area which is limited by water, an ecological environment with concentrated and heavy precipitation events can greatly stimulate soil respiration (Liu et al. 2016). Heavy precipitation played a decisive role in soil respiration during the concentrated precipitation period in the arid area. This effect is commonly found in arid and semi-arid regions with limited water conditions (Emmett et al. 2004;Lellei-Kovács et al. 2011;Sowerby et al. 2008;Zhang et al. 2010).

Soil respiration is the comprehensive result of autotrophic respiration mainly based on root activity and heterotrophic respiration related to soil organic matter decomposition (Wang et al. 2014). Drought can reduce the diffusion of organic matter substrates and extracellular enzyme activities, thereby inhibiting root growth and microbial activity (Liu et al. 2016) and impacting heterotrophic respiration. Our research shows that soil root biomass and fungi can affect soil respiration. Our study area was confined to an arid area, which limits its broader applications. Future climate change in precipitation may be mostly concentrated in a series of ecosystem responses caused by decreased precipitation and repeated samples should be taken in R50.

Conclusions

In summary, our research showed that seasonal precipitation changes caused significant seasonal changes in soil moisture, soil temperature, and soil respiration during the whole plant growing season in the study area. Increased and decreased precipitation reduced the root biomass of plants and increased the AGB/RB. Changes in precipitation significantly affected soil nutrients (SOC, TN and pH) at depths of 0-30 cm. Soil microbial diversity and richness were not sensitive to the response of precipitation changes, but were significantly affected by soil depths. Additionally, in arid grassland ecosystems where water is the limiting factor, soil moisture is the main factor affecting soil respiration.

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Figure 1

Rain-shelter construction and layout of the subplots at the study area.

Notes. Three precipitation treatments were applied: R50 (= 50% of ambient precipitation), R100 (ambient) and R150 (= 150% of ambient precipitation). The slope of three plots was considered to be a block.

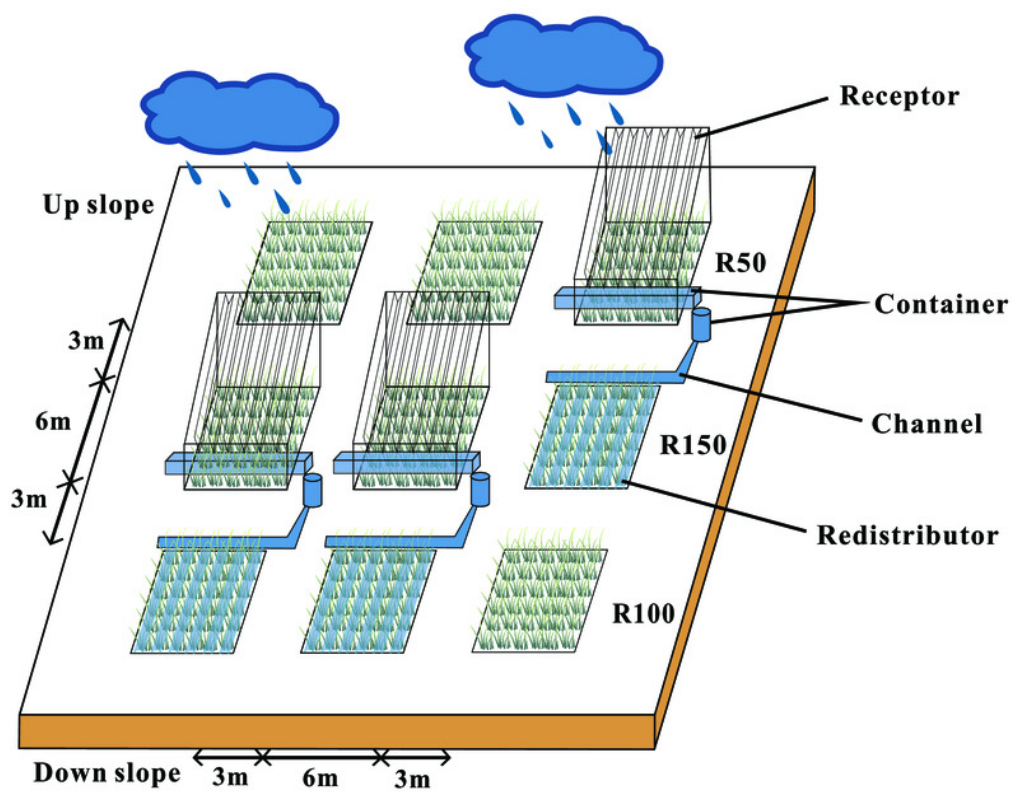

Figure 2

Monthly precipitation at the study site from 2017 to 2019 (bars) and average monthly temperature (T2019) in 2019 (black line and points).

Notes. Meteorological data were obtained from the National Meteorological Administration of China.

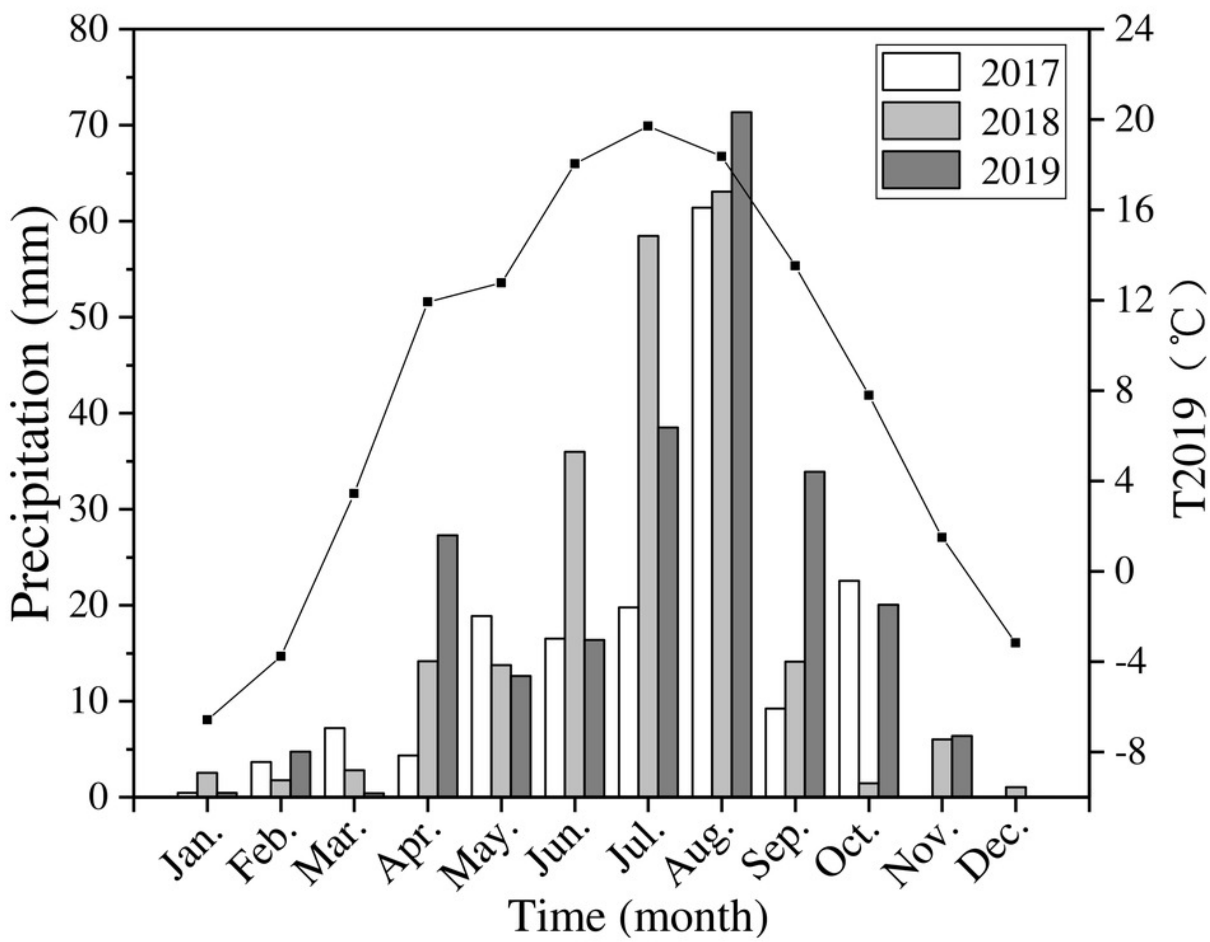

Figure 3

Biomass of shoot, litter, roots, total biomass (TB) and shoot/root ratio (AGB/RB) for each precipitation.

Notes. Different letters within each tissue type show significant differences ($P < 0.05$) between precipitation treatments according to one-way ANOVA (mean ± standard error, n = 3) . Shoot, litter, roots and total biomass use left the Y axis, and AGB/RB the right.

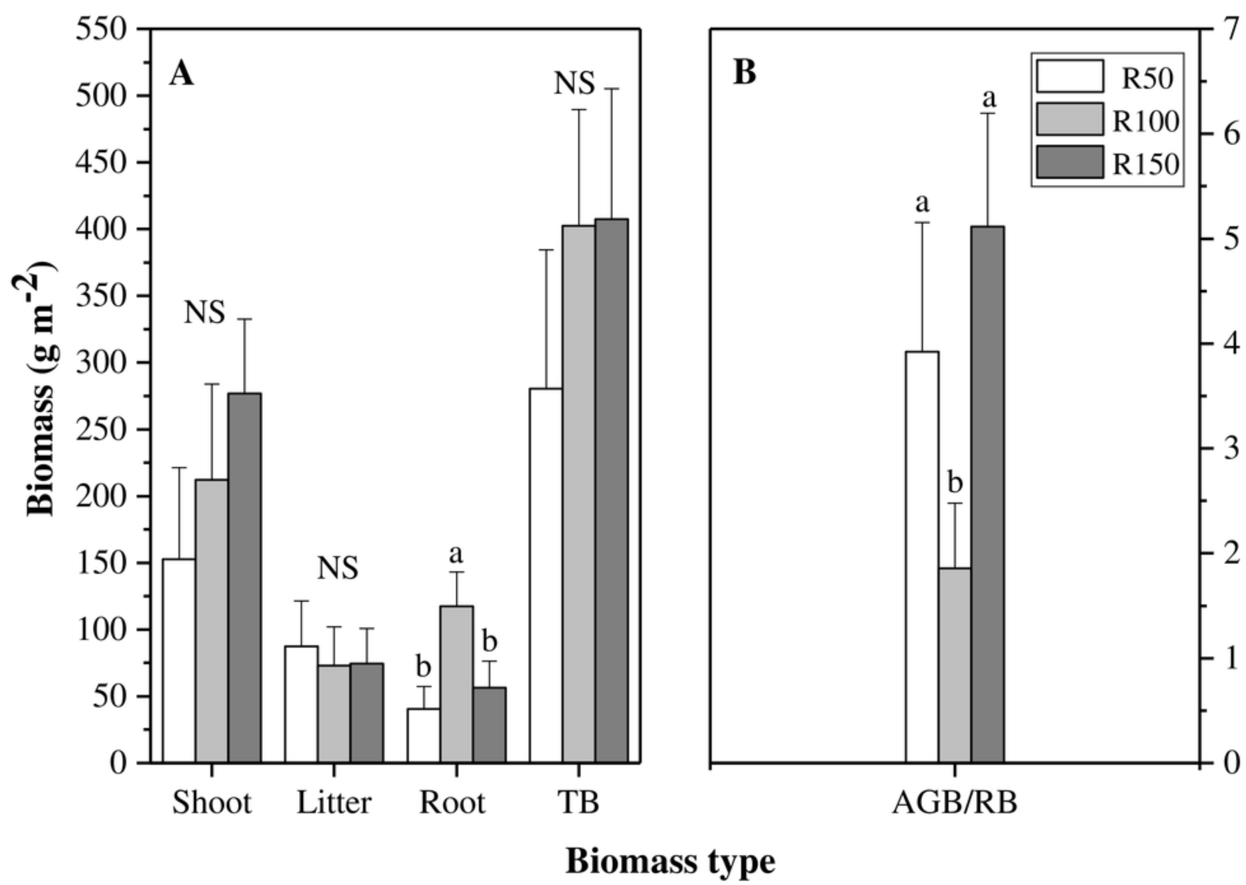

Figure 4

Root biomass at different soil depths in three precipitation treatments.

Notes. Different letters show significant differences ($P < 0.05$) between precipitation treatments and soil depths according to two-way ANOVA (mean ± standard error, n=3).

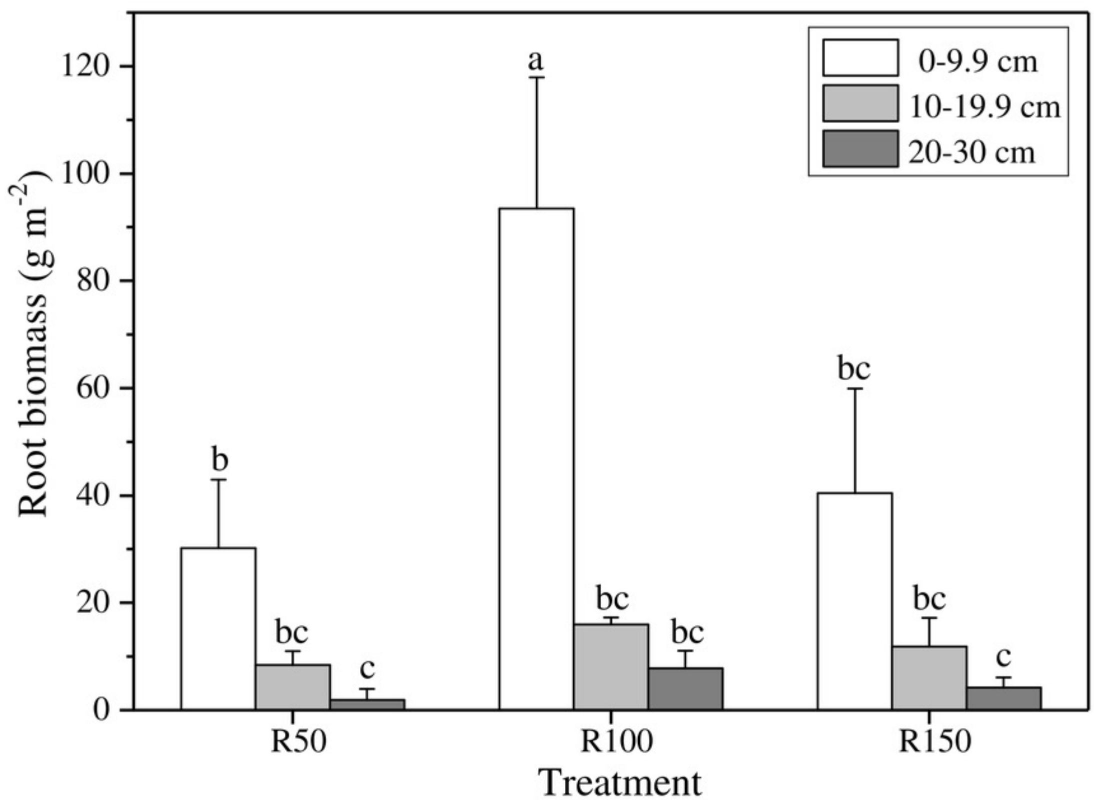

Figure 5

Interactions of precipitation treatments and times for soil moisture, soil temperature and soil CO_2 flux during the growing season.

Notes. Values are the mean ± standard error (n=3). There are three precipitation treatments, and each treatment has three repetitions. The whole test period is repeated for 11 times.

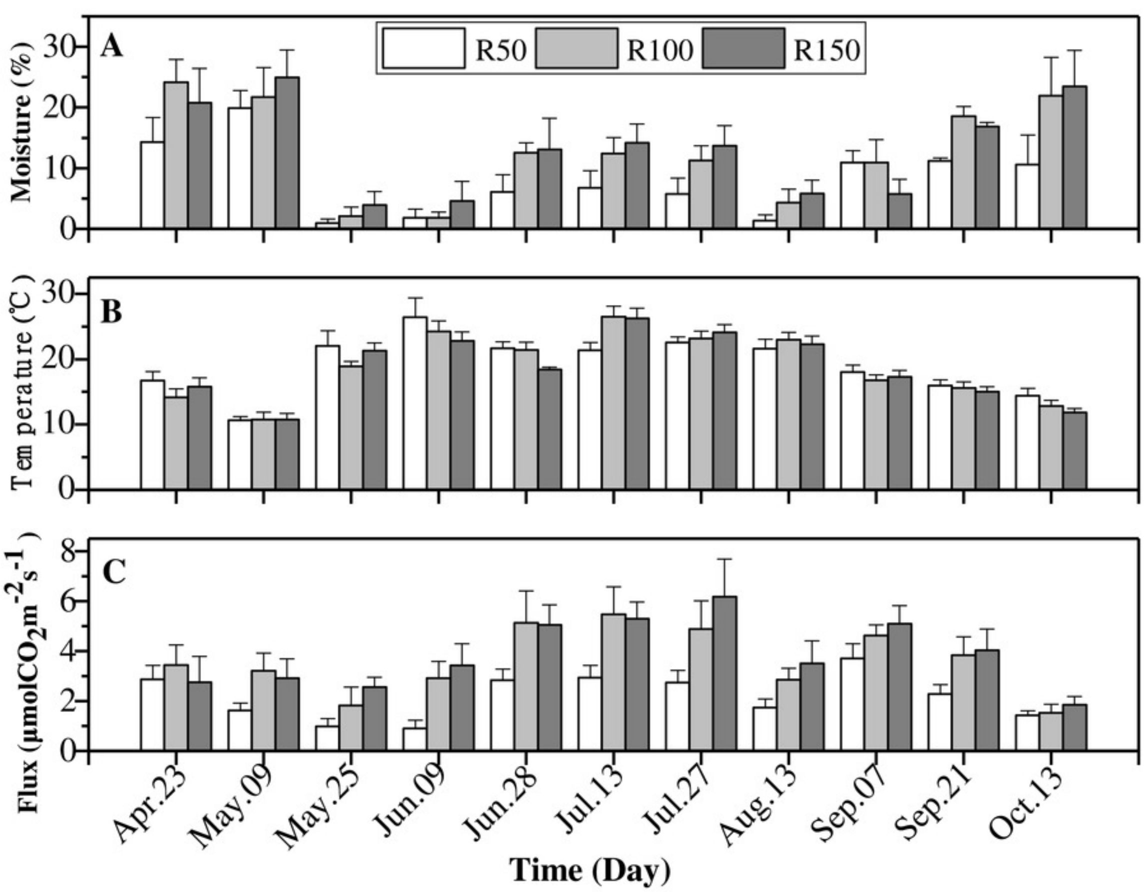

Table 1(on next page)

Soil nutrient content and soil pH in different precipitation treatments and soil depths.

Notes. Pre=Precipitation; SD=soil depth; SD1=soil depth 0-9.9cm; SD2=soil depth 10-19.9cm; SD3=soil depth 20-30cm; Pre* SD= Precipitation * soil depth . Values are mean ± standard error (n = 3).[a-b]Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA.

Variables	R50			R100			R150			P-Value		
	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3	Pre	SD	Pre*SD
TN (g kg-1)	2.3 ± 0.1 a	2.1 ± 0.1 b	1.9 ± 0.1 b	2.4 ± 0.0a	2.1 ± 0.0b	2.1 ± 0.1b	2.3 ± 0.1a	2.0 ± 0.1b	2.0 ± 0.0b	0.041	0.000	0.253
SOC (g kg-1)	8.1 ± 0.1a	6.1 ± 0.3bc	6.2 ± 0.2c	8.0 ± 0.0 a	7.0 ± 0.1 b	6.6 ± 0.3 bc	7.7 ± 0.3 a	6.8 ± 0.2bc	6.6 ± 0.3bc	0.045	0.000	0.049
TP (g kg-1)	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.231	0.687	0.213
pH	7.8 ± 0.1ab	7.8 ± 0.1ab	7.9 ± 0.1ab	7.8 ± 0.1 ab	8.0 ± 0.2a	8.1 ± 0.1a	7.7 ± 0.1b	7.9 ± 0.1 ab	8.0 ± 0.2 ab	0.022	0.003	0.626

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Table 2(on next page)

Alpha diversity of bacteria and fungi under different precipitation treatments and soil depths.

Notes. Pre=Precipitation; SD=soil depth; SD1=soil depth 0-9.9cm; SD2=soil depth 10-19.9cm; SD3=soil depth 20-30cm; B=Bacteria; F=Fungi; Community richness (Sobs' index), community diversity (Shannon's index) and community coverage (of OTUs) of bacteria and fungi under different precipitation treatments and soil depths. Different letters show significantly different values between depths within each precipitation as analyzed by two-way ANOVA ($P < 0.05$) .

Variables	R50			R100			R150			P-Value	
	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3	Pre	SD
B_Sobs	2529a	2183b	2037 bc	2615 a	2157 b	2024 bc	2530 a	2222 b	1880 c	0.46	0.00
B_Shannon	6.7 ab	6.3 d	6.2 d	6.7 a	6.4 cd	6.2 d	6.6 abc	6.4 bcd	6.2d	0.44	0.00
B_Coverage （%）	96.0	96.4	96.5	95.8	96.4	96.6	95.8	96.3	96.9	0.82	0.01
F_Sobs	725ab	528 abcd	491bcd	755a	458 cd	414 cd	632 abc	490bcd	352 d	0.11	0.00
F_Shannon	4.3 ab	3.4 ab	3.3 ab	4.5 ab	4.0 ab	3.2 b	4.7 a	3.8 ab	3.7 ab	0.33	0.00
F_Coverage （%）	99.8	99.8	99.9	99.8	99.9	99.9	99.9	99.9	99.9	0.02	0.00

Table 3(on next page)

ANOVAs for soil temperature, soil moisture and soil CO_2 flux between precipitation treatments and dates during the whole experimental period.

Notes. num DF: number of degrees of freedom; den DF: the number of degrees of freedom associated with the model errors.

	numDF	denDF	Soil moisture		Soil temperature		Soil CO_2 flux	
			F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
Intercept	1	60	822.1	<0.0001	17856	<0.0001	1626.0	<0.0001
Precipitations	2	6	42.7	0.0003	5.4	0. 046	55.9	0.0001
Dates	10	60	414.5	<0.0001	348.0	<0.0001	156.7	<0.0001
Precipitations * Dates	20	60	14.8	<0.0001	8.0	<0.0001	9.7	<0.0001

Table 4(on next page)

Precipitation treatments differences for soil moisture, soil temperature and soil CO_2 flux over the whole experimental period.

Notes. Values are the mean ± standard error (n=3). There are three precipitation treatments, and each treatment has three repetitions. The whole test period is repeated for 11 times. Different letters show significantly different between precipitation treatments (*P* < 0.05).

Precipitations	R50	R100	R150
Mean soil moisture (%)	8.1 ± 2.3 b	12.9 ± 2.9 ab	13.4 ± 3.5 a
Mean soil temperature (℃)	19.2± 1.3 a	18.9 ± 1.1 a	18.7 ± 1.1 a
Mean soil CO_2 flux (μ mol CO_2 m^{-2} s^{-1})	2.2 ± 0.4 b	3.6 ± 0.8 ab	3.9 ± 0.8 a

Table 5(on next page)

Seasonal differences in soil moisture, temperature and CO_2 flux among all precipitation treatments.

Notes. Values are the mean ± standard error (n=3).

Date	Soil moisture (%)	Soil temperature (℃)	Soil CO_2 flux (μmol CO_2 m^{-2} s^{-1})
Apr.23	19.7 ± 4.5	15.6 ± 1.3	3.02 ± 0.8
May.09	22.2 ± 4.1	10.7 ± 0.9	2.58 ± 0.6
May.25	2.3 ± 1.4	20.8 ± 1.4	1.78 ± 0.5
Jun.09	2.8 ± 1.9	24.5 ± 2.0	2.41 ± 0.6
Jun.28	10.6 ± 3.2	20.5 ± 0.9	4.33 ± 0.8
Jul.13	11.1 ± 2.9	24.7 ± 1.5	4.56 ± 0.8
Jul.27	10.2 ± 2.8	23.3 ± 1.1	4.59 ± 1.1
Aug.13	3.8 ± 1.8	22.3 ± 1.2	2.69 ± 0.6
Sep.07	9.2 ± 2.7	17.4 ± 0.9	4.48 ± 0.6
Sep.21	15.5 ± 0.9	15.5 ± 0.9	3.38 ± 0.7
Oct.13	18.7 ± 5.7	13.0 ± 0.9	1.59 ± 0.3

Table 6(on next page)

Predicting the model of soil CO_2 flux according to Stepwise regression.

Notes. It based on soil moisture and the most informative factors from the July measurements of each of the sets of soil properties, plant factors and soil microbial diversity (Shannon index) in three precipitation treatments.

Table 6 Stepwise regression to predict the main factors affecting soil respiration

Input variable	excluded Variables	R	Adjust R^2	Sig	Durbin-Watson
Soil moisture	RB 0-9.9cm, RB 0-9.9cm, Soil pH, Soil fungal diversity	0.941	0.870	0.00	2.727
Model	Soil CO_2 flux = 0.35 + 0.42 Soil moisture				