



1 **Impact of elevated precipitation, nitrogen deposition and warming on soil**
2 **respiration in a temperate desert**

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13

14 **Abstract**

15 Soil respiration (R_s) is the most important source of carbon dioxide emissions from soil to
16 atmosphere. However, it is unclear what the interactive response of R_s would be to environmental
17 changes such as elevated precipitation, nitrogen (N) deposition and warming, especially in unique
18 temperate desert ecosystems. To investigate this an *in situ* field experiment was conducted in the
19 Gurbantunggut Desert, northwest China, from September 2014 to October 2016. The results
20 showed that precipitation and N deposition significantly increased R_s , but warming decreased R_s ,
21 which was mainly through its impact on the variation of soil moisture at 5 cm depth. In addition,
22 the interactive response of R_s to combinations of the factors was much less than that of any
23 single-factor, and the main interaction being a positive effect, except interaction from increased



24 precipitation and high N deposition ($60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Although R_s was found to be a unimodal
25 change pattern with the variation of soil moisture, soil temperature and soil NH_4^+ -N content, and it
26 was significantly positively correlated to soil dissolved organic carbon (DOC) and pH, but from a
27 structural equation model found that soil temperature was the most important controlling factor.
28 Those results indicated that R_s was mainly interactively controlled by the soil multi-environmental
29 factors and soil nutrients, and was very sensitive to elevated precipitation, N deposition and
30 warming. But the interactions of multiple factors largely reduced between-year variation of R_s
31 more than any single-factor, suggesting that the carbon cycle in temperate deserts could be
32 profoundly influenced by positive carbon-climate feedbacks.

33 **Key words:** precipitation; nitrogen deposition; warming; soil respiration; temperate desert

34

35 **Highlights**

36 1. Impacts of rainfall, N addition and warming on R_s were studied in a temperate desert.

37 2. Rainfall and N deposition significantly increased R_s , but warming reduced it.

38 3. The interactive response of R_s was much lower than any single-factor.

39 4. Soil temperature was the most important controlling factor for R_s .

40

41 **1. Introduction**

42 Global climate warming, changes in precipitation patterns and increased atmospheric
43 nitrogen (N) deposition have all occurred since the industrial revolution, especially in temperate
44 regions (IPCC, 2013), which will be expected to significantly change soil respiration (R_s) that is
45 the most important source of carbon dioxide (CO_2) from soil to atmosphere (Wu et al., 2011): the



46 annual CO₂ flux from R_s was ten-fold that of fossil fuel emissions (Eswaran et al., 1993; Batjes,
47 1996; Gougoulias et al., 2014). Therefore, even a small change in R_s will profoundly affect
48 greenhouse gas balance and climate (Heimann and Reichstein, 2008). Although a number of
49 experiments of the effects of warming, precipitation, and N deposition on R_s have been conducted
50 in alpine grassland, tundra regions, peatlands and temperate forest (Lafleur and Humphreys, 2008;
51 Strong et al., 2017; Yang et al., 2017; Zhao et al., 2017), studies in temperate desert ecosystems are
52 scarce, especially the impact on R_s of the interactions of these changes. A field study of
53 multi-factor interactive effects on R_s was therefore conducted in a temperate desert ecosystem to
54 help in understanding the response of R_s to climate change and N deposition in future and
55 highlight the main driving factors.

56 R_s includes autotrophic respiration (R_A), which is mainly from plant roots and mycorrhizal
57 activities; and heterotrophic respiration (R_H), which is mainly from the activities of
58 microorganisms (Hanson et al., 2000). Soil moisture is an critical limiting factor for plant roots
59 and microbial activities in desert ecosystems (Huang et al., 2015a): R_s was significantly increased
60 by 47-70% in a degraded steppe in Inner Mongolia, China, by increasing precipitation (Chen *et al.*,
61 2013), with the effect especially strong in summer (Zhang et al., 2017). In addition, in arid
62 ecosystems, increasing precipitation significantly stimulated plant growth, enhanced soil microbial
63 activity and abundance (Huanget al., 2015a), and changed soil nutrient and substrate concentration,
64 such as dissolved organic carbon (DOC), inorganic nitrogen content, moisture and temperature
65 (Huang et al., 2015b).

66 Warming significantly increased soil temperature, another important controlling factor for
67 plants growth and microbial activity (Sheiket al., 2011; Huang et al., 2015a). R_s rates were



68 significantly increased in a forest soil and Tibetan Plateau grassland by warming (Chen et al.,
69 2017a), reducing R_s with decreasing soil moisture in the growing season, but increasing R_s in the
70 non-growing season (Fang et al., 2017; Li et al., 2017); no significant impact was observed from
71 warming (Liu et al., 2016a). Therefore, how R_s is affected by warming induced variations in the
72 soil environment is still unclear. In addition, low and short-term N deposition enhanced R_s , while
73 higher and long-term N deposition inhibited R_s due to changes in plant growth and microbial
74 activity (Zhu et al., 2017), but no impacts have also been reported (Luo et al., 2017; Zhang et al.,
75 2017). A meta-analysis showed that the effects of N enrichment on soil CO_2 fluxes depended on
76 temperature and soil properties (Zhong et al., 2016); desert soils may be even more sensitive to its
77 variation.

78 A nation-wide analysis showed that warming, elevated N deposition and precipitation
79 significantly increased R_s in China (Feng et al., 2017). Some studies have shown that the warming
80 effect on R_s mainly depended on the variation of soil moisture in a dry forest soil (Li et al., 2017).
81 Luo et al. (2008), using a modeling analysis, found that interactive effects became increasingly
82 weaker with increasing intensity of the factors, but a recent meta-analysis showed that interactive
83 effects were much greater than single factors (Zhou et al., 2016a). Thus how multi-factor
84 interactions impact R_s is still unclear. Therefore, an *in situ* experiment was carried out in the
85 Gurbantunggut Desert to (1) investigate the single-factor and interactive responses of R_s to
86 warming, precipitation and N deposition, and (2) identify the main controlling factors on R_s .

87

88 2. Materials and methods

89 2.1. Study site



90 A field experiment was carried out at the southern edge of the Gurbantunggut Desert (44°26'
91 N, 87°54'E and 436.8 m a.s.l.), northwest China, from September 2014 to October 2016. This is
92 the largest fixed/semi-fixed temperate desert in China. The mean annual temperature and
93 precipitation are 7.1 °C and 215.6 mm, respectively (Cui et al., 2017), and annual potential
94 evaporation exceeds 2000 mm. From late November to mid-March of the following year, a 20–35
95 cm depth of snow cover the whole desert (equivalent to 38–64 mm rainfall; Huang et al., 2015c).
96 The growing season is from April to October. This desert soil is of extremely low fertility and high
97 alkaline (Cui et al., 2017). Soil organic carbon, total N content, soil NO₃⁻-N, NH₄⁺-N contents and
98 C:N ratio are 2.21 ± 0.71 g kg⁻¹, 0.08 ± 0.003 g kg⁻¹, 4.49 ± 0.71 mg kg⁻¹, 1.38 ± 0.74 mg kg⁻¹ and
99 21.39 ± 1.84, respectively (Table 1; Cui et al., 2017). Plant species are dominated by *Haloxylon*
100 *ammodendron* and *Haloxylon persicum*, and the vegetation was extremely sparse, with only 30%
101 coverage, with some spring ephemeral plants (May–June), some annuals, and perennials
102 herbaceous plants (July–August; Liu et al., 2016). Spring ephemerals account for > 60% of the
103 community cover and 85% of the biomass. Summer ephemerals, annuals and perennials usually
104 account for only a small proportion of the community biomass before June, but dominate the
105 community after the die-back of the spring annuals (Huang et al., 2015c).

106

107 *2.2. Experimental treatments*

108 A striking N deposition rate (35.2 kg N ha⁻¹ yr⁻¹) has occurred in the Gurbantunggut Desert
109 due to the rapid development of agriculture and industry with main form of ammonium nitrate
110 (NH₄NO₃), and wet (19.6 kg N ha⁻¹ yr⁻¹) and dry (15.6 kg N ha⁻¹ yr⁻¹) deposition are almost half
111 (Song et al., 2015). In addition, according to the forecast of Galloway et al. (2008) that



112 atmospheric N deposition will double from the early 1990s to 2050, and the predictions of Liu et
113 al. (2010) that precipitation in this region would be increased by 30% in next 30 years. In June
114 2014, an *in situ* complete block interactive experiment was therefore conducted to study the
115 impact of N deposition and increased precipitation on R_s (Experiment 1). The three levels of N
116 deposition ($0 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (control, N0), $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (low, N1) and $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (high,
117 N2)) and two levels of precipitation ('natural' precipitation (W0) and an increase of 30% (an extra
118 60 mm precipitation annually (W1)) were applied (Cui et al., 2017). Therefore there were six
119 treatments (W0N0, W0N1, W0N2, W1N0, W1N1 and W1N2) with four replicates of each
120 treatment; each replicate plot was $10 \text{ m} \times 10 \text{ m}$ with a 5-m wide buffer zone. The additional
121 precipitation and N deposition (NH_4NO_3) were added twelve times in April, July and September,
122 equivalent to 5 mm precipitation and 2.5 or 5 kg N ha^{-1} per application over a week. The NH_4NO_3
123 was diluted in 50 L water (equal to 0.5 mm precipitation), and evenly applied following the
124 simulated precipitation. The same amount of water was applied to the control plots (W0N0).

125 Rapidly warming ($0.6 \text{ }^\circ\text{C}$ per decade), increasing precipitation ($3\text{-}5 \text{ mm yr}^{-1}$ since 1979) and
126 receiving high N deposition (3 kg N ha^{-1} since 1980) are affecting the Gurbantunggut Desert (Liu
127 et al., 2013; Li et al., 2015), which would be expected to affect rate of R_s . Therefore, another
128 interactive experiment was established at the same time, simulating the three most likely climate
129 scenarios in the future: (1) warming only (W0N0T1); (2) increased precipitation and N deposition
130 without warming (W1N1T0); (3) the interaction of increasing precipitation, N deposition and
131 warming (W1N1T1); all compared with the current climate (W0N0T0). Therefore, there were four
132 treatments (W0N0T1, W1N1T0, W1N1T1, W0N0T0) with four replicates (plots) of each
133 treatment. Open-top chambers (OTCs) were used to simulate warming. The OTCs were designed



134 with 5 mm transparent tempered glass and stainless steel angle iron to the ITEX standard (Marion
135 et al., 1997). They were 2 m high and 4 m in diameter, with each OTC area being 12 m². However,
136 the design was improved such that the top and bottom OTC areas were the same so that
137 precipitation and snowfall were the same as that to the surrounding environment; this also avoids
138 overheating inside the OTCs. The timings of applications of water and N were as in Experiment 1.

139

140 2.3. Measurements

141 R_s in all plots were measured twice or thrice a week (continuous measurements over 3 days
142 were made following simulated precipitation and N deposition) using gas chromatography and
143 static chambers (50 cm×50 cm×10 cm) at locations where grow only spring ephemeral plants
144 without any annuals and perennials in order to minimize the between-treatment spatial
145 heterogeneity due to sparse annuals, and perennials (Liu et al., 2012). Gas samples were collected
146 between 10:00-12:00 (GMT + 8) throughout the experimental period, which was detected in this
147 period were close to the diurnal averages (Fig.3b and 3d). Gas samples were collected from the
148 headspace of each chamber 0, 10, 20 and 30 min after closing the chamber per time. The gas
149 samples analyzed within three days using a gas chromatograph (GC; Agilent 7890A, Agilent
150 Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector for quantitative
151 R_s (Liu et al., 2012). R_s rates were calculated from four concentrations of the gas sample based on
152 a first order differential linear or non-linear equation and were temperature- and pressure-
153 corrected (Liu et al., 2012; Zhang et al., 2014). Soil samples were taken monthly from around the
154 static chambers to a depth of 10 cm using an auger (3.5 cm in diameter). Fine roots and small
155 stones were separated out using a 2 mm sieve. Dissolved organic carbon (DOC) was extracted



156 with deionized water (soil: water ratio = 1:10) by shaking on an orbital shaker at 10000 rpm for 5
157 min and analyzed using a TOC analyzer (multi N/C 3100, Jena, Germany; Jones and Willett,
158 2006). Brookes' (1985), Chloroform fumigation extraction was used to measure microbial
159 biomass carbon (MBC) and microbial biomass nitrogen (MBN). Soil organic carbon (SOC) and
160 pH were measured using the potassium dichromate method (Jiang et al., 2014), and soil NO₃⁻-N
161 and NH₄⁺-N analyzed as per Yue et al. (2016). Caipos Soil and Environment Monitoring Systems
162 (Caipos GmbH, Austria) were used to monitor soil moisture/temperature at 5 and 20 cm depth
163 every hour.

164

165 *2.4. Effects of each treatment on R_s*

166 The each treatment effect was analyzed using the following formula to better evaluating the
167 effect of precipitation, warming and N deposition on R_s (Yue et al., 2016).

$$168 \quad \text{The treatment effect} = (TR_s - CR_s) / CR_s \times 100\%,$$

169 Where the treatment effect is W0N1, W0N2, W1N0, W1N1, W1N2, W1N1T1 or W0N0T1
170 effect on R_s (a positive value shows that the treatment has increased R_s and a negative value shows
171 decrease of R_s), corresponding TR_s represents R_s from the W0N1, W0N2, W1N0, W1N1, W1N2,
172 W1N1T1 or W0N0T1 plots (mg C m⁻² h⁻¹) and CR_s indicates the R_s from the control plots (W0N0,
173 mg C m⁻² h⁻¹).

174

175 *2.5. Statistical analyses*

176 Treatments effect on soil organic carbon (SOC), NO₃⁻-N, NH₄⁺-N content, pH, DOC, MBC
177 and MBN were examined in each treatment by least significant difference LSD (p<0.05). The



178 single-factor and interaction effects of precipitation, warming and N deposition on R_s were
179 detected by multi-way analysis of variance (ANOVA), and the accumulated effect of precipitation,
180 warming and N deposition on R_s were tested by repeated measures ANOVA. In addition, the
181 relationships of R_s and DOC, MBC, MBN, soil temperature, soil moisture, NH_4^+ -N content, soil
182 NO_3^- -N, and pH were described using a linear or non-linear regression model. The factors of key
183 controls on R_s were used to analyze by structural equation models (SEMs). SPSS software
184 (version 20.0) was used to conduct all statistical analyses, and statistical significant differences
185 were set with $P < 0.05$. All Figures were created using the Sigmaplot software package (version
186 10.0), but SEMs analyses were carried out using AMOS 22.0 (Amos Development Corporation,
187 Chicago, IL, USA).

188

189 **3. Results**

190 *3.1. Treatments effects on soil environmental and properties*

191 Soil temperatures at 5 depth were mostly increased between 11:00 and 22:00 every day by
192 warming; the average annual soil temperatures at 5 and 20 cm depth were significantly increased
193 by 4.41 and 3.67 °C, respectively (Fig. 1a). Soil moisture at 5 cm depth was decreased by warming
194 by only 0.61v/v% (Fig. 1b), and a very small decrease of 0.01v/v% in soil moisture at 20 cm depth
195 was observed (Fig.1b). Soil moisture at 5 and 20 cm depth were largely increased by the
196 interaction of precipitation, N deposition and warming (Fig.1b). N deposition and warming
197 significant increased soil NH_4^+ -N and NO_3^- -N contents (Fig. 1c), but no significant change was
198 found from increased precipitation. Soil MBC and MBN were greatly increased by N deposition,
199 but significant negative effects on soil MBC and MBN were observed by warming and the



200 interaction of precipitation and N deposition (Fig. 1d). No significant change in SOC and DOC
201 was observed in any treatment (Fig. 1c and 1d).

202

203 3.2. Precipitation, warming and N deposition effects on R_s

204 In our study, a weak R_s emission rate (-2.46 to $50.26 \text{ mg C m}^{-2} \text{ h}^{-1}$) was observed at control
205 plots with an average emission rate of $12.18 \text{ mg C m}^{-2} \text{ h}^{-1}$ from September 2014 to October 2016
206 (Fig. 2b). Annual cumulative rate of R_s was $1090.11 \pm 450.78 \text{ kg C ha}^{-1}$, with non-growing season
207 account for 20.7% of the annual emission (Table 1). R_s was significantly enhanced by increasing
208 5-mm precipitation and N deposition from 12.18 to 16.23 and 14.97 $\text{mg C m}^{-2} \text{ h}^{-1}$ (average),
209 respectively ($P < 0.001$; Fig. 2b and 2c; Table 2), with annual R_s increased by 33.1% and
210 19.2-22.8%, respectively (Table 1). And the low N deposition effect on R_s was much higher than
211 that high N deposition (Fig. 2b and 2c). However, R_s was reduced mostly by warming, although
212 not significant ($P=0.084$; Table 2). And high temperatures at times of peak sunshine during the
213 diurnal variation significantly inhibited its emission rate (Fig 3a and 3b), but it was also
214 significantly increased by warming following rainfall that increased soil moisture (Fig. 3c and 3d).
215 The diurnal trend in R_s was consistent with that of soil temperature at 5 cm depth (Fig. 3). In
216 addition, the interactive responses of R_s to increasing precipitation, warming and N deposition
217 were much lower than that from any single-factor (Table 1), and with the interaction of 60 kg ha^{-1}
218 N and extra precipitation decreasing R_s by 4.25% (Table 1). Overall, annual R_s rates were
219 significantly impacted by precipitation, N deposition, and their interaction (Table 2), but no
220 significant net change was caused by warming (Table 2), although R_s rates were decreased by 9.99%
221 (Table 1).



222

223 *3.3. Temporal variation and its control*

224 The results of repeated measures ANOVA showed that significantly accumulated effects on
225 R_s were found by N deposition and interaction between N deposition and precipitation or warming
226 rather than alone increasing precipitation and warming (Table 2). A large between-year variation
227 in R_s was observed with a coefficient of variation (CV) up to 41.4% (a much higher R_s rate was
228 observed in 2016 than 2015), but variation was reduced by increasing precipitation, N deposition
229 and warming and their interaction, except with an increase in N deposition of 30 kg ha⁻¹ (Table 1).
230 The results of regression analysis showed that R_s was significantly increased by increases in pH
231 and DOC (Fig. 4e and 4f), but no significant relationships were found with MBC, MBN or
232 NO₃⁻-N content (Fig. 4a, 4b and 4c). In addition, thresholds in the impacts of increased soil
233 moisture, soil temperature and NH₄⁺-N content on R_s were found. Soil moisture was the most
234 important controlling factor when it was <2% or soil temperature was >37°C (Fig. 4g and 4h).
235 Secondly, soil temperature was the most important limiting factor when soil moisture was >20%
236 or soil temperature <12 °C (Fig. 4g and 4h). Thirdly, there was no significant impact on R_s when
237 soil NH₄⁺-N content was <6.3 mg N / kg. A significant increase in R_s occurred when soil NH₄⁺-N
238 content was between 6.3 and 12.6 mg N / kg, but R_s was inhibited when soil NH₄⁺-N content was
239 between 12.6 and 31.6 mg N / kg (Fig. 4d).

240

241 **4. Discussion**242 *4.1. Single-factors impacts of precipitation, N deposition and warming on R_s* 243 Annual R_s was 1090 kg C ha⁻¹ in this temperate desert, with the non-growing season



244 accounting for 20.7 % of the annual flux (Table 1). This is consistent with previous study in here
245 (Zhou et al., 2014; Huang et al., 2015a) because SOC content was very low (Fig. 1c), and
246 vegetation was very sparse in this desert (Liu et al., 2016b). Increasing precipitation significantly
247 increased R_s (Fig. 2b). It is also consistent with the results of a meta-analysis and previous study in
248 here (Huang et al., 2015a ; Liu et al., 2016c). This is because that the growth of desert plants and
249 microbial activity are significantly activated by increasing precipitation (Huang et al., 2015a), and
250 microbial biomass, mass-specific respiration, microbial biomass carbon (MBC) and nitrogen
251 (MBN), and microbial PLFAs were consistently significantly enhanced by increased precipitation
252 (Zhang et al., 2013; Huang et al., 2015a). However, R_s in our study was much higher in moderate
253 soil moisture conditions than with too little or too much soil moisture (Fig. 4g). This suggests that
254 R_s is mainly R_H rather than R_A in this desert, namely from soil microorganism, because (1) too
255 little or too much soil moisture could significantly inhibit microbial activity due to variation of
256 soil temperature and soil properties (Ma et al., 2013), while moderate soil moisture could
257 significantly enhance microbial activity (Skopp et al., 1990), and (2) the biomass of fine roots was
258 no significantly enhanced at our sites by increased precipitation (Cui et al., 2017). This is
259 consistent with results from a desert steppe in northern China where the contribution of R_H (78.1%)
260 was significantly higher than that of R_A (21.9%) under increasing precipitation (Liu et al., 2016a).

261 N deposition also significantly increased R_s , especially in low N deposition (Fig. 2c). This is
262 consistent with results from an alpine meadow and in the Loess Plateau (Fang et al., 2017; Zong et
263 al., 2017), and with a meta-analysis showing that N deposition increased R_s by 8.8% (Zhou et al.,
264 2016a). This is because N deposition, on the one hand, could increase fine root biomass, although
265 this was not significant in our study (Cui et al., 2017); on the other hand, increases microbial



266 activity and abundance by low N deposition (Huang et al., 2015b). But this was inconsistent with a
267 young *Cunninghamialanceolata* forest (Wang et al., 2017), and beneath shrubs of *H.*
268 *ammodendron*, soil high N content, has the opposite effect in our study site (Chen et al., 2013;
269 Huang et al., 2015b). What's more, the results of nonlinear regression analysis showed that higher
270 R_s rates occurred at moderate soil $\text{NH}_4^+\text{-N}$ contents (between 6.3 and 12.6 mg N / kg), while lower
271 R_s occurred in much lower (<6.3 mg N / kg) or much higher (>12.6 mg N / kg) soil $\text{NH}_4^+\text{-N}$
272 contents (Fig. 4d), but this effect of N deposition on R_s is not consistent with other ecosystems
273 (Burton et al., 2004; Chen et al., 2013; Liu et al., 2015; Chen et al., 2017b). This is because the
274 desert soil is extremely limited than other ecosystem (Adams, 2003), so low N deposition
275 enhanced plants growth and microbial activity, but high N inhibited microbial activity and
276 community composition, reduced R_s (Zhou et al., 2014; Huang et al., 2015b). Overall, soil
277 $\text{NH}_4^+\text{-N}$ content was an important controlling factor for R_s because microbial activity, abundance
278 and species diversity were regulated by soil $\text{NH}_4^+\text{-N}$ content in this desert, and R_s was very
279 sensitive to variation of N deposition.

280 Warming decreased R_s (Fig. 2e), although not significantly ($P=0.084$; Table 2), which was
281 consistent with results from a semi-arid alfalfa-pasture of the Loess Plateau (Fang et al., 2017). In
282 addition, a significant decrease in R_s was observed on a hot sunny day when soil moisture was
283 reduced, and sharply reduced R_s when soil temperature reached 37 °C (Fig. 3a and 3b). This is
284 because: (i) microbial activity is significantly inhibited by extreme temperatures and low soil
285 moisture, may reduce population size by 50-80% (Sheik et al., 2011); (ii) fine root growth is
286 inhibited in high temperature and low soil moisture. Others have noted this phenomenon as
287 occurring at about 16:00 each day (Ma et al., 2013), but in our study the effect was advanced to



288 14:00 by warming, which may reduce carbon emission from soil to atmosphere. However, this is
289 not consistent with results from a tundra ecosystem, subtropical forest or alpine regions where R_s
290 was significantly increased by warming due to the limitation of soil temperature in these
291 ecosystems, and no significant change in soil moisture (Noh et al., 2016; Wu et al., 2016; Zhou et
292 al., 2016b). In addition, a significant increase in R_s was found following enhanced precipitation
293 with warming (Fig. 3c and 3d), which indicates that soil moisture was the most important
294 controlling factor for R_s under a warming climate. This is consistent with other studies (Chen et al.,
295 2017a; Zhao et al., 2017). However, statistical analysis showed that no overall significant impact
296 on R_s was found during the experimental period by warming, although it was reduced by 9.99%.
297 This is because our gas samples were taken at 10:00 – 12:00 each day, when average soil
298 temperatures were increased about one degree. Thus mean annual R_s was not sensitive to
299 temperature changes this small in contrast to the very significant effects of short-term diurnal
300 changes in soil temperature, observed between 12:00 and 17:00 (Fig. 3a and 3c). Those results
301 indicated that R_s dependent mainly on variations of soil moisture and temperature in the context of
302 warming, and climate change is likely to have a very significant effect on temperate deserts.

303

304 4.2. The interactive effects of precipitation, N deposition and warming on R_s

305 Interactive responses of R_s were much lower than those of any single-factor, but still
306 increased R_s overall, except interaction between precipitation and high N deposition (Table 1).
307 This is consistent with results in dry ecosystems (Morillas et al., 2015; Martins et al., 2016), but
308 not with the results of a meta-analysis that precipitation and N deposition interactive experiments
309 were a greater extent positive effect on R_s (Zhou et al., 2016a). This can be explained in our study



310 that soil MBC or MBN were much less in interactive treatments than that of single-factor (Fig. 1d),
311 which showed that a number of microorganisms were much less in interactive treatments than that
312 of single-factor due to much stronger N effect. As we found that R_s was reduced with increasing N
313 deposition and precipitation by as much as 4.25% in W1N2 plots (Table 1), which showed that the
314 inhibiting effect of soil $\text{NH}_4^+\text{-N}$ content was much stronger when there was sufficient soil
315 moisture (Fig. 2d). This is consistent with the results in a *Populuseuphratica* community in a
316 desert ecosystem (He et al., 2015). This was because (i) microbial activity was inhibited by high
317 or low soil moisture and high soil $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$ content (Burton et al., 2004); and (ii) high N
318 tent to reduce extracellular enzyme activity and the fungal population (Maris et al., 2015). In
319 addition, the interactive effect of the three factors on R_s in this desert was much lower than
320 interaction of two factors of precipitation and N deposition (Table 1), and is consistent with the
321 results of modeled interactive effects, which showed that three-factor interactions were rare while
322 two-factor interactions were more common (Luo et al., 2008). Fortunately, the interactive effect of
323 three factors or two factors (precipitation, N deposition and warming) in this desert could largely
324 reduce between-year variation on R_s (Table 2), which may because (i) the limits of soil moisture,
325 soil temperature and soil N content were relieved for key biological processes by increasing
326 precipitation, N deposition and warming (Huang et al., 2015a; Liu et al., 2016b); (ii) various
327 factors antagonistic to each other (Zhou et al., 2016a). However, the variation in the growing
328 season on R_s can be increased by warming, elevated precipitation and N deposition because of
329 their dominant effects on plant growth and microbial activity (Huang et al., 2015b), but it was the
330 exact opposite in the non-growing season due to reduce the limit of temperature (Zeng et al.,
331 2016). Those results showed that R_s would be reduced under interactive effect of increasing



332 rainfall, temperature and N deposition in the future, and took place a positive carbon-climate
333 feedbacks.

334

335 *4.3. Temporal variation in treatments on R_s and controlling factors*

336 Significantly accumulated effects on R_s were found by elevated N deposition rather than
337 alone increasing precipitation and warming (Table 2). A previous study in here has showed that R_s
338 was decreased to N addition with experimental duration (Zhou et al., 2014), which was
339 inconsistent with our results (Fig. 2c) because in our study relatively lower rate of N addition than
340 that Zhou et al. (2014), and the composition of microbial community and soil propertie were
341 altered gradually by long-term and high N deposition (Fig. 1c and d; Huang et al., 2015b; Zong et
342 al., 2017). In addition, significantly accumulated effects in the interaction between N deposition
343 and precipitation or warming on R_s were also found (Table 2), and R_s was decreased by 4.25% by
344 interaction between increasing precipitation and high N deposition (Table 1), which indicated that
345 the response of R_s to N deposition largely dependent on soil moisture in desert soil. This may be
346 attributed to the antagonistic interaction between elevated N deposition and precipitation on R_s
347 (Zhou et al., 2016a). Those results indicated that N deposition produced strong accumulated
348 effects on R_s in this desert, and was enhanced largely with increasing soil moisture, which would
349 reduce carbon emission from soil to atmosphere.

350 Regression analysis shows that R_s exhibited a unimodal change pattern with variations of soil
351 $\text{NH}_4^+\text{-N}$ (Fig. 4d), mositure (Fig. 4g), and temperature (Fig. 4h), and R_s was signicantly postively
352 correlated to soil dissolved organic carbon (DOC) and pH (Fig. 4e and 4f). However, structural
353 equation modeling indicated that soil temperature was the most important controlling factor than



354 soil $\text{NH}_4^+\text{-N}$ and soil moisture (Fig. 5), unsupported our hypothesis, but it is consistent with most
355 research results (Wu et al., 2016; Zhou et al., 2016b; Chen et al., 2017a). In addition, large
356 inter-annual variation was observed ($\text{CV} = 41.4\%$) during our experiment (Table 1), while the
357 variation of annual precipitation and air temperature were only 4.41% and 7.78%, respectively
358 (Table 1), but close to the CV of spring root biomass of ephemeral plants (47.14%) with 24 times
359 of aboveground biomass of spring ephemeral plants in 2016 than that in 2015 (Cui et al., 2017),
360 which indicated that the increase of R_s in 2016 was mainly from the root respiration of ephemeral
361 plants. This is consistent with previous study that ephemeral plants mediated inter-annual variation
362 of carbon fluxes in this desert (Huang et al., 2015c; Liu et al., 2016). It is different from other
363 ecosystems where inter-annual variations of R_s were mainly dependent on variations in annual
364 precipitation and air temperature (Gerard et al., 1999; Asensio et al., 2007; Chen et al., 2012).
365 Overall, our results indicate that annual variation in R_s in this temperate desert is mainly
366 controlled by soil temperature, but between-year variation in R_s is mainly controlled by ephemeral
367 plants.

368

369 5. Conclusion

370 Climate change and elevated N deposition play important roles in controlling R_s in
371 temperate deserts. We found that increasing precipitation and N deposition significantly increased
372 R_s in the Gurbantunggut Desert, but warming reduced R_s , mostly because of the variation of soil
373 moisture. In addition, we found that the interactive responses of R_s was much lower to
374 precipitation, N deposition and warming than that any single factors. What's more, R_s are mainly
375 mediated by soil moisture, soil temperature and soil $\text{NH}_4^+\text{-N}$ content, but soil temperature are the



376 most important with between-year variation in R_s mainly controlled by ephemeral plants. Those
377 results showed that R_s is very sensitive to increasing precipitation, N deposition and warming, and
378 their interactive effects could reduce soil carbon emissions and so reduce the impacts of climate
379 change.

380

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385

386 **Inferences**

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558 **Fig. 1** Comparative effects of warming by Open Topped Chambers, increased precipitation, and N
559 deposition on soil temperature (a), soil moisture (b) at 5 and 20 cm depth; soil organic carbon
560 (SOC), NH_4^+ -N content, NO_3^- -N content and pH (c), dissolved organic carbon (DOC), microbial
561 biomass carbon (MBC) and microbial biomass nitrogen (MBN, d). The data are mean \pm SE, $n = 4$
562 in c and d, different letter indicate significant effect at $P < 0.05$.

563

564 **Fig. 2** Variation in rainfall (mm, a) and air temperature ($^{\circ}\text{C}$, a) from September 2014 to October
565 2016 at the Gurbantunggut Desert, and the response of R_s (mean, $n = 4$) to precipitation (b), N
566 deposition (c-d) and warming (e). W0 and W1 indicate under ambient precipitation (without water
567 addition) and 60 mm yr^{-1} precipitation addition; N0, N1, and N2 indicate 0, 30 and 60 kg N ha^{-1}
568 yr^{-1} nitrogen addition; while WIN0, WIN1, and WIN2 indicate 0, 30 and $60 \text{ kg N ha}^{-1} \text{ yr}^{-1}$
569 nitrogen addition under 60 mm yr^{-1} precipitation addition; WIN1T1, W0N0T1 and W0N0T0
570 indicate the interaction between increasing precipitation (60 mm yr^{-1}), N deposition (30 kg N ha^{-1}
571 yr^{-1}) and warming by OTCs, warming alone (without increasing precipitation and N deposition)
572 and control plots, respectively. Black arrows indicate simulated precipitation (5 mm per time) and
573 N deposition (0.25 or 0.5 g N m^{-2} per time). Each point represents the mean of four replications
574 (chambers). Standard deviations for R_s are not showed for figure clarity.

575

576 **Fig. 3** Post-rainfall diurnal variation in R_s (mean \pm SE, $n = 4$, b) with variation in soil temperature
577 and soil moisture (a), and a sunny day variation in R_s (mean \pm SE, $n = 4$, d) with variation in soil
578 temperature (T5, T20, c) and soil moisture at 5 (W5) or 20 (W20) cm depth caused by warming in
579 open topped chambers (OTCs). Positive values indicate increment by warming, and negative
580 values indicate decline. A red straight line indicates the average value of R_s inside the OTCs in (b)
581 and (d), and a green straight line represents the average value of R_s out of OTCs in (b) and (d).
582 Red *, ** and *** indicate significant effect at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

583

584 **Fig. 4** The relationship of soil respiration with microbial biomass carbon (MBC, a); microbial
585 biomass carbon (MBN, b); soil NO_3^- -N (c); NH_4^+ -N content (d); soil dissolved organic carbon
586 (DOC, e); pH (f); soil moisture (g) and soil temperature (h).

587



588 **Fig. 5** Structure equation modeling (SEM) test the multivariate (soil moisture, soil temperature,
589 soil $\text{NH}_4^+\text{-N}$ content, DOC and pH) effects on R_s (n=34). Single-headed arrows show that the
590 effect of different key controls on R_s were analyzed. The green arrows indicated positive effects,
591 and red arrows showed negative effects. And the width of the arrows indicate the strength of the
592 relationship. The numbers are standardized path coefficients, which can show the importance of
593 the variables in the model. Goodness-of-fit statistics for the model are shown below the model. *
594 indicate significant effect at $P < 0.05$.

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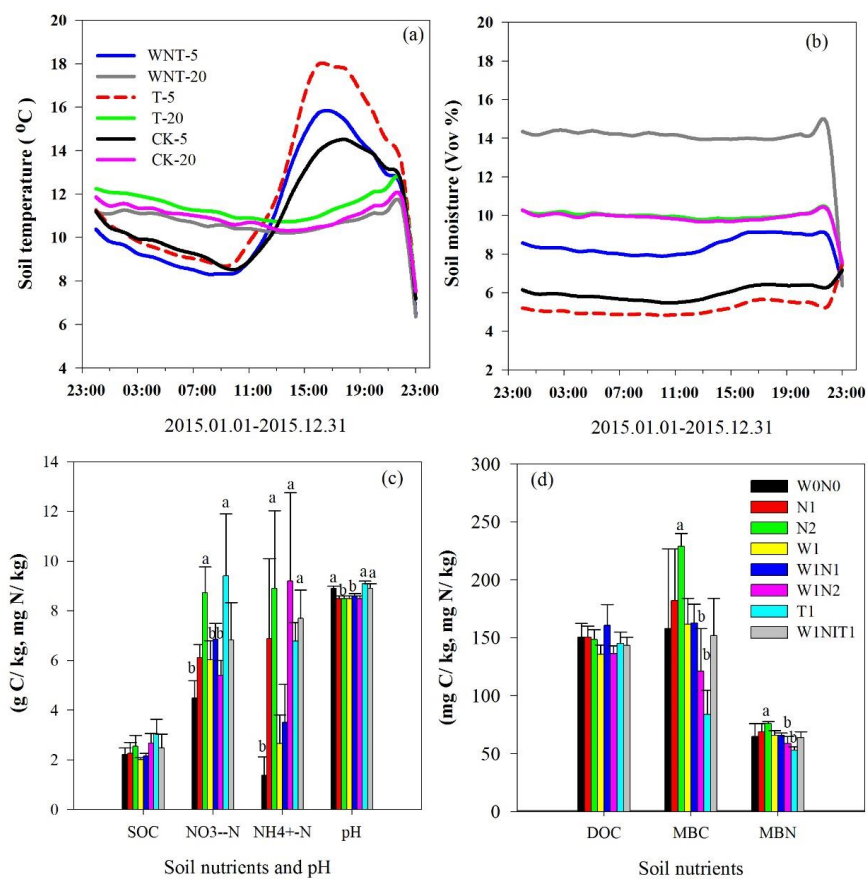
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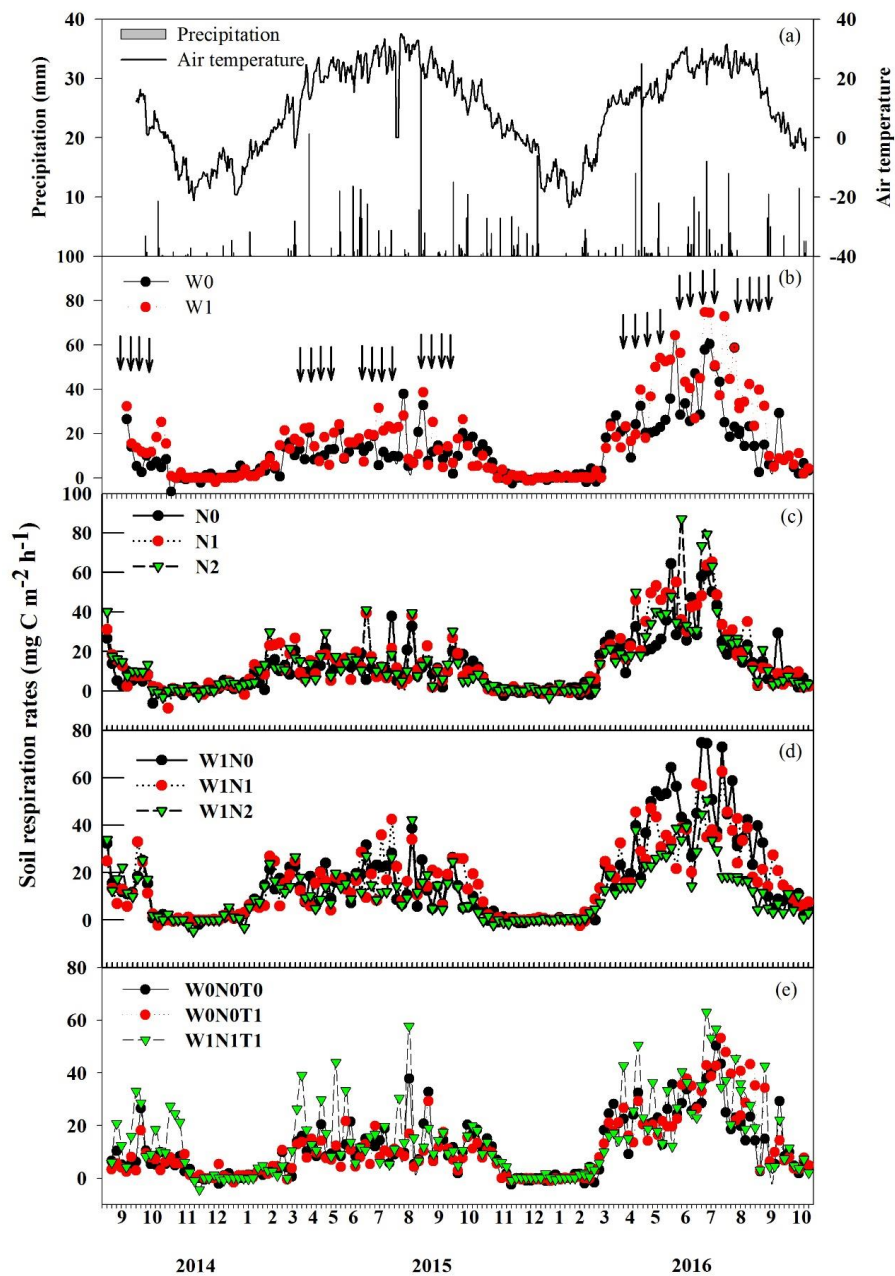
615 **Fig. 1**



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628 Fig. 2



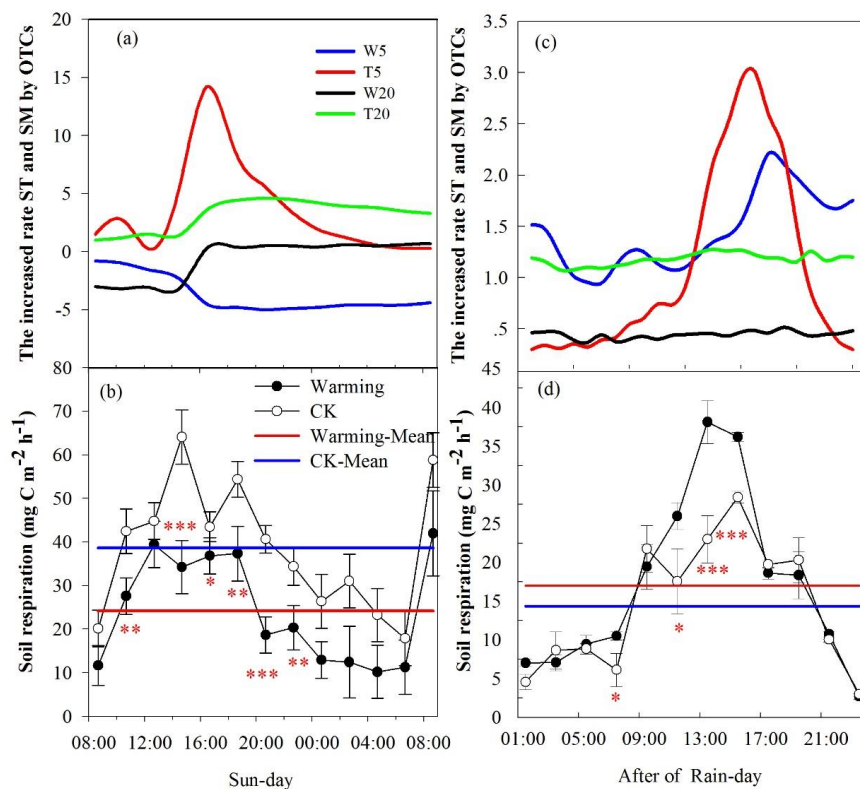
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631 Fig. 3



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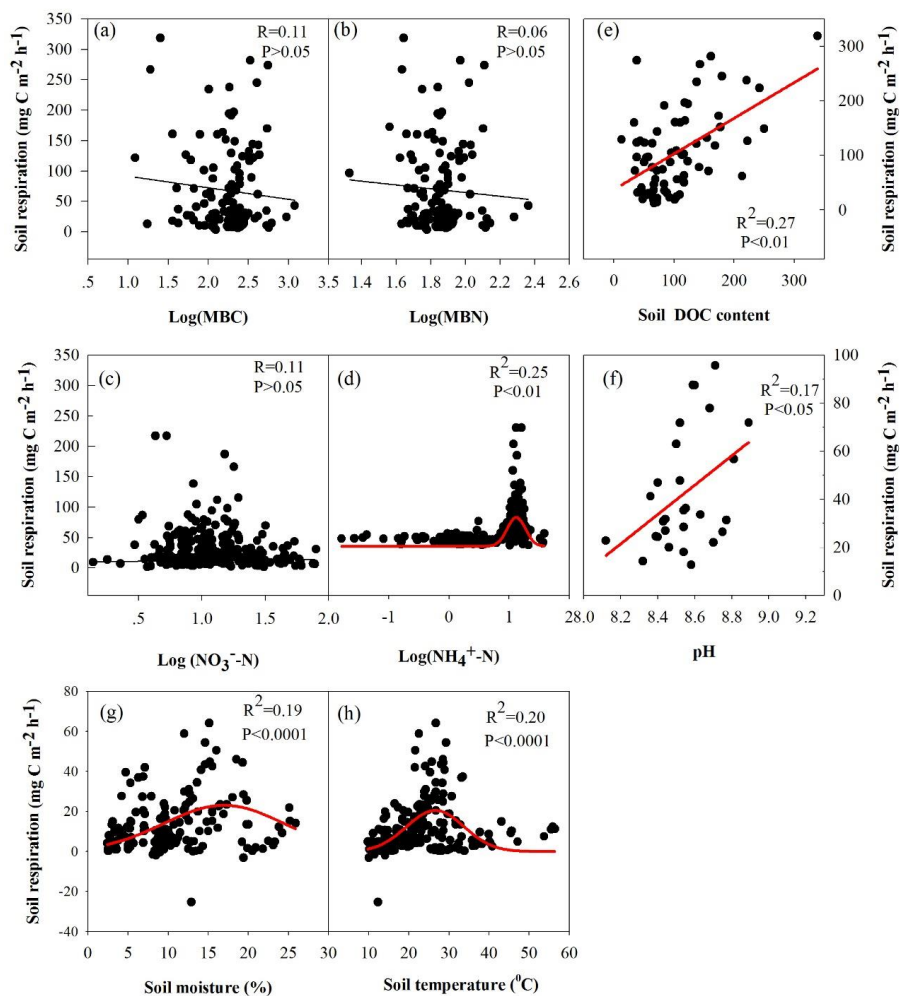
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647 **Fig. 4**



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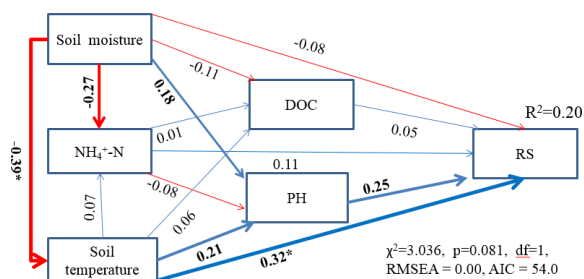
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657 **Fig. 5**



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681 **Table 1** The annual, growing season (GS), non-growing season (NGS), and between-year fluxes
 682 and variation of soil respiration (R_s) in September 2014 to September 2016 (mean \pm SE), including
 683 the contribution of GS and NGS, and the treatment effect. The positive values stand for increase
 684 R_s , and the negative value stand for reduced R_s .

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Treatments	Prec. mm	temp. °C	R_s rate (kg C ha ⁻¹)							
			W0N0	W0N1	W0N2	W1N0	W1N1	W1N2	W1N1T1	T1
Annual	175.75	4.63	1090.11	1338.26	1299.41	1450.78	1304.77	1043.77	1196.84	981.19
	± 7.75	± 0.36	± 450.78	± 599.12	± 537	± 543.70	± 383.29	± 233.23	± 334.31	± 371.34
CV (%)	4.41	7.78	41.35	44.77	41.33	37.48	29.37	22.34	27.93	37.85
Treatments effect (%)	---	---	---	22.76	19.20	33.09	19.69	-4.25	9.79	-9.99
Growing season										
2014.9-2015.8	120.5	14.67	508.30	561.95	570.38	650.66	669.93	562.04	561.10	425.35
2015.9-2016.8	114.5	21.18	1220.27	1546.62	1506.88	1641.62	1274.93	1052.22	1183.86	1089.51
CV (%)	3.61	25.68	46.18	52.03	50.96	48.30	32.79	33.21	50.47	62.00
Non-Growing season										
2014.9-2015.8	47.5	-6.13	131.03	177.09	192.03	256.43	251.55	248.50	301.43	184.49
2015.9-2016.8	69	-11.2	320.62	390.84	329.54	352.85	413.12	224.79	347.29	262.02
CV (%)	26.10	41.37	59.37	53.23	37.29	22.38	34.38	7.09	10.00	24.82
NGS Contribution	---	---	20.65	22.07	21.57	22.98	25.89	24.13	27.10	22.80

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700 **Table 2** Tests of significance of year (Y), warming (T), precipitation (W) and nitrogen addition
 701 (N) on soil respiration (R_s) by multivariate ANOVA (F and P values). The accumulated effect of
 702 precipitation, N deposition and warming on R_s in 2015 and 2016 (F and P values) as assessed by
 703 repeated measures ANOVA. *, ** and *** indicate significant effect at $P < 0.05$, $P < 0.01$, and $P <$
 704 0.001 , respectively.

Three-way ANOVA	n	F	P
Y	2	26.171	<0.001***
N	424	7.709	<0.001***
W	565	17.124	<0.001***
W×N	424	9.392	<0.001***
W×Y	424	6.899	<0.001***
N×Y	424	5.561	0.004**
Y×W×N	424	5.963	0.003**
T	424	2.320	0.084
T×Y	424	0.536	0.464
Repeated measures ANOVA			
Y	2	30.487	<0.000***
N	383	12.887	<0.000***
W	281	2.934	0.087
T	142	0.965	0.326
W×N	281	12.755	<0.000***
T×W×N	281	39.927	<0.000***

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