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

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Background. Precipitation influences the vulnerability of grassland ecosystems, especially upland grasslands, and soil respiration is critical for carbon cycling in arid grassland ecosystems which typically experience more droughty conditions. **Methods.** We used three precipitation treatments to understand the effect of precipitation on soil respiration of a typical arid steppe in the Loess Plateau in north-western 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 by all precipitation treatments. Shoot biomass was greater, though non-significantly, as precipitation increased. However, both increase and decrease of precipitation significantly reduced root biomass. 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 partial least squares regression, and soil moisture was the best of these. **Conclusion .** Our study highlights the importance of increased precipitation on soil respiration in drylands. Precipitation changes can cause significant alterations in soil properties, microbial fungi, and root biomass, and any surplus or transpired moisture is fed back into the climate, thereby affecting the rate of soil respiration in the future.

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2 **Loess Plateau, China**

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28 **Abstract**

29 **Background.** Precipitation influences the vulnerability of grassland ecosystems, especially

30 upland grasslands, and soil respiration is critical for carbon cycling in arid grassland ecosystems

31 which typically experience more droughty conditions.

32 **Methods.** We used three precipitation treatments to understand the effect of precipitation on soil

33 respiration of a typical arid steppe in the Loess Plateau in north-western China. Precipitation was

34 captured and relocated to simulate precipitation rates of 50%, 100%, and 150% of ambient

35 precipitation.

36 **Results and Discussion.** Soil moisture was influenced by all precipitation treatments. Shoot

37 biomass was greater, though non-significantly, as precipitation increased. However, both

38 increase and decrease of precipitation significantly reduced root biomass. There was a positive

39 linear relationship between soil moisture and soil respiration in the study area during the summer

40 (July and August), when most precipitation fell. Soil moisture, soil root biomass, pH, and fungal

41 diversity were predictors of soil respiration based on partial least squares regression, and soil

42 moisture was the best of these.

43 **Conclusion.** Our study highlights the importance of increased precipitation on soil respiration in

44 drylands. Precipitation changes can cause significant alterations in soil properties, microbial

45 fungi, and root biomass, and any surplus or transpired moisture is fed back into the climate,

46 thereby affecting the rate of soil respiration in the future.

47 **Introduction**

48 Recent changes in global temperatures and precipitation patterns have occurred due to the

49 increase in greenhouse gases (Gao et al. 2016). Temperatures are expected to gradually increase

50 in most parts of the world and extremes are anticipated to become more frequent (Garrett et al.

51 2006). Global warming is expected to cause atmospheric water vapor to increase significantly

52 and affect the hydrological cycle (O'Gorman & Schneider 2009), impacting global precipitation

53 patterns and causing regional precipitation changes (Pall et al. 2006). The intensity of

54 precipitation events is expected to increase, and extreme precipitation events will occur more

55 frequently according to the forecast of the Intergovernmental Panel on Climate Change (IPCC

56 2001).

57   Previous studies have indicated that changes in precipitation affect the dynamics of the

58 terrestrial carbon cycle and terrestrial carbon pools (Ahlström et al. 2015; Felton et al. 2019;

59 Frank et al. 2015; Wu et al. 2011). Water is a driving factor for chemical and biological

60 processes in ecosystems, including plant survival, photosynthesis and respiration, heterotrophic

61 respiration (Gerten et al. 2008; Zhang et al. 2019a), soil nutrient dynamics (Yuan et al. 2017),

62 terrestrial ecosystem functioning (Wu et al. 2009), and microbial activity, diversity, and

63 respiration (Classen et al. 2015; Felton et al. 2019; Frank et al. 2015). Moreover, changes in

64 global precipitation patterns exert profound effects on the nature of vegetation (Gao et al. 2016),

65 especially in arid and semiarid regions where water is the main limiting factor for plant growth

66 (Jing et al. 2010; Knapp et al. 2002).

67   Global emissions of CO_2 from soil are considered to be one of the largest causes of flux in the

68 global carbon cycle and small changes in soil respiration may have large impacts on atmospheric

69 CO_2 concentrations (Schlesinger & Andrew 2000). Soil CO_2 flux is sensitive to higher

70 temperatures, since root exudates, root mycorrhizae, plant detritus, and other part of the plant

71 have different temperature sensitivities to fluctuations in soil CO_2 levels (Boone et al. 1998) ,

72 which themselves strongly impact the terrestrial carbon cycle (Fischlin et al. 2007; Frank et al.

73 2015). However, we still have a poor understanding of the response of soil respiration as related

74 to climate change (Fernandez et al. 2006).

75   Carbon fluxes have recently been studied in alpine meadows and show that only underground

76 biomass and soil moisture have a direct effect on soil respiration (Geng et al. 2012). Studies on

77 tropical forests have shown that higher elevation decreases in root and litter samples resulted in

78 increases in soil respiration, with microbial respiration more closely related to soil moisture

79 levels (Zimmermann et al. 2010). In arid temperate grasslands, soil respiration has a positive

80 response to extreme precipitation events, affecting the ecosystem's carbon cycle (Thomey et al.

81 2011). However, there are few studies on semi-arid ecosystems which account for about 15% of

82 the terrestrial area of the globe (Huang et al. 2015). They are especially sensitive to precipitation

83 changes (Niu et al. 2019) . Often occurring in semi-arid areas, grasslands play a key role in the

84 carbon cycle (Li et al. 2017; Poulter et al. 2014; Zhang et al. 2019b), while being very sensitive

85 to large-scale climate change (Feng et al. 2006).

86   The upland Loess Plateau in northwestern China is a critical transition zone for semi-arid

87 ecosystems in China (Zhao et al. 2014). It is predicted that temperature and precipitation will

88 increase significantly in this area (Zhao et al. 2014), where we conducted field work to simulate

89 increased and decreased precipitation exploring the factors affecting soil CO_2 flux. Our study has

90 great scientific and practical implications for the effects of precipitation on soil respiration and

91 plant productivity and their roles in regional and global terrestrial carbon cycles. We sought to

92 evaluate the differences in soil properties, plant biomass, and microbial diversity at different soil

93 depths using different precipitation treatments, to determine the main factors affecting soil

94 respiration in the study area.

95 **Materials & Methods**

96 **Study sites**

97 The study was conducted at the Agriculture Experimental Station of Ningxia University ,

98 Yinchuan Province of China, in Yunwu Mountain of Guyuan, Ningxia (106°21′E-106°27′ E,

99 36°10′ N-36°17′ N). The climate of the area is temperate, continental monsoon. The annual

100 average precipitation is about 439 mm and varied from 282 mm in 1982 to 706 mm in 2013.

101 More than 50% of the annual precipitation occurs in the summer months (June to August). The

102 average annual temperature is 7.2 ℃, and varied from 5.3 ℃ in 1984 to 8.7 ℃ in 2013. The

103 average monthly minimum temperature for the coldest month (January) was -7.2 ℃, and the

104 average monthly maximum temperature for the warmest month (July) was 19.6 ℃. The annual

105 evaporation is 1300-1640 mm, and the annual duration for sunshine can reach 2500 h, with a

106 frost-free period of 112-140 days. The annual potential evapotranspiration is 1625 mm

107 (meteorological data from 1981 to 2017 is from the National Meteorological Administration of

108 China). The soils are grey-cinnamon and dark loessial, as classified by the Chinese soil

109   classification system (National Soil Survey Office 1993). The vegetation is typical steppe and the

110   main plant species are *Stipa bungeana*, *Artemisia gmelinii*, *Stipa grandis*, *Artemisia frigida*,

111   *Potentilla acaulis* and *Agropyron michnoi* (Wang et al. 2020).

112   **Experimental design**

113   Our research site was located in a semi-arid natural grassland that was left ungrazed for 19 years.

114   The study area was at 2077 m, with a 7-10° slope, and a south-facing, sunny aspect. Annual

115   precipitation in 2019 was 592 mm, which was 20% higher than average. We set three blocks

116   with three 6×6 m plots in each block, to make a total of 9 plots. Each trio of plots down the

117   gradient was considered to be a block (Fig. 1). According to local multi-year meteorological data

118   from the study area, the mean maximum and minimum precipitation levels were about 50% and

119   150% of the average annual precipitation. A Rain shelter was set up on each block. Each rain-

120   shelter was fixed to the ground by steel pillars, and transparent polyethylene plates were fixed in

121   "V" shapes to intercept precipitation and channel it off the plot using the natural slope of the

122   mountain, while forming a stable and well-ventilated structure (Afreen & Singh 2019). Rain

123   shelters intercepted half of the natural precipitation to form a reduced precipitation treatment

124   (R50). The intercepted water was piped to an adjacent plot to form an increased precipitation

125   area (R150). The remaining three plots were the controls (R100). Snow was collected from the

126   rain shelters after each snowfall (R50) and was sprinkled evenly into the R150 plots. Plastic

127   barriers were used to prevent surface runoff or leakage of soil moisture between plots. Each

128   barrier was buried at a depth of 1.1 m and projected 10 cm above ground. Our study ran from

129   May 2017 to May 2019.

130   **Environmental factors**

131   We collected field data in July 2019 which corresponded to the annual period of peak biomass.

132   Three soil sample replicates were collected at depths of 0-9.9 cm, 10-19.9 cm, and 20-30 cm in

133   each plot, after the litter was discarded. The soil samples were separated into two parts: one part

134   was kept moist to determine the microbial diversity of the soil, and the other was air-dried for

135   measurement of soil properties.

136   Soil organic carbon (SOC) was measured by potassium dichromate-sulfuric acid digestion,

137   with ammonium ferrous sulfate titration. Total nitrogen (TN) was determined using an Elementar

138   analyzer (Elementar, Vario EL III, Germany). Total phosphorus (TP) was measured using

139   Olsen's method (Bremner & Mulvaney 1982). Soil pH was measured using a PHS-3C pH Meter

140   in a 1:5 ratio of fresh soil: water slurry (Huakeyi, Beijing, China).

141   A 1 m^2 quadrat was randomly selected in each subplot to determine the plant biomass. The

142   litter was raked and bagged and the shoots of the plants were cut at ground level. Root biomass

143   (RB) was sampled to 30 cm in three intervals of equal depth and the soil carefully brushed off

144   the roots. All plant samples were dried at 65℃ in an oven for 75 hours and then weighed.

145   Soil microbial diversity was determined based on the Illumina HiSeq sequencing platform of

146   the Majorbio Cloud Platform (www.majorbio.com). The bacterial primer was 338F_806R and

147   the fungal primer was ITS1F_ITS2R. Sobs' and Shannon's indices were used to indicate the

148   alpha diversity of bacteria and fungi. Coverage index, as defined by Good (Good 1953) indicates

149   the percentage of operational taxonomic units (OTUs) sampled in a microbial community (i.e.

150   recovered per sample) as a percent of all OTUs found on the site (Chao 1984; Lemos et al. 2011).

151   **Measurements of soil respiration**

152   Soil respiration (release of CO_2) was measured every 14-16 days by the LI-8100A portable gas

153   exchange system from April 2019 to October 2019 (LiCor, Lincoln NE, USA; chamber 8100-

154   103, diameter of 20 cm). Polyvinyl chloride (PVC) collars were set permanently in place in the

155   soil one week before the first measurement to minimize soil disturbance. The height of each

156   collar was 12 cm and the above-ground height was 3 cm, with the soil surface area and volume

157   within the collar being 317.8 cm^2 and 953.4 cm^3, respectively. Five collars were placed randomly

158   in each plot, giving a total of 45 collars. The above-ground parts of the plants inside the collar

159   were removed before taking each flux reading and the roots were left in place (Afreen & Singh

160   2019). Soil fluxes were measured about every 16 days, between 9 am and 1 pm, based on

161   weather conditions. The flux from each collar was measured for 100 s. Soil moisture was

162   measured at a depth of 5 cm with a GS-1 Licor sensor, and the temperature was measured

163   concurrently at a depth of 10 cm using the Licor sensor 6000-09 TC. To avoid pseudo-replication,

164   the five values per plot were averaged for each variable to get a single datum for each

165   measurement timepoint.

166   **Statistical analysis**

167   Statistical analysis was conducted using IBM SPSS (IBM, Chicago, USA). One-way ANOVA

168   was used to process the aboveground and underground biomass of plants under different

169   precipitation treatments. A two-way ANOVA was used to process root biomass, soil nutrient

170   content, soil pH, and the microbial diversity index under different precipitation treatments and

171   different soil depths. Microbial diversity was calculated by Mothur (Version v.1.30). Origin

172   (Origin Lab 2017, Microcal, Massachusetts, USA) was used for figures. The different dates

173   formed temporal pseudoreplication, so the significance was explored using nlme (https://svn.r-

174   project.org/R-packages/trunk/nlme; Author Pinheiro J) in R (R Core Team 2013; Version 3.0.2)

175   for soil temperature, soil moisture, and soil CO_2 flux (using the format lme: flux ~ Precipitations

176   * Date, random = ~1 | plot, weights = varIdent (form = ~1 | Date). The varIdent function in

177   package Predictmeans v1.0.2 (https://www.rdocumentation.org/packages/predictmeans; Authors

178   Luo D, Ganesh S, and Koolard J) was used to allow each timepoint to have a different variance.

179   Package ggplot2 (https://www.rdocumentation.org/packages/ggplot2; Authors Wickham H. et al)

180   was used for correlations of soil CO_2 flux and all other factors to filter out some variables. For

181   example, if the correlation between Soil SOC and Soil TN was high, we only selected one of

182   these variables, and then performed sPLS analysis (Partial Least Squares

183   regression)(http://mixomics.org/methods/spls/). By contrast, if the correlation between the two

184   was poor, we performed sPLS on both variables. Finally, we considered including soil moisture,

185   RB 0-19.9, soil pH, soil fungi in a sPLS analysis to select variables influencing the predictive

186   model of soil CO_2 flux. Stepwise regression was used to model of the main factors affecting soil

187   respiration.

188   **Results**

189   **Precipitation and temperature during the trial**

190 The annual precipitation was 420 mm, 550 mm, and 592 mm in 2017-2019. In 2018 and 2019

191 precipitation was 30% higher than the average level of precipitation for the last 40 years.

192 Precipitation in 2019 from April to October (the growing season) was 562 mm, which was about

193 95% of the average annual precipitation (Fig. 2). The average annual air temperature was 7.9 ℃

194 in 2019, which was 9% higher than the average temperatures from 1980 to 2019. The highest

195 temperature was 19.7 ℃ in July, and the lowest was -6.6 ℃ in January in 2019 (Fig. 2).

196 **Soil properties**

197 Two-way ANOVA results showed that, except for TP, soil properties had significant differences

198 under precipitation treatments and at different soil depths (P <0.05). Precipitation and soil depth

199 gave the only significant interaction in SOC. The rest of the soil properties had their highest

200 value under R100 in soil 0-30 cm deep (Table 1), with the exception of the soil TP. SOC was 23%

201 greater under R50 as the depth of the soil layer was greater, followed by R100 (18%) and R150

202 (14%). SOC at R50 increased just 1% in the topsoil (0-9.9 cm) and decreased about 6% in soil

203 depths of 20-30 cm, compared with R100, while R150 had same value with R100 in 20-30 cm

204 soil depths but decreased about 3% in the 0-9.9 cm soil band. As the depth of the soil layer

205 increased, soil pH gradually increased. Soil TN and SOC were highest in the topsoil (0-9.9 cm),

206 and decreased with soil depth.

207 **Biomass of shoot, litter and root**

208 There were no significant differences in shoot, litter, and total biomass (TB= sum of shoots, litter,

209 and RB 0-30) under different precipitation treatments according to one-way ANOVA's results.

210 Nevertheless, shoot biomass was greatest in R150, and was lower at lower precipitation. Litter

211 biomass was greatest in R50. By contrast, root biomass (RB) 0-30 cm showed significant

212 differences between precipitation treatments according to one-way ANOVA , with R100 having

213 the most root biomass, and R150 and R50 being significantly lower by 52% and 65% when

214 compared with R100, respectively (Fig. 3A). The total biomass under R50 was lower than for

215 treatments R100 and R150. The shoot/root ratio (Aboveground biomass/RB), was largest in

216 R150, and was significantly lower by 64% in R100 (P < 0.05) (Fig. 3B).

217   Different precipitation treatments and soil depths caused significant differences in root

218   biomasses according to two-way ANOVA. All values were lower at greater soil depths (Fig. 4).

219   The highest value of root biomass appeared at 0-9.9 cm in R100; values in R50 and R150 at 0-

220   9.9 cm were significantly lower by 68% and 57%, respectively. Both increased precipitation and

221   reduced precipitation significantly reduced the root biomass in the topsoil (0-9.9 cm) (Fig. 4).

222   **Microbial richness and diversity**

223   More than 95% of operational taxonomic units (OTUs) found in the microbial community at the

224   study site were present in each soil sample examined. There was no significant difference in soil

225   microbial richness via Sobs' index and diversity via Shannon's index under different precipitation

226   treatments and for the precipitation*soil depth interaction. However, there was a significant

227   difference in soil microbial richness and diversity with soil depth, and the value was greatest at

228   0-9.9 cm (Table 2). The Sobs' index and Shannon's index of bacteria and fungi gradually

229   decreased at greater soil depth.

230   **Soil moisture, soil temperature and soil CO_2 flux**

231   Soil moisture, soil temperature and soil CO_2 flux showed significant differences between

232   precipitation treatments over the entire experimental period (April - October) (Table 3).

233   Measurements over the growing season also differed significantly for all variates and were

234   significantly affected by precipitation levels ($P <0.05$) (Table 3).

235   Soil moisture and CO_2 flux were greater at higher levels of precipitation while soil

236   temperature was lower (Table 4). The response of soil moisture to the decreased precipitation

237   treatment (-37.2 % of R100) was greater than to increased precipitation (3.9 % of R100). Mean

238   soil CO_2 flux under R100 was higher by 38.9 % than that for R50, while for R150 the increase

239   relative to R100 was only 8.3 % (Table 4).

240   All variables (soil moisture, soil temperature and soil CO_2 flux) were strongly influenced by

241   seasonality (Table 3). Soil moisture was highest in April and May, then dropped to rise again in

242   October, giving a "W"-shaped relationship with time. Soil temperatures peaked in summer

243   (June-August). The soil CO_2 flux showed an upward trend from April to July, and peaked before

244   decreasing to its lowest levels in October (Table 5).

245   Soil moisture was typically greatest in the R150 treatment, with the exception of April and

246   September (Fig. 5A). The highest soil temperature values were typically seen in R50 (Table 4),

247   but this situation was reversed in June and July (summer; Fig. 5B), when precipitation was high

248   (Fig. 2). Overall, the soil temperature first rose and then decreased during the growing season.

249   Soil CO_2 flux showed the same trends as soil moisture for most months. The soil CO_2 flux was

250   lower at the lower end of the precipitation gradient and was highest at R150 and lowest at R50.

251   However, across the summer months, early May and early July, with enhanced precipitation

252   (R150), the soil CO_2 flux   was slight lower about 1%-10% , compared to R100 (Fig 5C). By

253   contrast, precipitation had little effect on soil CO_2 flux in the autumnal month of October.

254   **Modeling soil CO_2 flux**

255   Based on the results of the correlation analysis (Fig. S1), sPLS analysis was performed and

256   showed that the factors most closely correlated with soil respiration were soil moisture, root

257   biomass (RB 0-9.9, RB 10-19.9), soil pH, and fungal diversity (Fig. S2). Stepwise regression

258   showed that soil moisture was the only input factor that significantly affected soil CO_2 flux ($P <$

259   0.001, R = 0.94, adjusted R^2=0.870), and all other variables were excluded (Table 6).

260   **Discussion**

261   Soil organic carbon (SOC), total soil nitrogen (TN) and pH were significantly different in the

262   three precipitation treatments, having their highest values in R100, while both increased and

263   decreased precipitation reduced these values. Nutrients in the soil can accumulate through the

264   degradation of plants, litter, and root secretions (Qiu et al. 2009; Zhang et al. 2016). Our study

265   showed that different precipitation treatments had no significant impact on biomass of shoots and

266   litter, but there was a significant effect on the root biomass. Increases and decreases in

267   precipitation also significantly reduced root biomass, which was significantly higher in R100,

268   especially in the topsoil (0-9.9cm). Changes in precipitation may influence a plant's growth as

269   related to the balanced growth hypothesis (Shipley & Meziane 2002). Plants will preferentially

270   distribute any acquired water to the root in semi-arid areas (Afreen & Singh 2019). Therefore,

271   soil properties under different precipitation treatments may be more affected by the root biomass,

272   as root decomposition and roots' secretions will cause differences in SOC, TN and pH. We found

273   that as precipitation decreased (R50) in the study area, the water requirements for grass growth

274   were no longer met, and root biomass was significantly lower compared with that in R100. As

275   precipitation increased in the summer, the root biomass was higher in R150 than R50 and R100.

276   The indices for soil microbial diversity and richness were unresponsive to the three

277   precipitation treatments, but were responsive to different soil depths. While previous studies

278   have suggested that water supply is positively correlated with the richness and diversity of the

279   community (Hawkins et al. 2003), this rule does not necessarily apply to soil microorganisms

280   (Bachar et al. 2010), which may instead be determined by physical isolation of microbial

281   populations (Treves et al. 2003). In addition, short-term precipitation changes of 1 or 2 years

282   have little effect on microbial diversity (Wang et al. 2020).

283   Soil moisture, soil temperature, and soil respiration showed significant seasonal differences

284   under different precipitations throughout the experiment. Soil moisture and soil respiration

285   increased with the precipitation gradient, but soil temperature showed the opposite, downward,

286   trend. Soil temperature may be affected by coverage of surface vegetation (Kang et al. 2000),

287   which increases with increasing precipitation in semi-arid environments; the canopy intercepts

288   solar radiation and reduces evaporation, thus lowering soil and air temperatures. Plant biomass is

289   lowest in R50, which had the largest area of bare land and thus probably experienced more

290   evaporation from the bare soil. The higher the soil moisture, the greater the specific heat capacity,

291   so that occurrence of more soil water may lower the soil temperature.

292   Generally, changes of soil temperature reflected atmospheric temperature in the study area

293   except at very high temperatures, and more precipitation had a weakening effect on the

294   relationship between air and soil temperatures. Temperature regulates soil respiration by

295   changing the rate at which organisms process carbon and nutrients (Crowther & Bradford 2013).

296   Our study showed there was no significant correlation between soil respiration and soil

297   temperature during vigorous plant growth, which may be related to our brief experimental period,

298   only two years. Studies indicate that the temperature sensitivity of soil respiration will decrease

299   under continuous global warming (Peng et al. 2009). In addition, an increasing number of studies

300   show that soil respiration responds more strongly to precipitation pulses rather than soil warming

301   pulses in regions limited by water (Almagro et al. 2009).

302   Changes in soil moisture content and precipitation were not closely correlated, but showed

303   high values in spring and autumn and trended in a "W" shape during the growing season. In the

304   spring melting snow replenished the soil moisture, and lower evapotranspiration kept the soil

305   moist. However, high temperature with high evapotranspiration reduced the soil moisture in the

306   study area in summer and limited growth. Precipitation events in water-limited areas usually only

307   affect the topsoil and this water is easily lost by direct evaporation (Schwinning & Sala 2004),

308   resulting in no soil moisture gains. Generally, soil moisture accumulates as air temperature drop

309   and evapotranspiration losses are decreased in autumn (Felton et al. 2019; Maes & Steppe 2012;

310   Wang et al. 2013). We found that, under increased precipitation, soil moisture was increased in

311   May and then decreased towards September.

312   Literature has indicated that other non-negligible factors affecting soil moisture include soil

313   permeability, surface runoff, evaporation, and evapotranspiration (Wang et al. 2012). We found

314   that surface run-off could be negated with the use of a plastic barrier with an underground depth

315   of 110 cm and a ground height of 10 cm. A bigger root system can improve soil permeability,

316   aeration, and porosity (Ozalp et al. 2016). In our experiment, the greater root biomass caused by

317   increased precipitation may have resulted in improved soil permeability and moisture storage,

318   increasing soil moisture. At the same time, the higher above-ground biomass and canopy

319   coverage under the increased precipitation treatment may have had an inhibiting effect on

320   surface evaporation, which is intense in arid areas, while the rain-reduction treatment

321   experienced the opposite effect. Soil moisture is one of the main environmental factors affecting

322   soil CO_2 flux. Soil moisture affects the physiological performance of microorganisms and

323   nutrient diffusion (Yuste et al. 2007), especially in arid areas with limited water conditions

324   (Emmett et al. 2004; Lellei-Kovács et al. 2011; Zhang et al. 2010). A clear threshold effect links

325   soil respiration with soil moisture (Balogh et al. 2011), and especially so in arid areas (Niu et al.

326   2019). Our study demonstrated that the total soil CO_2 flux in R150 was higher than in R50

327   throughout the test period, but in April, June, and July when the soil moisture content was higher

328   than in other months, the soil CO_2 flux of R50 was almost equal to or even slightly above R150.

329   Spring-time soil moisture replenishment from ice and snow melt caused the soil moisture in R50

330   to reach the maximum threshold for soil respiration in the study area. Increased precipitation

331   produced little or no effect on soil respiration.

332   Soil respiration is the comprehensive result of autotrophic respiration mainly based on root

333   activity and heterotrophic respiration related to soil organic matter decomposition (Wang et al.

334   2014). In our experiment, despite other contributory factors including root biomass, pH, and

335   fungal Shannon diversity, multiple regression analysis showed that soil moisture was the best

336   predictor of soil respiration. Stepwise regression showed a significant positive linear correlation

337   between soil moisture and soil respiration throughout the plant growth period, with the strongest

338   correlation at the end of July (peak of summer) ($R^2 = 0.87$). In arid areas, concentrated and heavy

339   precipitation events can greatly stimulate soil respiration (Liu et al. 2016), which our results

340   support (Alwyn Sowerby et al. 2008; Emmett et al. 2004; Lellei-Kovács et al. 2011; Zhang et al.

341   2010). Drought can reduce the diffusion of organic substrates and decrease extracellular enzyme

342   activity, thereby inhibiting root growth and microbial activity and impacting heterotrophic

343   respiration (Liu et al. 2016). Our study area was confined to an arid area, which may limit the

344   broader applications of our responses. Future climate change in precipitation may be mostly

345   concentrated in a series of ecosystem responses caused by decreased precipitation and repeated

346   samples should be taken in R50 and at even lower soil moisture levels.

347   **Conclusions**

348   In summary, our research showed that both increased and decreased precipitation treatments

349   reduced the root biomass of plants and increased the shoot/root ratio. Precipitation changes

350   caused significant changes in soil moisture, soil temperature, and soil respiration during the

351   whole plant growing season in the study area, but moisture became non-limiting during heavy

352   precipitation episodes at the height of the growing season. Changes in precipitation treatments

353   significantly affected soil nutrients (SOC, TN and pH) across all soil depths, especially in the

354   topsoil. Though significantly affected by soil depth, microbial diversity and richness were not

355   sensitive to precipitation treatments. Overall, in arid grassland ecosystems where water is the

356   limiting factor, the ecological response to changes in soil moisture caused by precipitation

357   changes should be a key research topic to deepen our understanding of soil respiration in the

358   future.

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362

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523

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).

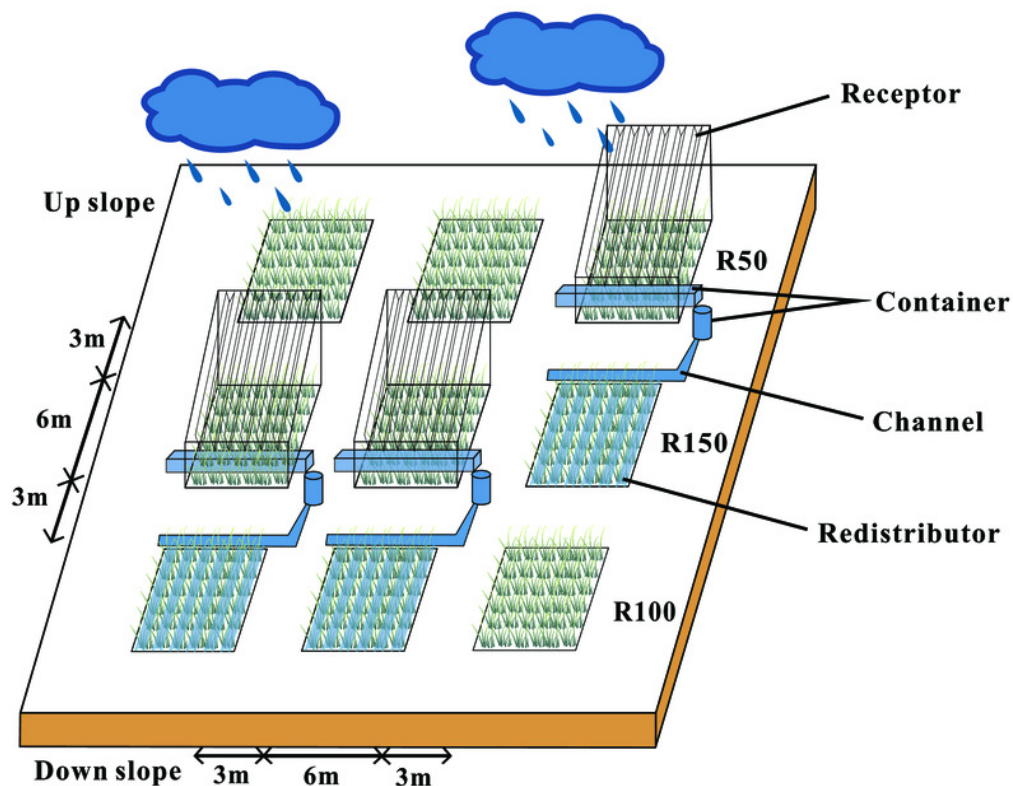



Figure 2

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.

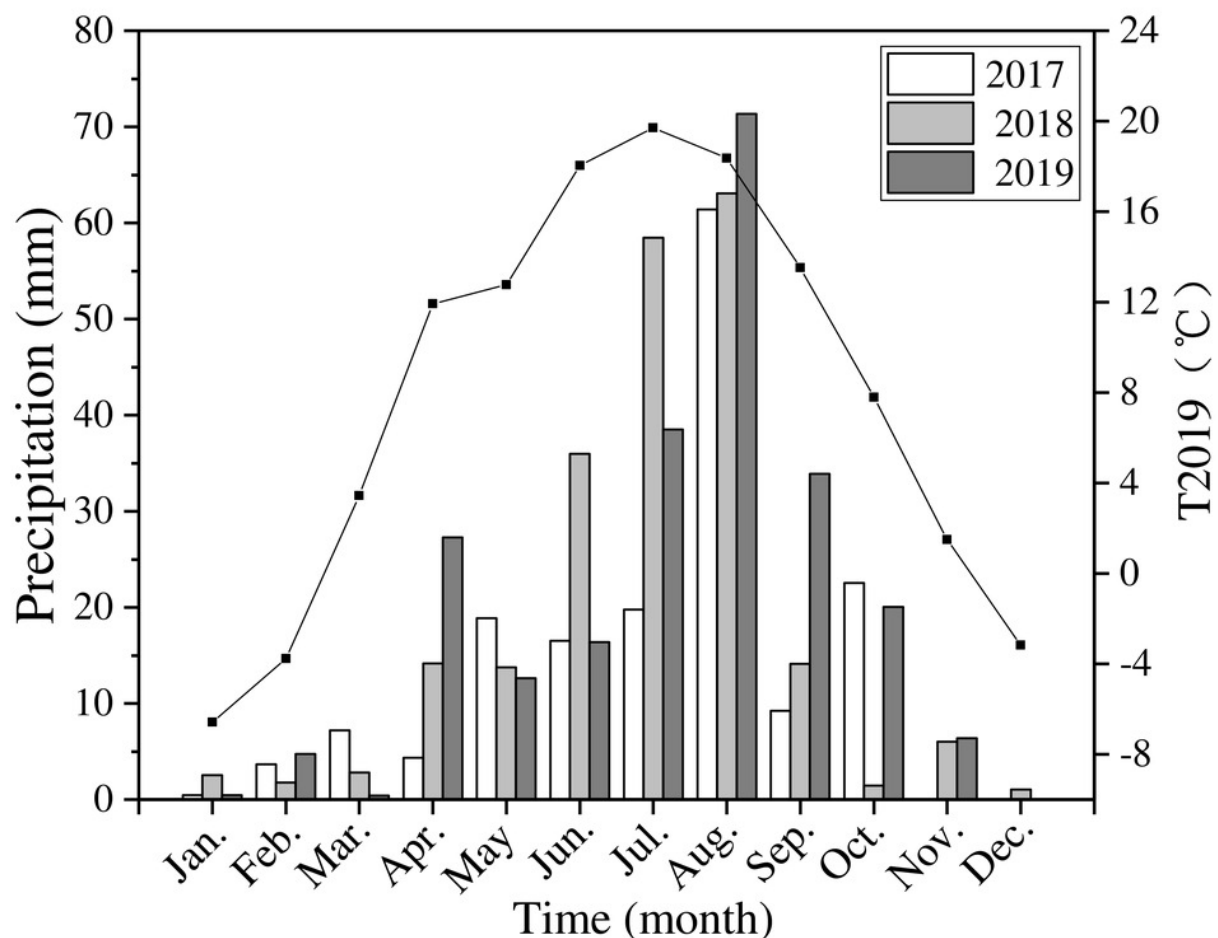



Figure 3

Biomass of shoot, litter, roots, total biomass (TB) and shoot/root ratio 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 TB use the left Y axis, and shoot/root the right.

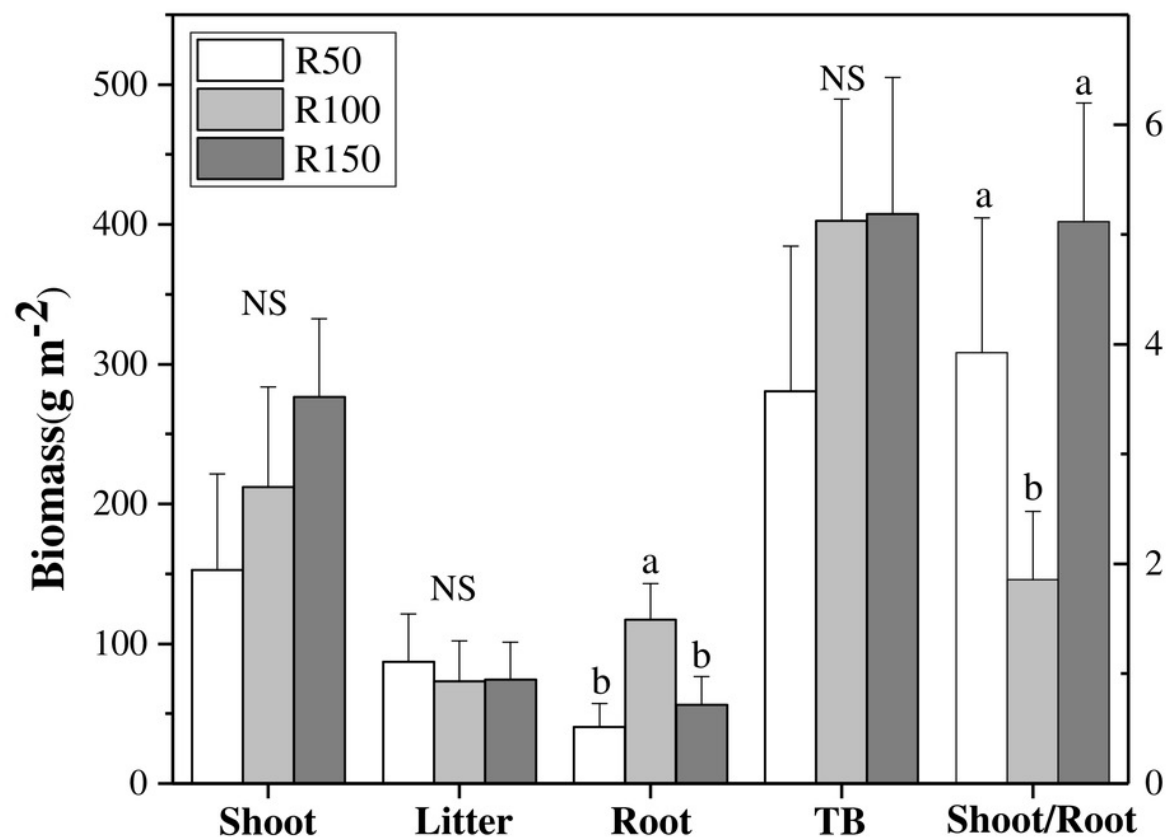



Figure 4

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).

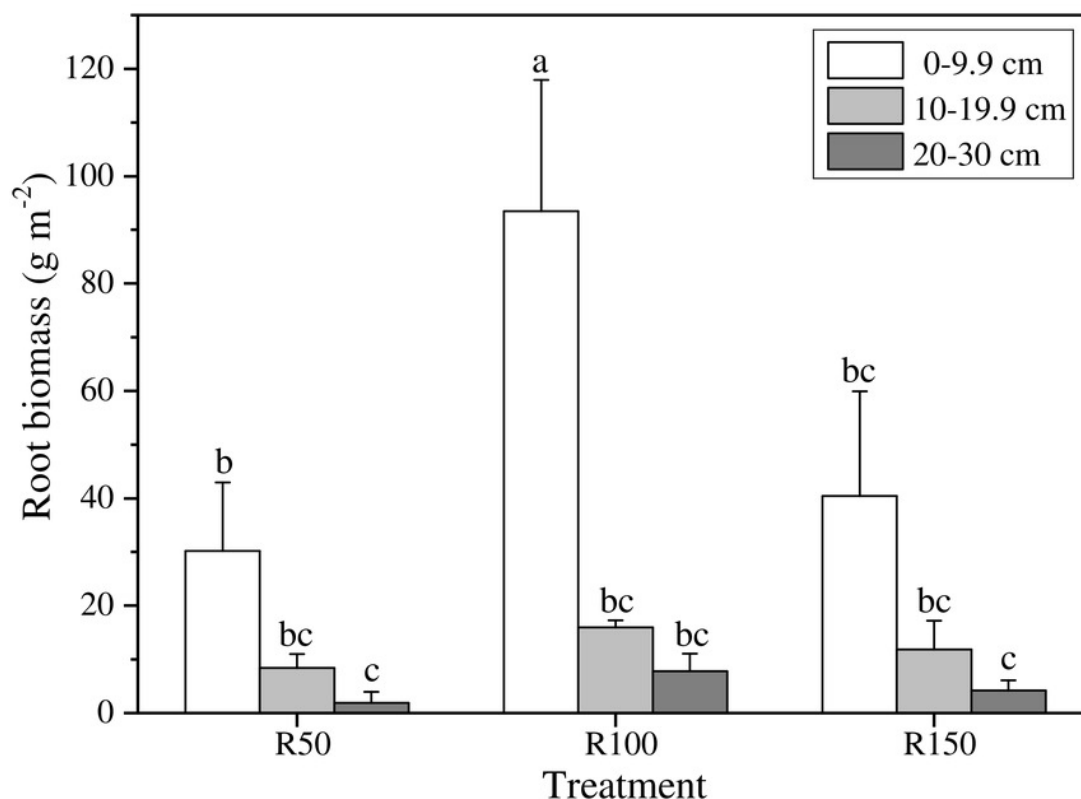
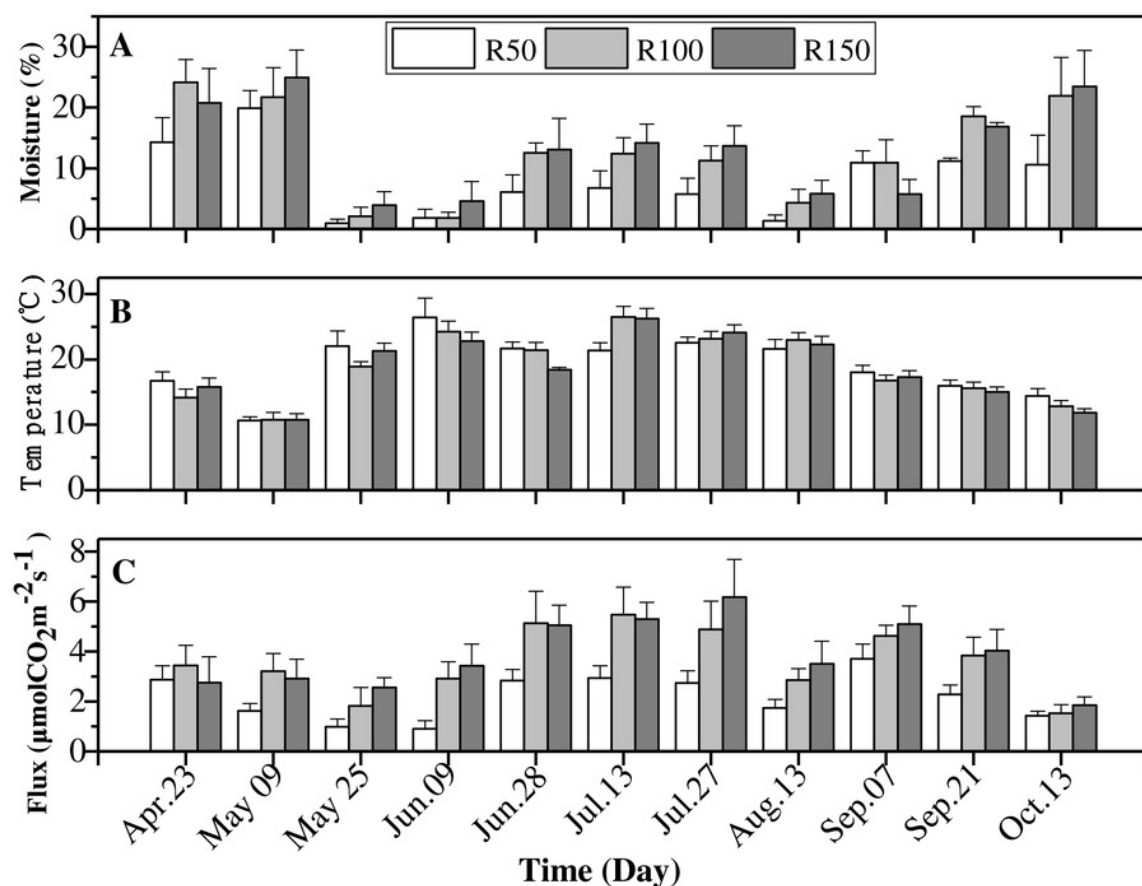



Figure 5

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).

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).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±0.0 a	2.1±0.0 b	2.1±0.1 b	2.3±0.1 a	2.0±0.1 b	2.0±0.0 b	**0.041**	**0.000**	0.253
SOC (g kg-1)	8.1± 0.1 a	6.1±0.3 bc	6.2± 0.2 c	8.0±0.0 a	7.0±0.1 b	6.6± 0.3 bc	7.7±0.3 a	6.8±0.2 bc	6.6±0.3 bc	**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±0.1 ab	7.8±0.1 ab	7.9±0.1 ab	7.8±0.1 ab	8.0±0.2 a	8.1±0.1 a	7.7±0.1 b	7.9±0.1 ab	8.0±0.2 ab	**0.022**	**0.003**	0.626

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

Notes. B=Bacteria; cov=coverage; 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 treatment.

| Variables | R50 | | | R100 | | | R150 | | | P-Value | |
	SD1	SD2	SD3	SD1	SD2	SD3	SD1	SD2	SD3	Pre	SD
B_Sobs	2529 a	2183 b	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	6.2 d	0.44	**0.00**
B_Cov(%)	96.0	96.4	96.5	95.8	96.4	96.6	95.8	96.3	96.9	0.82	**0.01**
F_Sobs	725 ab	528 abcd	491 bcd	755 a	458 cd	414 cd	632 abc	490 bcd	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_Cov(%)	99.8	99.8	99.9	99.8	99.9	99.9	99.9	99.9	99.9	**0.02**	**0.00**

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
Pre treatments	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
Pre treatments * Dates	20	60	14.8	<0.0001	8.0	<0.0001	9.7	<0.0001

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 replicates. Sampling was conducted 11 times during the growing season. 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

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

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

Date	Soil moisture (%)	Soil temperature (°C)	Soil CO_2 flux ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)
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

The Predictive 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.

1 **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				