

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

Yutao Wang¹, Yingzhong Xie¹, Jill Rapson², Hongbin Ma¹, Le Jing¹, Yi Zhang¹, Juan Zhang¹, Jianping Li^{Corresp. 1}

¹ School of agriculture, Ningxia University, Yinchuan, Ningxia, China

² School of Agriculture and Environment, Massey University, Palmerstone North, New Zealand

Corresponding Author: Jianping Li
Email address: lijianpingsas@163.com

Background. Precipitation changes induced by global climate change profoundly impact on the vulnerability of dry ecosystems, such as upland grasslands. In dry climates, which probably will experience greater drought, soil respiration is a critical factor in carbon cycling. **Methods.** To understand the effect of precipitation on soil respiration of typical steppe (i.e., dry, low-density upland grassland) in the Loess Plateau of China, three precipitations were applied. Precipitation was captured and relocated to simulate precipitation of 50%, 100% and 150% of incident. **Results and Discussion.** Across precipitations, soil moisture was correlated with applied precipitation. Above-ground biomass was strongly positively related to applied precipitation, but root biomass was highest at 100% of precipitation. There is a positive linear relationship between soil moisture and soil respiration in the summer (July and August), when most precipitation falls in the study area. Soil temperature also demonstrated a positive, though weak, relationship with soil respiration over the whole plant growing season (April – October). Soil moisture, soil root biomass, pH and fungal diversity are indicators of soil respiration, among them, soil moisture is the best predictor. **Conclusion .** Our study highlights the importance of increased precipitation on soil respiration in drylands, more precipitation can produce more plant above-ground biomass and root biomass, which will feed back to climate, enhancing the rate of soil respiration in the future.

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Jianping Li¹, *

¹ School of agriculture, Ningxia University, Yinchuan, Ningxia, China

² School of Agriculture and Environment, Massey University, Private Bag 11-222 Palmerstone
North 4442, New Zealand

*Corresponding Author:

Jianping Li

Ningxia University, Helanshan West Road, Xixia District, Yinchuan, Ningxia, 750021, China

Email address: lijianpingsas@163.com

Abstract

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Methods. To understand the effect of precipitation on soil respiration of typical steppe (i.e., dry, low-density upland grassland) in the Loess Plateau of China, three precipitations were applied. Precipitation was captured and relocated to simulate precipitation of 50%, 100% and 150% of incident.

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Conclusion. Our study highlights the importance of increased precipitation on soil respiration in drylands, more precipitation can produce more plant above-ground biomass and root biomass, which will feed back to climate, enhancing the rate of soil respiration in the future.

Subjects Agricultural Science, Ecology, Soil Science

Key words Climate change, Respiration, Precipitation, Soil respiration, Typical steppe, Loess Plateau

Introduction

In recent years, global changes in temperature and precipitation have occurred due to the emission of greenhouse gases (Gao et al. 2016). Over the next decades, temperatures are expected to gradually increase in most parts of the world, and extremes will also become more frequent (Garrett et al. 2006). Global warming will cause atmospheric water vapor content to

increase significantly and thus affect the hydrological cycle (O’Gormana & Schneider 2009), affecting the global distribution of precipitation to generate regional changes (Pall et al. 2006). Thus the intensity of precipitation events is expected to increase, and extreme precipitation events will occur more frequently according to the IPCC forecast (IPCC 2001).

Many previous studies indicate that changes in precipitation affect the dynamics of terrestrial carbon cycle, and terrestrial carbon pools (Wu et al. 2011; Ahlström et al. 2015; Frank et al. 2015; Felton et al. 2019a). Water is a driving factor for chemical and biological processes in ecosystems, including plant survival, photosynthesis, and 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; Frank et al. 2015; Felton et al. 2019a). 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 (Knapp et al. 2002; Jing et al. 2010).

Several studies have suggested that soil CO₂ flux is more sensitive to higher temperatures, since that roots exudates, root mycorrhizae, plant detritus and other part of plant have different temperature sensitivities to soil CO₂ flux (Richard D. Boone et al. 1998), and it will strongly impacts the terrestrial carbon cycle, and thus the soil CO₂ flux (Fischlin 2007; Frank et al. 2015).

Global emissions of CO₂ from soil are considered to be one of the largest fluxes in the global carbon cycle, and so small changes in soil respiration may have large impacts on the concentration of CO₂ in the atmosphere (Schlesinger & A. Andrew 2000). However, our understanding of the response of soil respiration to climate change is inadequate (Fernandez et al. 2006).

Recently, carbon fluxes have been studied in alpine meadows demonstrated 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 are due to elevation caused changes of roots and litter resulted in the increases of soil respiration (Zimmermann 2010), and in arid areas of temperate grasslands, it 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). Of these, grasslands play a key role in the carbon cycle (Poulter et al. 2014; Li et al. 2017; 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 both climate and ecosystems in China (Zhao et al. 2014). It is predicted to be one of the regions where temperature and precipitation will increase significantly in the future (Zhao et al. 2014). Here, we conduct field work to simulate increased and decreased precipitation to explore factors affecting soil CO₂ 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 linkages to terrestrial carbon cycles from the regional to the global.

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, located in the Guyuan Yunwu Mountain of Ningxia in the hinterland of the Loess Plateau, at an altitude of 1700—2148m. Climate is temperate, continental monsoon. The annual average precipitation is about 439 mm, ranging from a minimum of 282 mm in 1982 to a maximum of 706 mm in 2013, and greater than 50% is received in summer (June to August). The average annual temperature is 7.2 °C, ranging from a minimum of 5.3 °C in 1984 to a maximum of 8.7 °C in 2013. The average monthly minimum for the coldest month (January) is -7.2 °C, and the average monthly maximum for the warmest month (July) is 19.6 °C. Annual evaporation is 1300—1640 mm, annual sunshine duration can reach 2500 h, with a frost-free period of 112—140 days, and 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 type is typical

steppe and the main plant species are *Stipa bungeana*, *Artemisia gmelinii*, *Stipa grandis*,
Artemisia frigida, *Potentilla acaulis* and *Agropyron michnoi*.

Experimental design

In the hinterland of the Loess Plateau, semi-arid natural grassland which was ungrazed for 19 years was used as the research site. The altitude is 2077m, the slope 7—10°, and the aspect southern (sunny). During the experiment, annual precipitation for 2019, at 592 mm, was 20% higher than average. According to local multi-year meteorological data of the study area, the maximum and minimum precipitation is about 50% and 150% of the average annual precipitation. Therefore, selected three randomly blocks containing 3 plots, each 6×6 m, three precipitations were applied, i.e., 50% (R50), 100% (R100) and 150% (R150) of the natural precipitation, using rain-shelters. 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. The intercepted water was piped to the adjacent plot to form the R150 (Fig. 1). After snowfalls, snow was collected from the rain shelters (R50) and sprinkled evenly into the R150. In order to prevent surface runoff or leakage of soil moisture between plots, a 1.2 m deep plastic barrier was used, buried at a depth of 1.1 m, and projecting 10 cm above ground. The experiment ran from May 2017 to May 2019.

Environmental factors

In July, 2019, corresponding to the annual period of peak biomass, field data were collected under the three precipitations. Three replicate soil samples were collected at 0—9.9 cm, 10—19.9 cm and 20—30 cm depth in each plot, discarding litter. All soil samples were separated into two parts. One part was kept moist to determine soil microbial diversity, 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 by elemental analyzer (Elementar, Vario EL III, Germany). Total phosphorus (TP) was measured by the

method of Olsen (Olsen et al. 1982). Soil pH was measured using PHS-3C pH audiometer in a 1:5 of fresh soil /water slurry (Huakeyi, Beijing, China).

Avoiding collars (see below), 1m² quadrat was randomly selected in each subplot to determine plant biomass. The litter was raked up and bagged (Litter), and the shoot parts of the plants were cut as shoot (Shoot). Root biomass (RB) was sampled to 30 cm deep in three intervals of equal depth, with the soil being brushed off the roots. All plant samples were dried at 65°C in an oven for 75 hours and weighed.

Soil microbial diversity was determined based on the Illumina HiSeq sequencing platform of 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 alpha diversity of bacteria and fungi. Coverage index, as defined by Good (GOOD 1953) measures how well an environment is sampled, and here 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

From April 2019 to October 2019, soil respiration (release of CO₂) was measured every 14—16 days by a LI-8100A portable gas exchange system (LiCor, Lincoln NE, USA; chamber 8100-103 has a 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 3 cm, so the soil surface area within the collar was 317.8 cm². Five collars were placed randomly in each plot, giving a total of 45 collars. Above-ground parts of plants inside the collar were removed before taking each flux reading, though roots were left in place (Talat & Hema 2019). Soil fluxes were measured about every 16 days, between 9 am to 1 pm, based on weather conditions. The flux from each collar was measured for 100 s. Measurements of soil moisture at a depth of 5 cm (the Licor sensor is GS-1) and temperature at a depth of 10 cm (using the Licor sensor 6000-09 TC) were taken at the same time. To avoid pseudo-replication, the five values per plot were averaged for each variate to get a single datum for each

163 measurement time.

164 **Statistical analysis**

165 Statistical analysis was conducted using IBM SPSS (SPSS Inc, Chicago, USA) with ANOVA for
 166 modeling precipitation-based differences in soil properties, plant biomass and microbial diversity.
 167 Microbial diversity was calculated by Mothur (Version v.1.30). Origin (Origin Lab 2017,
 168 Microcal, Massachusetts, USA) was used for figures. Since the different dates formed temporal
 169 pseudoreplication, significance was explored by using nlme ([https://svn.r-project.org/R-](https://svn.r-project.org/R-packages/trunk/nlme)
 170 [packages/trunk/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
 171 and soil CO₂ flux (using the format lme: flux ~ Precipitations * Date, random = ~1 | plot, weights
 172 = varIdent (form = ~1 | Date). R was used to carry out Tukey HSD analysis on significant effects.
 173 The varIdent function in package Predictmeans v1.0.2
 174 (<https://www.rdocumentation.org/packages/predictmeans>) was used to allow each precipitation
 175 to have different variances. Package ggplot2 (<https://www.rdocumentation.org/packages/ggplot2>)
 176 was used for analyzing the correlation relationships of soil CO₂ flux and all other factors. If the
 177 correlation between two factors are high, ie, the correlation between soil organic carbon and soil
 178 total nitrogen is high (R = 0.95, P < 0.01), we only selected one of them (soil organic carbon) for
 179 subsequent analysis, then excluded AGB, Litter, soil TN, soil TP, soil C / N, total biomass, RB
 180 20—30, RB 0—30, AGB / RB. In addition to always including soil moisture, we used SPLS
 181 (<http://mixomics.org/methods/spls/>) to select normal variables to provide us with the main
 182 influencing factors of the soil CO₂ flux prediction model .Then we used Akaike's information
 183 criteria for small sample sizes (AICc, <https://www.rdocumentation.org/packages/AICcmodavg>)
 184 to compare and rank all candidate models composed of the main factors selected by SPLS. The
 185 smaller the AICc score, the better the model.

186 **Results**

187 **Precipitation and temperature during the trial**

188 Annual precipitation was 420 mm, 550 mm, and 592 mm during the period 2017—2019. That in
 189 2018 and 2019 was 30% higher than the average level of precipitation for the last 40 years.

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

Soil properties

Except for soil TP, the rest of the soil properties have their highest value under R100 in soil 0—30 cm deep (Table 1). SOC decreased the most under R50 (23%) as the depth of the soil layer deepens, then is R100 (18%), and R150 (14%). Compared with R100, SOC at R50 slightly increased just 1% in topsoil and decreased about 6% in 20-30 cm, while R150 has same value with R100 in 20—30 cm but decreased about 3% in 0—9.9 cm. Soil pH was greater at depth in all three precipitations. Soil TN and SOC were the highest in the topsoil, and decreased with depth.

Biomass of shoot, litter and root

Shoot biomass was largest in R150, and was 23% lower, though non-significantly, in R100, and significantly lower by 45% in R50. Litter biomass did not differ between precipitations, though it was greatest in R50. Root biomass 0—30 cm (RB) showed significant differences between the three precipitations ($P < 0.05$) according to ANOVA, with R100 having the most root biomass, and R150 and R50 being lower by 52% and 65%, respectively (Fig. 3A). Total biomass (TB: sum of shoot, Litter and RB 0—30) under R50 was significantly lower (about 30%) than R100 and R150 ($P < 0.05$). The shoot/root ratio (AGB/RB), was largest in R150, and was significantly lower by 64% in R100 and not significantly lower by 23% in R50 (Fig. 3B).

Different soil depths differed in the volume of roots they hosted ($P < 0.05$) according to ANOVA, all values being lower with depth (Fig. 4). Compared with R100 at 0—9.9 cm, R50 and R150 are significantly lower by 68% and 57%, respectively. Although the 10—19.9 cm deep layer did not differ between precipitations, the lowest layer was similar to the top layer, with R50 and R150 being significantly lower than R100 by 75% and 46%, respectively ($P < 0.05$) (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. Microbial richness via Sobs' index and diversity via Shannon's index are not significantly different in the different precipitations (Table 2). However, there were significant differences with soil depth, richness being highest at 0—10 cm under ambient precipitation, and reducing, though by only about 3% —16%, when precipitation is modified. With deepening of the soil layers, the Sobs index for both bacteria and fungi is gradually decreased.

Soil moisture, soil temperature and soil CO₂ flux

Soil moisture, soil temperature and soil CO₂ flux showed significant differences between precipitations over the whole experimental period (April — October) (Table 3). Measurements over the growing season also differ significantly for all variates, and these interact significantly with precipitations (Table 3).

Soil moisture and CO₂ 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 that to increased precipitation (3.9 % of R100). Mean soil CO₂ 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 variates (soil moisture, soil temperature and soil CO₂ flux) exhibit strong seasonality (Table 5). Soil moisture is highest in April and May, dropping to rise again in October. It also has medium values in July, showing a 'W-shaped' relationship with time. Soil temperatures peaked in summer (June—July—August). Though fluctuating, soil CO₂ flux showed an upwards trend from April to July, to peak before showing a decrease to be lowest in October (Table 5).

The three precipitations and 11 sampling times (from April to October) showed significant interactions for means of soil moisture, temperature and CO₂ flux (Table 3).

At most times, soil moisture was greater in R150, but this did not apply at April 23 and September (Fig. 5A). Generally highest soil temperature value showed in R50 (Table 4), but it reversed in June and July (Fig. 5B), when precipitation is at summer highs (Fig. 2). However the

norm was re-established during August, the period of maximum precipitation (Fig. 5B). Overall, the soil temperature rises first and then decreases with the measurement date.

Soil CO₂ flux showed the same trends as soil moisture for most months, that is, soil CO₂ flux was lower at the lower end of the precipitation gradient, with the highest at R150 and the lowest at R50. However, across the summer months, at early May, late June to early July, soil CO₂ flux with enhanced precipitation (R150) was lower than R50 and R100 (Fig 5C). Precipitations had little effect on soil CO₂ flux in October (autumn in study area).

Modeling soil CO₂ flux

Based on the results of the correlation analysis (Fig. S1), one of the normally distributed and strongly correlated factors is selected for SPL analysis. SPL (Fig. S2) analysis show that the factors most tightly correlated with soil respiration are soil moisture, RB 0—9.9, RB 10—19.9, soil pH and Fungal diversity.

Soil moisture is most highly correlated with soil CO₂ flux ($P < 0.001$, $R = 0.94$) (Fig. 6), which has a linear relationship with CO₂ flux. So other factors were also analyzed using a linear model. Soil moisture is the most important governing variate having the lowest AICc (AICc = 24, $R^2 = 0.87$) (Table 6). The other "best" models from the groups of variates (Table 6) have much higher Akaike scores and so are unacceptable as alternatives, as generally, only models with AICc < 2 of the preferred model are acceptable.

Discussion

While soil organic carbon, total nitrogen and pH in the soil did not change significantly in the three precipitations, soil SOC, TN and pH showed the highest value under R100. Both enhanced and decreased precipitation reduced these soil parameters. Nutrients in the soil can accumulate through the degradation of plants, litter and the secretion of roots (Qiu et al. 2009; Zhang et al. 2016). Our study showed that different precipitation did not have a significant impact on aboveground biomass and litter, but there is a positive correlation between aboveground biomass and actual precipitation. However, the precipitation had a significant effect on the root biomass, both of increased and decreased precipitation significantly reduced the root biomass in the study

area, and it is significantly higher under R100, especially in topsoil (0—9.9cm). In arid study areas, the impact of changes in precipitation on plants is influenced by the plant's 'balanced growth hypothesis'. Plant will preferentially distribute the obtained water to the root. In low-moisture areas, the proportion of plant roots that obtain moisture is higher than other parts of the plant (Talat & Hema 2019). Compared with the other two precipitations, higher biomass under R100 maybe effect the factors such as the degradation of plants and the secretion of roots and thus resulted in higher soil nutrient levels. However, compared with the evaporation of about 1500 mm per year on the Loess Plateau with limited precipitation, the increase in precipitation by 50% has little impact and effect on soil nutrients.

In the study, the soil microbial diversity index is unresponsive to three precipitations. Existing studies suggested that although available water is manifested a positive correlation with the richness and diversity of the community in the ecological process (Hawkins et al. 2003), this rule does not necessarily hold for 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. The Journal of Acta Ecologica Sinica (in Chinese) has studied one-year precipitation changes and the two-year precipitation changes in this study did not cause interference with microbial diversity. It may be that although the precipitation changes, the evaporation is large (1300—1640 mm) in the study area, after the precipitation changes, the soil temperature and humidity will quickly return to an original or a given value, resulting in a more stable environment.

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 gradient of precipitation, but soil temperature showed the opposite downward trend. The reason may be that the soil temperature is affected by the surface vegetation coverage (S. Kang 2000), vegetation coverage increases with increasing precipitation, the areas with higher vegetation coverage have more vegetation canopy can intercept solar radiation and reduce

evaporation, and thus lower soil temperature and local microclimate temperature; While the plant biomass is lowest at R50, with largest area of bare land, which causes more evaporation of the bare soil.

The change of soil temperature reflected to atmospheric temperature in the study area, but the highest value of soil temperature did not appear correspondingly when the atmospheric temperature was the highest, more precipitation at this time has a weakening effect on 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 is no significant correlation between soil respiration and soil temperature during vigorous plant growth. The reason may be that it was carried out for two years. Therefore, changes in soil temperature have limited effects on soil respiration. In addition, more and more studies showed that in arid regions limited by water, soil respiration responds stronger to precipitation pulses rather than soil temperature (Almagro et al. 2009).

Changes in soil moisture content and precipitation do not correlate that closely, except that it showed high values in spring and autumn, so its trend showed a "W" shape; in spring, melting snow replenishes the soil moisture, and lower evapotranspiration also helps to leave the soil moister. Besides, although precipitation is higher in summer, the plant growing season results in high evapotranspiration in the study area, so there is no peak of soil moisture, which would limit growth. Moreover, precipitation events in water-limit area 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 temperatures and evapotranspiration losses decreased in autumn (September to November) (Maes & Steppe 2012; Wang et al. 2013; Felton et al. 2019b). In this experiment, soil moisture under increased precipitation is significantly higher than decreased, which may be mainly caused by the soil permeability and the evaporation of the bare surface. Some literature indicates that the non-negligible factors affecting soil moisture include soil

permeability, surface runoff and evaporation and evapotranspiration (Wang et al. 2012). In our experiment, surface run-off can be ignored because of a plastic barrier with an underground depth of 110 cm and a ground height of 10 cm was designed. A better root system can improve soil permeability, aeration, and porosity (Ozalp et al. 2016). More root biomass in R100 and R150 resulted in soil permeability is improved and the soil can store moisture and increase humidity. In addition, the aboveground biomass under the increased precipitation, with high plant coverage can have a blocking effect on the evaporation of soil moisture, is higher than decreased.

As we all know, soil moisture is one of the main environmental factors affecting soil CO₂ flux by affecting 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; Zhang et al. 2010; Lellei-Kovács et al. 2011). Soil moisture affects the metabolic activity of fine roots and microorganisms, because the diffusion of nutrients and substrates occurs in the aqueous medium. Therefore, the restrictions on soil metabolic activities will increase as the soil moisture decreases (Curiel Yuste et al. 2007). Besides, it is considered that soil respiration is not only affected by soil moisture (Balogh et al. 2011), but has a clear threshold of soil moisture content for soil respiration. Our study suggested that the total soil CO₂ flux in R150 is higher than in R50 throughout the test period, but in April, June and July when the soil moisture content is higher than other months, the soil CO₂ flux of R150 is approximately equal or slightly below R50. The reason may be that in arid area, when the atmospheric precipitation is lacking, the soil moisture content has a greater impact on soil respiration; when atmospheric precipitation increases and the source of soil moisture replenishment such as ice and snow melting increases, the soil moisture in R50 has reached the maximum threshold of soil moisture for soil respiration in the study area. Increased precipitation produces little or invalid on soil respiration.

Akaike analysis showed that soil moisture is the best predictor of soil respiration; other influential factors include root biomass, pH and fungal Shannon diversity based on SPLS. There is a significant positive linear correlation between soil moisture and soil respiration throughout

the plant growth period, with the strongest correlation at the end of July ($R^2 = 0.87$). In this 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). Therefore, during the concentrated precipitation period in the arid area, heavy precipitation played a decisive role in soil respiration. This has been commonly found in arid and semi-arid regions with limited water conditions (Emmett et al. 2004; Sowerby et al. 2008; Zhang et al. 2010; Lellei-Kovács et al. 2011).

Soil respiration is a 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 enzymes activities, thereby inhibiting root and microbial activity (Liu et al. 2016). In turn, it has a certain impact on heterotrophic respiration. Our research results also show that soil root biomass and fungi can affect soil respiration. The shortcoming of this experiment is that the study area is an arid area, and the future climate change in precipitation may be mostly concentrated on a series of ecosystem responses caused by decreased precipitation, so there should be more repeated samples to explore 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. Increasing and decreasing precipitation reduce the root biomass of plants and increase the shoot/root ratio. Both increased and decreased precipitation can reduce, although not significantly, soil nutrients (SOC, TN and pH) of 0—30 cm and the bacterial and fungal richness of the top soil. Soil respiration affected by environmental factors include soil moisture, root biomass and soil pH, and biological factors include fungal diversity, where soil moisture plays a decisive role.

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Figure 1

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

Three precipitations were applied: R50 (= 50% of ambient precipitation), R100 (ambient) and R150 (= 150% of ambient precipitation).

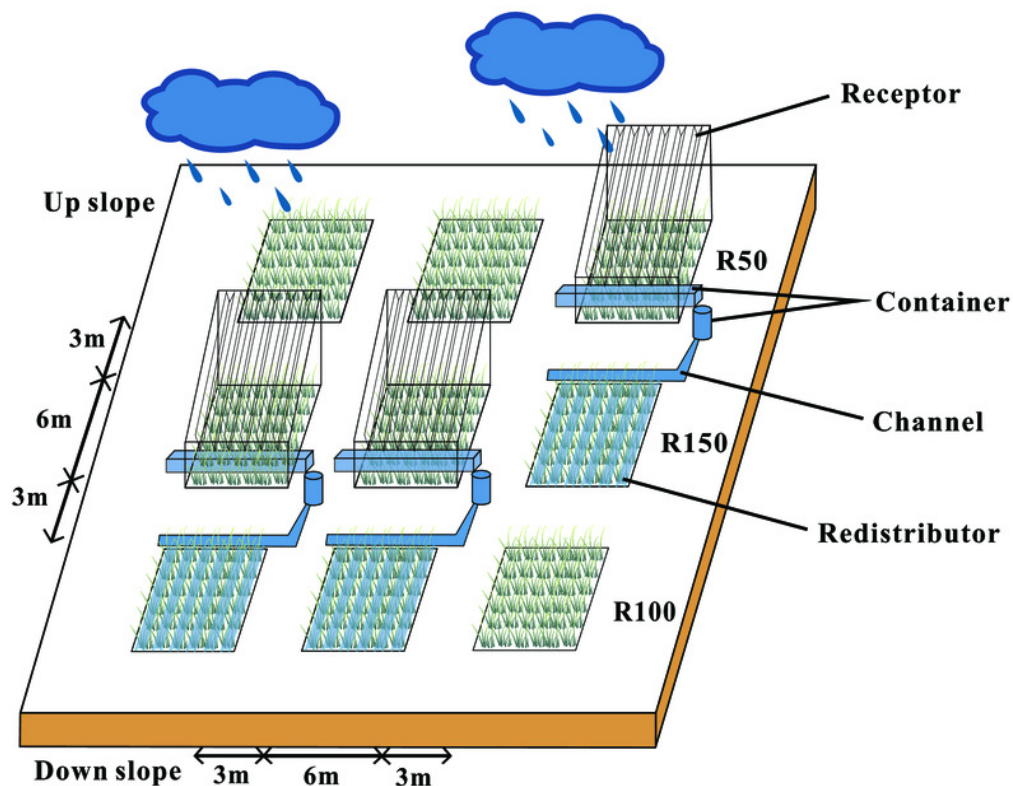


Figure 2

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

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

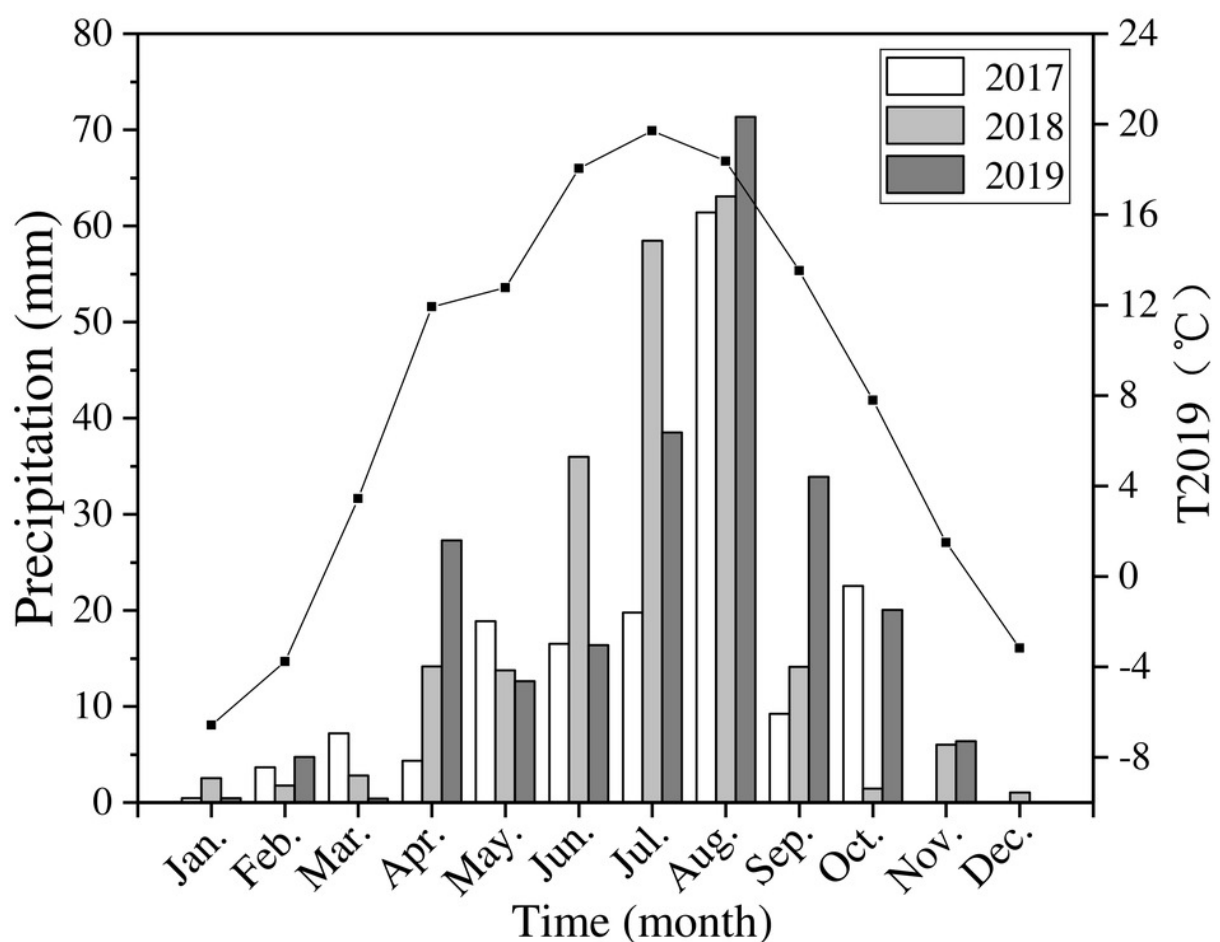


Figure 3

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

(A) Biomass of shoot, litter, roots, total biomass (TB); (B) shoot/root ratio. Different letters within each tissue type show significant differences ($P < 0.05$) between precipitations ($n = 3$, mean \pm standard error) according to ANOVA. Shoot, litter, roots and total biomass use left the Y axis, and AGB/RB the right.

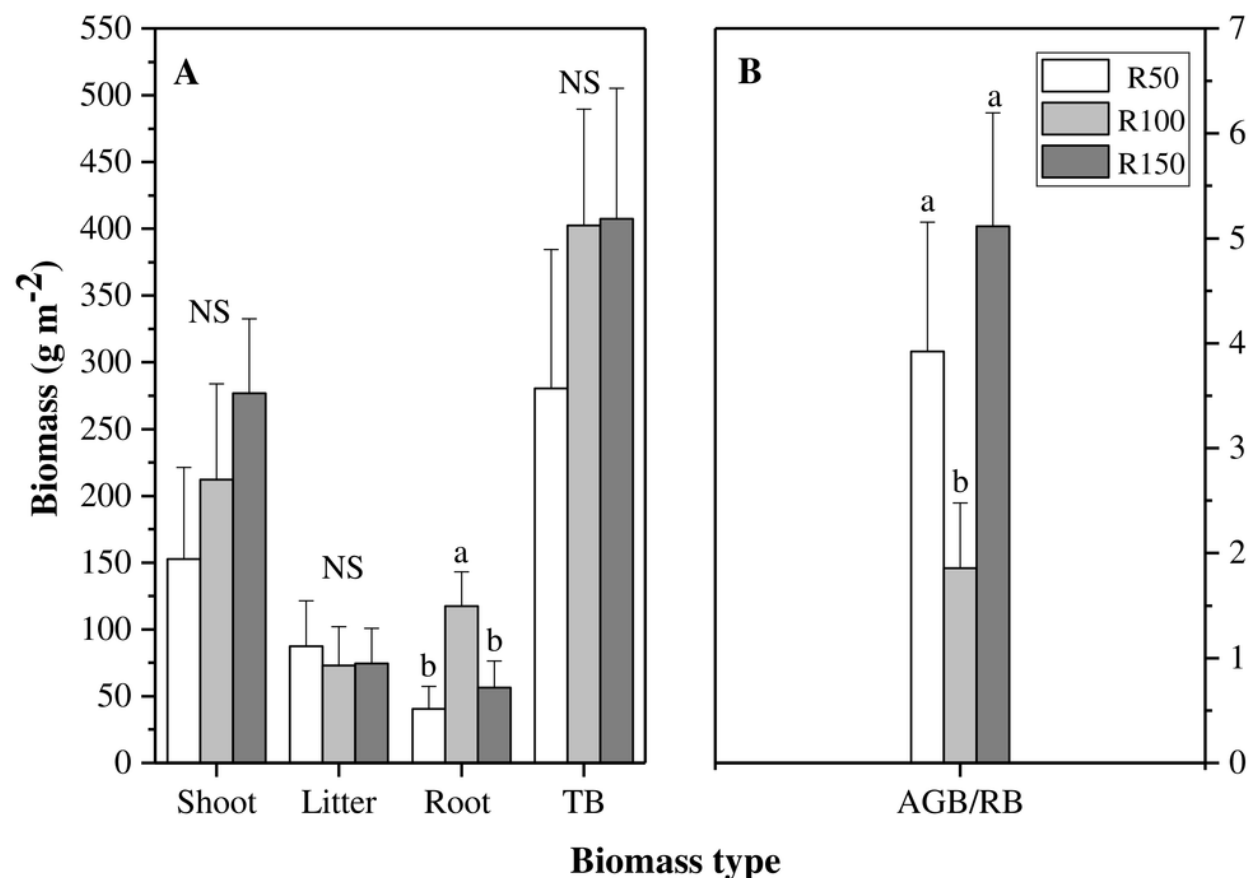


Figure 4

Root biomass (mean \pm standard error) at different soil depths in three precipitations.

Different letters show significant differences between precipitations and soil depths according to ANOVA.

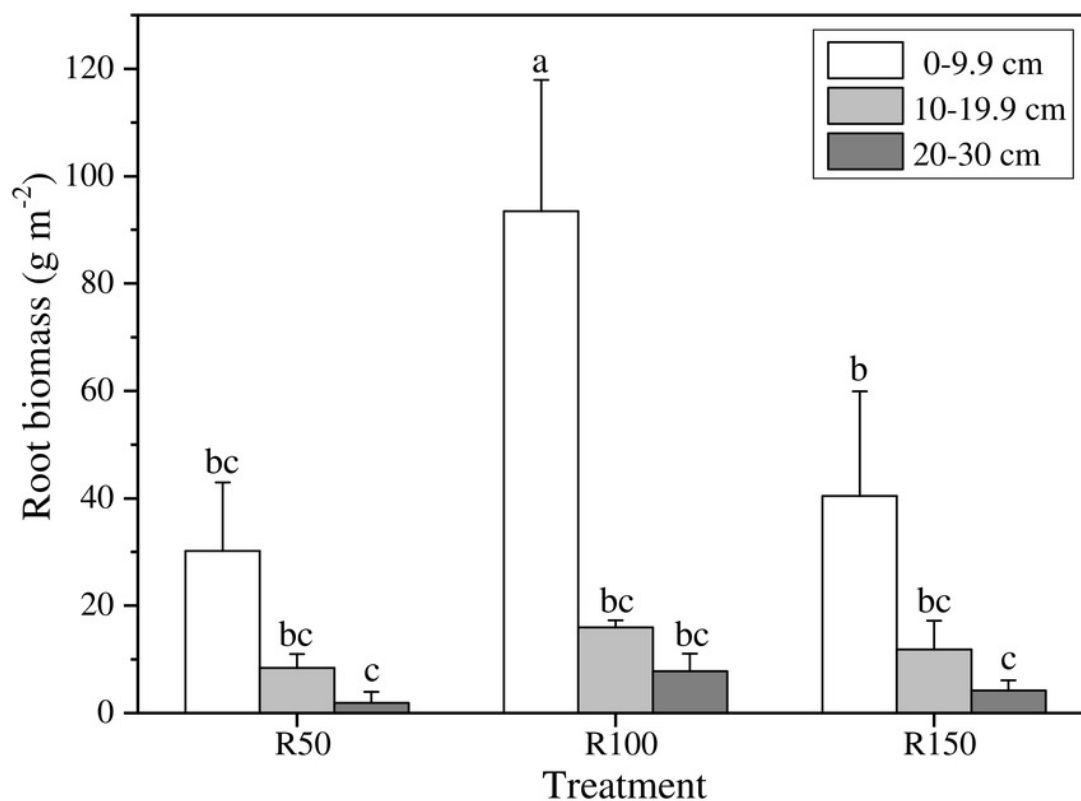


Figure 5

Interactions of precipitations R50, R100 and R150 and time for soil moisture, soil temperature and soil CO₂ flux during the growing season.

(A) Interactions of precipitations and time for soil moisture; (B) Interactions of precipitations and time for soil temperature ; (C) Interactions of precipitations and time for soil CO₂ flux.

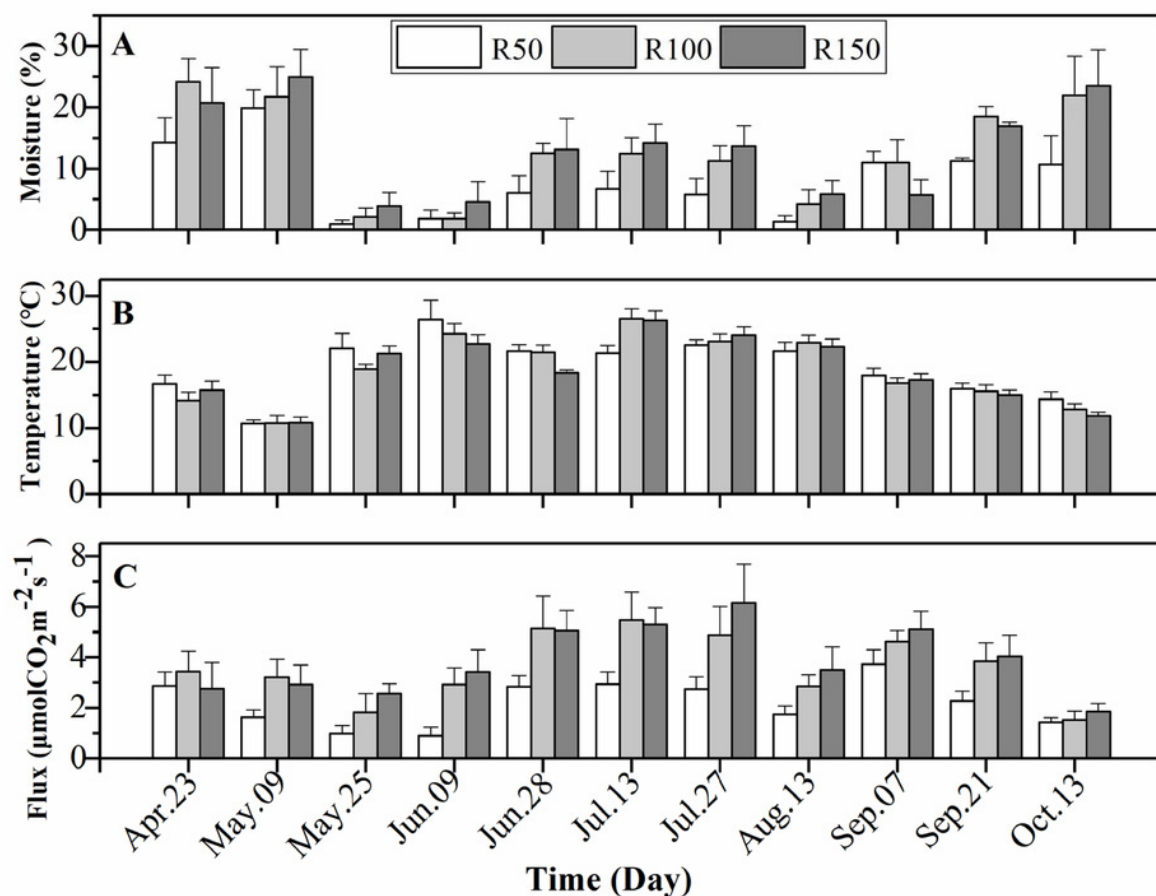


Table 1(on next page)

Soil nutrient content and soil pH (mean \pm standard error) in different precipitations and precipitation means and soil depths.

Precipitation	Soil depth (cm)	Soil TN (g kg ⁻¹)	Soil SOC (g kg ⁻¹)	Soil TP (g kg ⁻¹)	Soil pH
R50	0-9.9	2.3 ± 0.1 a	8.1 ± 0.1 a	0.7 ± 0.0 a	7.8 ± 0.1 b
R50	10-19.9	2.1 ± 0.1 b	6.1 ± 0.3 c	0.7 ± 0.0 a	7.8 ± 0.1 b
R50	20-30	1.9 ± 0.1 c	6.2 ± 0.2 c	0.7 ± 0.0 a	7.9 ± 0.1 b
R50	0-30	2.1	6.8	0.7	7.8
R100	0-9.9	2.4 ± 0.0 a	8.0 ± 0.0 a	0.7 ± 0.0 a	7.8 ± 0.1 b
R100	10-19.9	2.1 ± 0.0 b	7.0 ± 0.1 b	0.8 ± 0.1 a	8.0 ± 0.2 ab
R100	20-30	2.1 ± 0.1 b	6.6 ± 0.3 c	0.7 ± 0.0 a	8.1 ± 0.1 a
R100	0-30	2.2	7.2	0.7	8.0
R150	0-9.9	2.3 ± 0.1 a	7.7 ± 0.3 a	0.7 ± 0.0 a	7.7 ± 0.1 b
R150	10-19.9	2.0 ± 0.1 bc	6.8 ± 0.2 bc	0.7 ± 0.0 a	7.9 ± 0.1 ab
R150	20-30	2.0 ± 0.0 bc	6.6 ± 0.3 bc	0.7 ± 0.0 a	8.0 ± 0.2 ab
R150	0-30	2.1	7.0	0.7	7.9

1 Notes.

2 Different lower-case letters in each column indicate significant ($P < 0.05$) differences between the three depths of
 3 soil samples in each of the three precipitations (down columns) according to ANOVA.

Table 2(on next page)

Alpha diversity of bacteria and fungi under different precipitations and soil depths.

Precipitation	Soil depth (cm)	Bacteria			Fungi		
		Sobs	Shannon	Coverage (%)	Sobs	Shannon	Coverage (%)
R50	0-9.9	2528 a	6.7 a	96.0 a	724.7 a	4.3 ab	99.8 a
R50	10-19.9	2183 bc	6.4 bc	96.4 a	528.0 b	3.4 b	99.8 a
R50	20-30	2037 c	6.2 c	96.5 a	490.7 bc	3.3 b	99.9 a
R50	0-30	2249	6.4	96.3	581.1	3.7	99.8
R100	0-9.9	2615 a	6.7 a	95.8 a	754.7 a	4.5 ab	99.8 a
R100	10-19.9	2157 bc	6.4 b	96.4 a	458.3 bc	4.0 ab	99.9 a
R100	20-30	2024 c	6.3 bc	96.6 a	413.7 bc	3.2 b	99.9 a
R100	0-30	2265	6.5	96.3	542.2	3.9	99.9
R150	0-9.9	2529 a	6.64 a	95.8 a	632.0 ab	4.7 a	99.9 a
R150	10-19.9	2222 b	6.41 b	96.3 a	489.7 bc	3.8 b	99.9 a
R150	20-30	1880 c	6.16 c	96.9 a	352.0 c	3.7 b	99.9 a
R150	0-30	2210	6.4	96.3	491.2	4.1	99.9

- 1 Notes.
- 2 Community richness (Sobs' index), community diversity (Shannon's index) and community coverage (of OTUs)
- 3 of bacteria and fungi under different precipitations. Different letters show significantly different values between
- 4 depths within each precipitation (down columns) by ANOVA.

Table 3(on next page)

ANOVAs for soil temperature, soil moisture and soil CO₂ flux between precipitations and dates during the whole experimental period.

	numDF	denDF	Soil moisture		Soil temperature		Soil CO ₂ flux	
			F-value	<i>P</i> -value	F-value	<i>P</i> -value	F-value	<i>P</i> -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

1 Notes.

2 num DF: number of degrees of freedom; den DF: the number of degrees of freedom associated with the model

3 errors.

Table 4(on next page)

Precipitation differences for soil moisture, soil temperature and soil CO₂ flux over the whole experimental period.

Precipitations	R50	R100	R150
Mean soil moisture (%)	8.1 ± 2.3	12.9 ± 2.9	13.4 ± 3.5
Mean soil temperature (°C)	19.2± 1.3	18.9 ± 1.1	18.7 ± 1.1
Mean soil CO ₂ flux (μ mol CO ₂ m ⁻² s ⁻¹)	2.2 ± 0.4	3.6 ± 0.8	3.9 ± 0.8

1 Note.

2 Values are the mean ± standard error (n=33).

Table 5(on next page)

Seasonal differences in soil moisture, temperature and CO₂ flux among all precipitations.

Date	Soil moisture (%)	Soil temperature (°C)	Soil CO ₂ flux (μmol CO ₂ m ⁻² s ⁻¹)
Apr.23	19.7 ± 4.5 ab	15.6 ± 1.3 cd	3.02 ± 0.8 bcd
May.09	22.2 ± 4.1 a	10.7 ± 0.9 e	2.58 ± 0.6 cd
May.25	2.3 ± 1.4 f	20.8 ± 1.4 b	1.78 ± 0.5 d
Jun.09	2.8 ± 1.9 f	24.5 ± 2.0 a	2.41 ± 0.6 cd
Jun.28	10.6 ± 3.2 cd	20.5 ± 0.9 b	4.33 ± 0.8 ab
Jul.13	11.1 ± 2.9 cd	24.7 ± 1.5 a	4.56 ± 0.8 a
Jul.27	10.2 ± 2.8 cd	23.3 ± 1.1 ab	4.59 ± 1.1 a
Aug.13	3.8 ± 1.8 ef	22.3 ± 1.2 ab	2.69 ± 0.6 cd
Sep.07	9.2 ± 2.7 de	17.4 ± 0.9 c	4.48 ± 0.6 ab
Sep.21	15.5 ± 0.9 bc	15.5 ± 0.9 cd	3.38 ± 0.7 abc
Oct.13	18.7 ± 5.7 ab	13.0 ± 0.9 de	1.59 ± 0.3 d

1 Notes.

2 Values are the mean ± standard error (n=45). Different letters show significantly different values between each
 3 sampling date ($P < 0.05$; columns) according to Tukey's HSD.

Table 6(on next page)

Regression equations for predicting soil CO₂ flux according to Akaike (AICc).

Equation	AIC _c	R ²	P
Flux = 0.35 + 0.42 moisture	24.0	0.87	<0.001
Flux = 0.44 + 0.43 moisture - 0.004 RB 0-9.9	30.6	0.86	<0.005
Flux = 0.59 + 0.44 moisture - 0.04 RB 10-19.9	30.3	0.86	<0.005
Flux = 5.41 + 0.41 moisture - 0.6 pH	31.1	0.85	<0.005
Flux = -1.54 + 0.39 moisture + 0.56 Fungal diversity	30.1	0.87	<0.005

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