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

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**Abstract**

Soil respiration (Rs) is the most important source of carbon dioxide emissions from soil to atmosphere. However, it is unclear what the interactive response of Rs would be to environmental changes such as elevated precipitation, nitrogen (N) deposition and warming, especially in unique temperate desert ecosystems. To investigate this an *in situ* field experiment was conducted in the Gurbantunggut Desert, northwest China, from September 2014 to October 2016. The results showed that precipitation and N deposition significantly increased Rs, but warming decreased Rs, which was mainly through its impact on the variation of soil moisture at 5 cm depth. In addition, the interactive response of Rs to combinations of the factors was much less than that of any single-factor, and the main interaction being a positive effect, except interaction from increased precipitation and high N deposition (60 kg N ha-1 yr-1). Although Rs was found to be a unimodal change pattern with the variation of soil mositure, soil temperature and soil NH4+-N content, and it was signicantly postively correlated to soil dissloved organic carbon (DOC) and pH, but from a structural equation model found that soil temperature was the most important controlling factor. Those results indicated that Rs was mainly interactively controlled by the soil multi-environmental factors and soil nutrients, and was very sensitive to elevated precipitation, N deposition and warming. But the interactions of multiple factors largely reduced between-year variation of Rs more than any single-factor, suggesting that the carbon cycle in temperate deserts could be profoundly influenced by positive carbon-climate feedbacks.

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

**Highlights**

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

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

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

4. Soil temperature was the most important controlling factor for Rs.

1. **Introduction**

Global climate warming, changes in precipitation patterns and increased atmospheric nitrogen (N) deposition have all occurred since the industrial revolution, especially in temperate regions (IPCC, 2013), which will be expected to significantly change soil respiration (Rs) that is the most important source of carbon dioxide (CO2) from soil to atmosphere (Wu et al., 2011): the annual CO2 flux from Rs was ten-fold that of fossil fuel emissions (Eswaranet al., 1993; Batjes, 1996; Gougoulias et al., 2014). Therefore, even a small change in Rs will profoundly affect greenhouse gas balance and climate (Heimann and Reichstein, 2008). Although a number of experiments of the effects of warming, precipitation, and N deposition on Rs have been conducted in alpine grassland, tundra regions, peatlands and temperate forest (Lafleur and Humphreys, 2008; Strong et al., 2017; Yang et al., 2017; Zhao et al., 2017), studies in temperate desert ecosystems are scarce, especially the impact on Rs of the interactions of these changes. A field study of multi-factor interactive effects on Rs was therefore conducted in a temperate desert ecosystem to help in understanding the response of Rs to climate change and N deposition in future and highlight the main driving factors.

Rs includes autotrophic respiration (RA), which is mainly from plant roots and mycorrhizal activities; and heterotrophic respiration (RH), which is mainly from the activities of microorganisms (Hanson et al., 2000). Soil moisture is an critical limiting factor for plant roots and microbial activities in desert ecosystems (Huang et al., 2015a): Rs was significantly increased by 47-70% in a degraded steppe in Inner Mongolia, China, by increasing precipitation (Chenet al., 2013), with the effect especially strong in summer (Zhang et al., 2017). In addition, in arid ecosystems, increasing precipitation significantly stimulated plant growth, enhanced soil microbial activity and abundance (Huang et al., 2015a), and changed soil nutrient and substrate concentration, such as dissloved organic carbon (DOC), inorganic nitrogen content, moisture and temperature (Huang et al., 2015b).

Warming significantly increased soil temperature, another important controlling factor for plants growth and microbial activity (Sheiket al., 2011; Huang et al., 2015a). Rs rates were significantly increased in a forest soil and Tibetan Plateau grassland by warming (Chen et al., 2017a), reducing Rs with decreasing soil moisture in the growing season, but increasing Rs in the non-growing season (Fang et al., 2017; Li et al., 2017); no significant impact was observed from warming (Liu et al., 2016a). Therefore, how Rs is affected by warming induced variations in the soil environment is still unclear. In addition, low and short-term N deposition enhanced Rs, while higher and long-term N deposition inhibited Rs due to changes in plant growth and microbial activity (Zhu et al., 2017), but no impacts have also been reported (Luo et al., 2017; Zhang et al., 2017). A meta-analysis showed that the effects of N enrichment on soil CO2 fluxes depended on temperature and soil properties (Zhong et al., 2016); desert soils may be even more senstive to its variation.

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

1. **Materials and methods**

*2.1. Study site*

A field experiment was carried out at the southern edge of the Gurbantunggut Desert (44º26’ N, 87º54’E and 436.8 m a.s.l.), northwest China, from September 2014 to October 2016. This is the largest fixed/semi-fixed temperate desert in China. The mean annual temperature and precipitation are 7.1°C and 215.6 mm, respectively (Cui et al., 2017), and annual potential evaporation exceeds 2000 mm. From late November to mid-March of the following year, a 20–35 cm depth of snow cover the whole desert (equivalent to 38–64 mm rainfall；Huang et al., 2015c). The growing season is from April to October. This desert soil is of extremely low fertility and high alkaline (Cui et al., 2017). Soil organic carbon, total N content, soil NO3--N, NH4+-N contents and 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 21.39 ± 1.84, respectively (Table 1; Cui et al*.,* 2017). Plant species are dominated by *Haloxylon ammodendron* and *Haloxylon persicum*, and the vegetation was extremely sparse, with only 30% coverage, with some spring ephemeral plants (May–June), some annuals, and perennials herbaceous plants (July–August; Liu et al., 2016). Spring ephemeralsaccount for > 60% of the community cover and 85% of the biomass. Summer ephemerals, annuals and perennials usually account for only a small proportion of the community biomass before June, but dominate the community after the die-back of the spring annuals (Huang et al., 2015c).

*2.2. Experimental treatments*

A striking N deposition rate (35.2 kg N ha-1 yr-1) has occurred in the Gurbantunggut Desert due to the rapid development of agriculture and industry with main form of ammonium nitrate (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 (Song et al., 2015). In addition, according to the forecast of Galloway et al. (2008) that atmospheric N deposition will double from the early 1990s to 2050, and the predictions of Liu et al. (2010) that precipitation in this region would be increased by 30% in next 30 years. In September 2014 to August 2016, an *in situ* complete block interactive experiment was therefore conducted to study the impact of N deposition and increased precipitation on Rs (Experiment 1). The three levels of N deposition (0 kg N ha-1 yr-1 (control, N0), 30 kg N ha-1 yr-1 (low, N1) and 60 kg N ha-1 yr-1 (high, N2)) and two levels of precipitation (‘natural’ precipitation (W0) and an increase of 30% (an extra 60 mm precipitation annually (W1)) were applied (Cui et al., 2017). Therefore there were six treatments (W0N0, W0N1, W0N2, W1N0, W1N1 and W1N2) with four replicates of each treatment; each replicate plot was 10 m × 10 m with a 5-m wide buffer zone. The additional precipitation and N deposition (NH4NO3) were added twelve times in April, July and September, equivalent to 5 mm precipitation and 2.5 or 5 kg N ha−1 per application over a week. The NH4NO3 was diluted in 50 L water (equal to 0.5 mm precipitation), and evenly applied following the simulated precipitation. The same amount of water was applied to the control plots (W0N0).

Rapidly warming (0.6 oC per decade), increasing precipitation (3-5 mm yr-1 since 1979) and receiving high N deposition (3 kg N ha-1 since 1980) are affecting the Gurbantunggut Desert (Liu et al., 2013; Li et al., 2015), which would be excepted to affect rate of Rs. Therefore, another interactive experiment was established at the same time, simulating the three most likely climate scenarios in the future: (1) warming only (W0N0T1); (2) increased precipitation and N deposition without warming (W1N1T0); (3) the interaction of increasing precipitation, N deposition and warming (W1N1T1); all compared with the current climate (W0N0T0). Therefore, there were four treatments (W0N0T1, W1N1T0, W1N1T1, W0N0T0) with four replicates (plots) of each treatment. Open-top chambers (OTCs) were used to simulate warming. The OTCs were designed with 5 mm transparent tempered glass and stainless steel angle iron to the ITEX standard (Marion et al., 1997). They were 2 m high and 4 m in diameter, with each OTC area being 12 m2. However, the design was improved such that the top and bottom OTC areas were the same so that precipitation and snowfall were the same as that to the surrounding environment; this also avoids overheating inside the OTCs. The timings of applications of water and N were the same as in Experiment 1.

*2.3. Measurements*

Rs in all plots were measured twice or thrice a week (continuous measurements over 3 days were made following simulated precipitation and N deposition) using gas chromatography and static chambers (50 cm×50 cm×10 cm) at locations where grow only spring ephemeral plants without any annuals and perennials in order to minimize the between-treatment spatial heterogeneity due to sparse annuals, and perennials (Liu et al., 2012). Gas samples were collected between 10:00-12:00 (GMT + 8) throughout the experimental period, which was detected in this period were close to the diurnal averages (Fig.3b and 3d, Fig. 1S). Gas samples were collected from the headspace of each chamber 0, 10, 20 and 30 min after closing the chamber per time. The gas samples analyzed within three days using a gas chromatograph (GC; Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector for quantitative Rs (Liu et al., 2012). Rs rates were calculated from four concentrations of the gas sample based on a first order differential linear or non-linear equation and were temperature- and pressure- corrected (Liu et al., 2012; Zhang et al., 2014). Soil samples were taken monthly from around the static chambers to a depth of 10 cm using an auger (3.5 cm in diameter). Fine roots and small stones were separated out using a 2 mm sieve. Dissolved organic carbon (DOC) was extracted with deionized water (soil: water ratio = 1:10) by shaking on an orbital shaker at 10000 rpm for 5 min and analyzed using a TOC analyzer (multi N/C 3100, Jena, Germany; Jones and Willett, 2006). Brookes’ (1985), Chloroform fumigation extraction was used to measure microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). Soil organic carbon (SOC) were measured using the potassium dichromate method (Jiang et al., 2014), and soil NO3--N and NH4+-N analyzed as per Yue et al. (2016). Soil pH was measured on a 1:5 soil: deionized water suspension using a pH Meter (Seven Easy, Mettler-Toledo, Switzerland). Caipos Soil and Environment Monitoring Systems (Caipos GmbH, Austria) were used to monitor soil moisture/temperature at 5 and 20 cm depth every hour.

*2.4. Effects of each treatment on Rs*

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

The treatment effect = (TRs - CRs) / CRs × 100%,

Where the treatment effect is W0N1, W0N2, W1N0, W1N1, W1N2, W1N1T1 or W0N0T1 effect on Rs (a positive value shows that the treatment has increased Rs and a negative value shows decrease of Rs), corresponding TRs represents Rs from the W0N1, W0N2, W1N0, W1N1, W1N2, W1N1T1 or W0N0T1 plots (mg C m−2 h−1) and CRs indicates the Rs from the control plots (W0N0, mg C m−2 h−1).

*2.5. Statistical analyses*

Treatments effect on soil organic carbon (SOC), NO3--N, NH4+-N content, pH, DOC, MBC and MBN were examined in each treatment by least significant difference LSD (p<0.05). The single-factor and interaction effects of precipitation, warming and N deposition on Rs were detected by multi-way analysis of variance (ANOVA), and the accumulated effect of precipitation, warming and N deposition on Rs were tested by repeated measures ANOVA. In addition, the relationships of Rs and DOC, MBC, MBN, soil temperature, soil moisture, NH4+-N content, soil NO3-- N, and pH were described using a linear or non-linear regression model. The factors of key controls on Rs were used to analyze by structural equation models (SEMs). SPSS software (version 20.0) was used to conduct all statistical analyses, and statistical significant differences were set with P<0.05. All Figures were created using the Sigmaplot software package (version 10.0), but SEMs analyses were carried out using AMOS 22.0 (Amos Development Corporation, Chicago, IL, USA).

1. **Results**

*3.1. Treatments effects on soil environmental and properties*

Soil temperatures at 5 depth were mostly increased between 11:00 and 22:00 every day by warming; the average annual soil temperatures at 5 and 20 cm depth were significantly increased by 4.41 and 3.67 0C, respectively (Fig. 1a). Soil moisture at 5 cm depth was decreased by warming by only 0.61v/v% (Fig. 1b), and a very small decrease of 0.01v/v% in soil moisture at 20 cm depth was observed (Fig.1b). Soil moisture at 5 and 20 cm depth were largely increased by the increased precipitation (Fig.1b and 2b). N deposition and warming significant increased soil NH4+-N and NO3--N contents (Fig. 1c), but no significant change was found from increased precipitation. Soil MBC and MBN were greatly increased by N deposition, but significant negative effects on soil MBC and MBN were observed by warming and the interaction of precipitation and N deposition (Fig. 1d). No significant change in SOC and DOC was observed in any treatment (Fig. 1c and 1d).

*3.2. Precipitation, warming and N deposition effects on* Rs

In our study, a weak Rsemission rate (-2.46 to 50. 26 mg C m-2 h-1) was observed at control plots with an average emission rate of 12.18 mg C m-2 h-1 from September 2014 to October 2016(Fig. 2c). Annual cumulative rate of Rs was 1090.11±450.78 kg C ha-1, with non-growing season account for 20.7% of the annual emission (Table 1). Rs was significantly enhanced by increasing 5-mm precipitation and N deposition from 12.18 to 16.23 and 14.97 mg C m-2 h-1 (average), respectively (P< 0.001; Fig. 2c and 2d; Table 2), with annual Rs increased by 33.1% and 19.2-22.8%, respectively (Table 1). And the low N deposition effect on Rs was much higher than that high N deposition (Fig. 2c and 2d). However, Rs was reduced mostly by warming, although not significant (P=0.084; Table 2). And high temperatures and low humidity at times of peak sunshine during the diurnal variation significantly inhibited its emission rate (Fig 3a and 3b, Fig.2S), but it was also significantly increased by warming following extremely rainfall events that increased soil moisture (Fig. 3c and 3d). The diurnal trend in Rs was consistent with that of soil temperature at 5 cm depth (Fig. 3). In addition, the interactive responses of Rs to increasing precipitation, warming and N deposition were much lower than that from any single-factor (Table 1), and with the interaction of 60 kg ha-1 N and extra precipitation decreasing Rs by 4.25% (Table 1). Overall, annual Rs rates were significantly impacted by precipitation, N deposition, and their interaction (Table 2), but no significant net change was caused by warming (Table 2), although Rs rates were decreased by 9.99% (Table 1).

*3.3. Temporal variation and its control*

The results of repeated measures ANOVA showed that significantly accumulated effects on Rs were found by N deposition and interaction between N deposition and precipitation or warming rather than alone increasing precipitation and warming (Table 2). A large between-year variation in Rs was observed with a coefficient of variation (CV) up to 41.4% (a much higher Rs rate was observed in 2016 than 2015), but variation was reduced by increasing precipitation, N deposition and warming and their interaction, except with an increase in N deposition of 30 kg ha-1(Table 1). The results of regression analysis showed that Rs was significantly increased by increases in pH and DOC (Fig. 4e and 4f), but no significant relationships were found with MBC, MBN or NO3--N content (Fig. 4a, 4b and 4c). In addition, different response characteristics of Rs in the impacts of increased soil mositure, soil temperature and NH4+-N content were found. Soil mositure was the most important controlling factor when it was <4.2 % and soil temperature was >26.50C (Fig. 4g and 4h, Fig. 2S). Secondly, soil temperature was the most important limiting factor when soil moisture was >15.9 % and soil temperature <2.7 0C (Fig. 4g and 4h, 2S). Thirdly, there was no significant impact on Rs when soil NH4+-N content was <6.3 mg N / kg. A significant increase in Rs occurred when soil NH4+-N content was between 6.3 and 12.6 mg N / kg, but Rs was inhibited when soil NH4+-N content was between 12.6 and 31.6 mg N / kg (Fig. 4d).

1. **Discussion** 
   1. *Single-factors impacts of precipitation, N deposition and warming on Rs*

Annual Rs was 1090 kg C ha−1 in this temperate desert, with the non-growing season accounting for 20.7 % of the annual flux (Table 1). This is consistent with previous study in here (Zhou et al., 2014; Huang et al., 2015a) because SOC content was very low (Fig. 1c), and vegetation was very sparse in this desert (Liu et al., 2016b). Increasing precipitation significantly increased Rs(Fig. 2c). It is also consistent with the results of a meta-analysis and previous study in here (Huang et al., 2015a ; Liu et al., 2016c). This is because that the growth of desert plants and microbial activity are significantly activated by increasing precipitation (Huang et al., 2015a), and microbial biomass, mass-specific respiration, microbial biomass carbon (MBC) and nitrogen (MBN), and microbial PLFAs were consistently significantly enhanced by increased precipitation (Zhang et al., 2013; Huang et al., 2015a). However, Rs in our study was much higher in moderate soil moisture conditions than with too little or too much soil moisture (Fig. 4g). This suggests that Rs is mainly RH rather than RA in this desert, namely from soil microorganism, because (1) too little or too much soil moisture could significantly inhibit microbial activity due to variation of soil temperature and soil properties (Ma et al., 2013), while moderate soil moisture could significantly enhance microbial activity (Skopp et al., 1990), and (2) the biomass of fine roots was no significantly enhanced at our sites by increased precipitation (Cui et al., 2017). This is consistent with results from a desert steppe in northern China where the contribution of RH (78.1%) was significantly higher than that of RA (21.9%) under increasing precipitation (Liu et al., 2016a).

N deposition also significantly increased Rs, especially in low N deposition (Fig. 2d). This is consistent with results from an alpine meadow and in the Loess Plateau (Fang et al., 2017; Zong et al., 2017), and with a meta-analysis showing that N deposition increased Rs by 8.8% (Zhou et al., 2016a). This is because N deposition, on the one hand, could increase fine root biomass, although this was not significant in our study (Cui et al., 2017); on the other hand, increases microbial activity and abundance by low N deposition (Huang et al., 2015b). But this was inconsistent witha young *Cunninghamialanceolata* forest (Wang et al., 2017), and beneath shrubs of *H. ammodendron*, soil high N content, has the opposite effect in our study site (Chen et al., 2013；Huang et al., 2015b). What’s more, the results of nonlinear regression analysis showed that higher Rs rates occurred at moderate soil NH4+-N contents (between 6.3 and 12.6 mg N / kg), while lower Rs occurred in much lower (<6.3 mg N / kg) or much higher ( >12.6 mg N / kg) soil NH4+-N contents (Fig. 4d), but this effect of N deposition on Rs is not consistent with other ecosystems (Burton et al., 2004; Chen et al., 2013; Liu et al., 2015; Chen et al., 2017b). This is because the desert soil is extremely limited than other ecosystem (Adams, 2003), so low N deposition enhanced plants growth and microbial activity, but high N inhibited microbial activity and community composition, reduced Rs (Zhou et al., 2014; Huang et al., 2015b). Overall, soil NH4+-N content was an important controlling factor for Rs because microbial activity, abundance and species diversity were regulated by soil NH4+-N content in this desert, and Rs was very sensitive to variation of N deposition.

Warming decreased Rs (Fig. 2f), although not significantly (P=0.084; Table 2), which was consistent with results from a semi-arid alfalfa-pasture of the Loess Plateau (Fang et al., 2017). In addition, a significant decrease in Rs was observed on an extreme drought or hot sunny day when soil moisture was reduced, and sharply reduced Rs when soil temperature reached 37 0C (Fig. 3a and 3b, 1S). This is because: (i) microbial activity is significantly inhibited by extreme temperatures and low soil moisture, may reduce population size by 50-80% (Sheik et al., 2011); (ii) fine root growth is inhibited in high temperature and low soil moisture. Others have noted this phenomenon as occurring at about 16:00 each day (Ma et al., 2013), but in our study the effect was advanced to 14:00 by warming, which may reduce carbon emission from soil to atmosphere. However, this is not consistent with results from a tundra ecosystem, subtropical forest or alpine regions where Rs was significantly increased by warming due to the limitation of soil temperature in these ecosystems, and no significant change in soil moisture (Noh et al., 2016; Wu et al., 2016; Zhou et al., 2016b). In addition, a significant increase in Rs was found following enhanced precipitation with warming (Fig. 3c and 3d), which indicates that soil moisture was the most important controlling factor for Rs under a warming climate. This is consistent with other studies (Chen et al., 2017a; Zhao et al., 2017). However, statistical analysis showed that no overall significant impact on Rs was found during the experimental period by warming, and it was reduced by 9.99%. This is because our gas samples were taken at 10:00 – 12:00 each day, when average soil temperatures were increased by about one degree. Thus mean annual Rs was not sensitive to temperature changes this small in contrast to the very significant effects of short-term diurnal changes in soil temperature, observed between 12:00 and 17:00 (Fig. 3a and 3c). However, gas samples during 10:00-12:00 in this study could catch the meanly warming effects on Rs, except some extremely precipitation and drought events in summer (Fig.1S), which will require further systematic evaluation. Those results indicated that Rs depends mainly on variations of soil moisture and temperature in the context of warming, and climate change is likely to have a very significant effect on temperate deserts.

* 1. *The interactive effects of precipitation, N deposition and warming on Rs*

Interactive responses of Rs were much lower than those of any single-factor, but still increased Rs overall, except interaction between precipitation and high N deposition (Table 1). This is consistent with results in dry ecosystems (Morillas et al., 2015; Martins et al., 2016), but not with the results of a meta-analysis that precipitation and N deposition interactive experments were a greater extent positive effect on Rs (Zhou et al., 2016a). This can be explained in our study that soil MBC or MBN were much less in interactive treatments than that of single-factor (Fig. 1d), which showed that a number of microorganisms were much less in interactive treatments than that of single-factor due to much stronger N effect. As we found that Rs was reduced with increasing N deposition and precipitation by as much as 4.25% in W1N2 plots (Table 1), which showed that the inhibiting effect of soil NH4+-N content was much stronger when there was sufficient soil moisture (Fig. 2e). This is consistent with the results in a *Populuseuphratica* community in a desert ecosystem (He et al., 2015). This was because (i) microbial activity was inhibited by high or low soil moisture and high soil NH4+-N or NO3--N content (Burton et al., 2004); and (ii) high N tent to reduce extracellular enzyme activity and the fungal population (Maris et al., 2015). In addition, the interactive effect of the three factors on Rs in this desert was much lower than interaction of two factors of precipitation and N deposition (Table 1), and is consistent with the results of modeled interactive effects, which showed that three-factor interactions were rare while two-factor interactions were more common (Luo et al., 2008). Fortunately, the interactive effect of three factors or two factors (precipitation, N deposition and warming) in this desert could largely reduce between-year variation on Rs (Table 2), which may because (i) the limits of soil moisture, soil temperature and soil N content were relieved for key biological processes by increasing precipitation, N deposition and warming (Huang et al., 2015a; Liu et al., 2016b); (ii) various factors antagonistic to each other (Zhou et al., 2016a). However, the variation in the growing season on Rs can be increased by warming, elevated precipitation and N deposition because of their dominant effects on plant growth and microbial activity (Huang et al., 2015b), but it was the exact opposite in the non-growing season due to reduce the limit of temperature (Zeng et al., 2016). Those results showed that Rs would be reduced under interactive effect of increasing rainfall, temperature and N deposition in the future, and took place a positive carbon-climate feedbacks.

* 1. *Temporal variation in treatments on Rs and controlling factors*

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

Regression analysis shows that Rs exhibited a unimodal change pattern with variations of soil NH4+-N (Fig. 4d), mositure (Fig. 4g), and temperature (Fig. 4h), and Rs was signicantly postively correlated to soil dissloved organic carbon (DOC) and pH (Fig. 4e and 4f). However, structural equation modeling indicated that soil temperature was the most important controling factor than soil NH4+-N and soil moisture (Fig. 5), unsupported our hypothesis, but it is consistent with most research results (Wu et al., 2016; Zhou et al., 2016b; Chen et al., 2017a). In addition, large inter-annual variation was observed (CV = 41.4%) during our experiment (Table 1), while the variation of annual precipitation and air temperature were only 4.41% and 7.78%, respectively (Table 1), but close to the CV of spring root biomass of ephemeral plants (47.14%) with 24 times of aboveground biomass of spring ephemeral plants in 2016 than that in 2015 (Cui et al., 2017), which indicated that the increase of Rs in 2016 was mainly from the root respiration of ephemeral plants. This is consistent with previous study that ephemeral plants mediated inter-annual variation of carbon fluxes in this desert (Huang et al., 2015c; Liu et al., 2016). It is different from other ecosystems where inter-annual variations of Rs were mainly dependent on variations in annual precipitation and air temperature (Gerard et al., 1999; Asensio et al., 2007; Chen et al., 2012). Overall, our results indicate that annual variation in Rs in this temperate desert is mainly controlled by soil temperature, but between-year variation in Rs is mainly controlled by ephemeral plants.

1. **Conclusion**

Climate change and elevated N deposition play important roles in controlling Rs in temperate deserts. We found that increasing precipitation and N deposition significantly increased Rs in the Gurbantunggut Desert, but warming reduced Rs, mostly because of the variation of soil moisture. In addition, we found that the interactive responses of Rs was much lower to precipitation, N deposition and warming than that any single factors. What’s more, Rs are mainly mediated by soil moisture, soil temperature and soil NH4+-N content, but soil temperature are the most important with between-year variation in Rs mainly controlled by ephemeral plants. Those results showed that Rs is very sensitive to increasing precipitation, N deposition and warming, and their interactive effects could reduce soil carbon emissions and so reduce the impacts of climate change.

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**Fig. 1** Comparative effects of warming by Open Topped Chambers, increased precipitation, and N deposition on soil temperature (a), soil moisture (b) at 5 and 20 cm depth; soil organic carbon (SOC), NH4+-N content, NO3--N content and pH (c), dissolved organic carbon (DOC), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN, d). The data are mean ± SE, n = 4 in c and d, different letter indicate significant effect at P < 0.05.

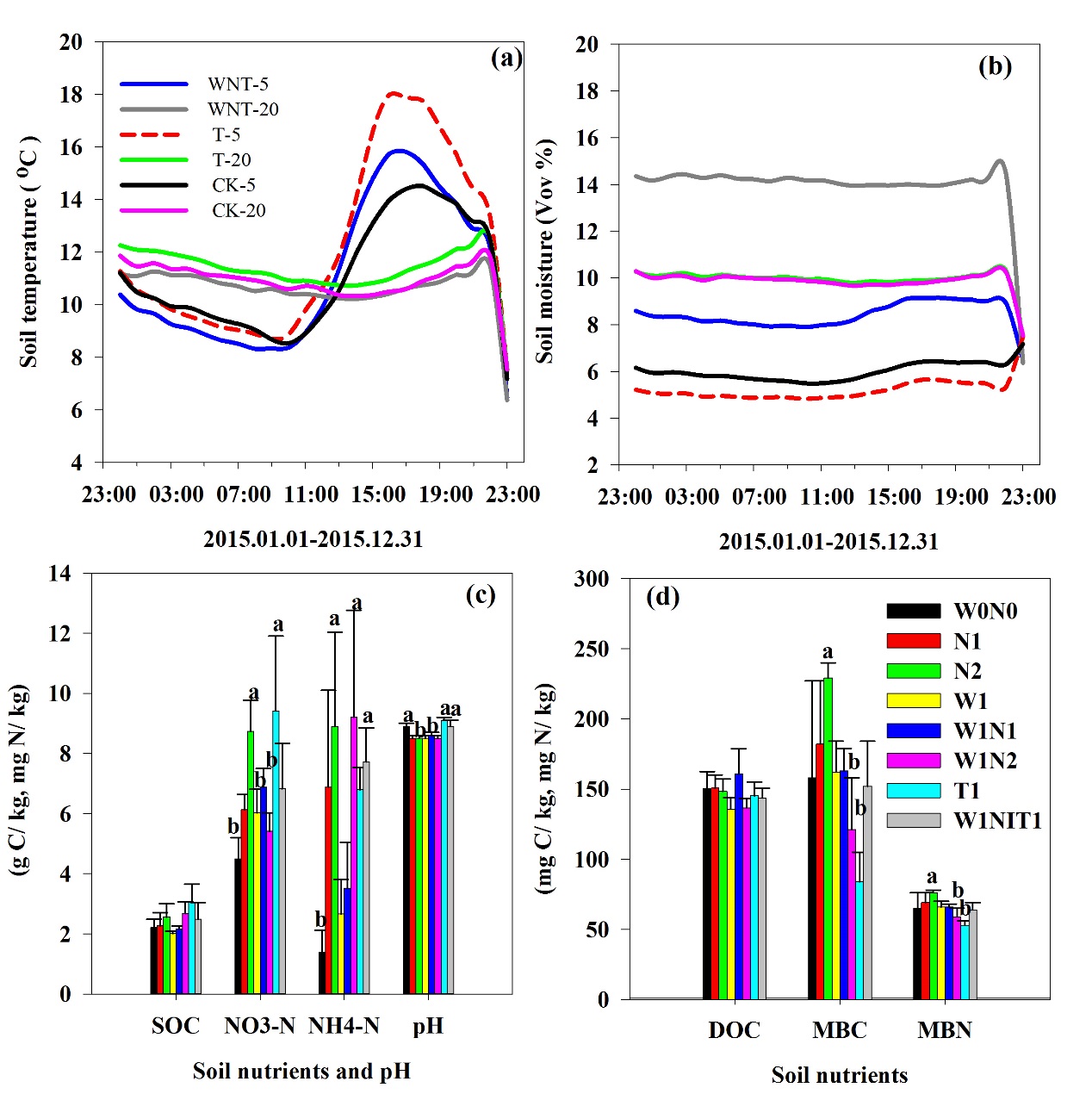
**Fig. 2** Variation in rainfall (mm, a), and air temperature (°C, a) from September 2014 to October 2016 at the Gurbantunggut Desert, and the soil moisture and temperature to increasing precipitation and warming (b), and the response of Rs (mean, n = 4) to precipitation (c), N deposition (d-e) and warming (f). W0 and W1 indicate under ambient precipitation (without water addition) and 60 mm yr−1 precipitation addition; N0, N1, and N2 indicate 0, 30 and 60 kg N ha−1 yr−1 nitrogen addition; while W1N0, W1N1, and W1N2 indicate 0, 30 and 60 kg N ha−1 yr−1 nitrogen addition under 60 mm yr−1 precipitation addition; W1N1T1, W0N0T1 and W0N0T0 indicate the interaction between increasing precipitation (60 mm yr−1), N deposition (30 kg N ha−1 yr−1) and warming by OTCs, warming alone (without increasing precipitation and N deposition) and control plots, respectively. Black arrows indicate simulated precipitation (5 mm per time) and N deposition (0.25 or 0.5 g N m-2 per time). Each point represents the mean of four replications (chambers). Standard deviations for Rs are not showed for figure clarity.

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

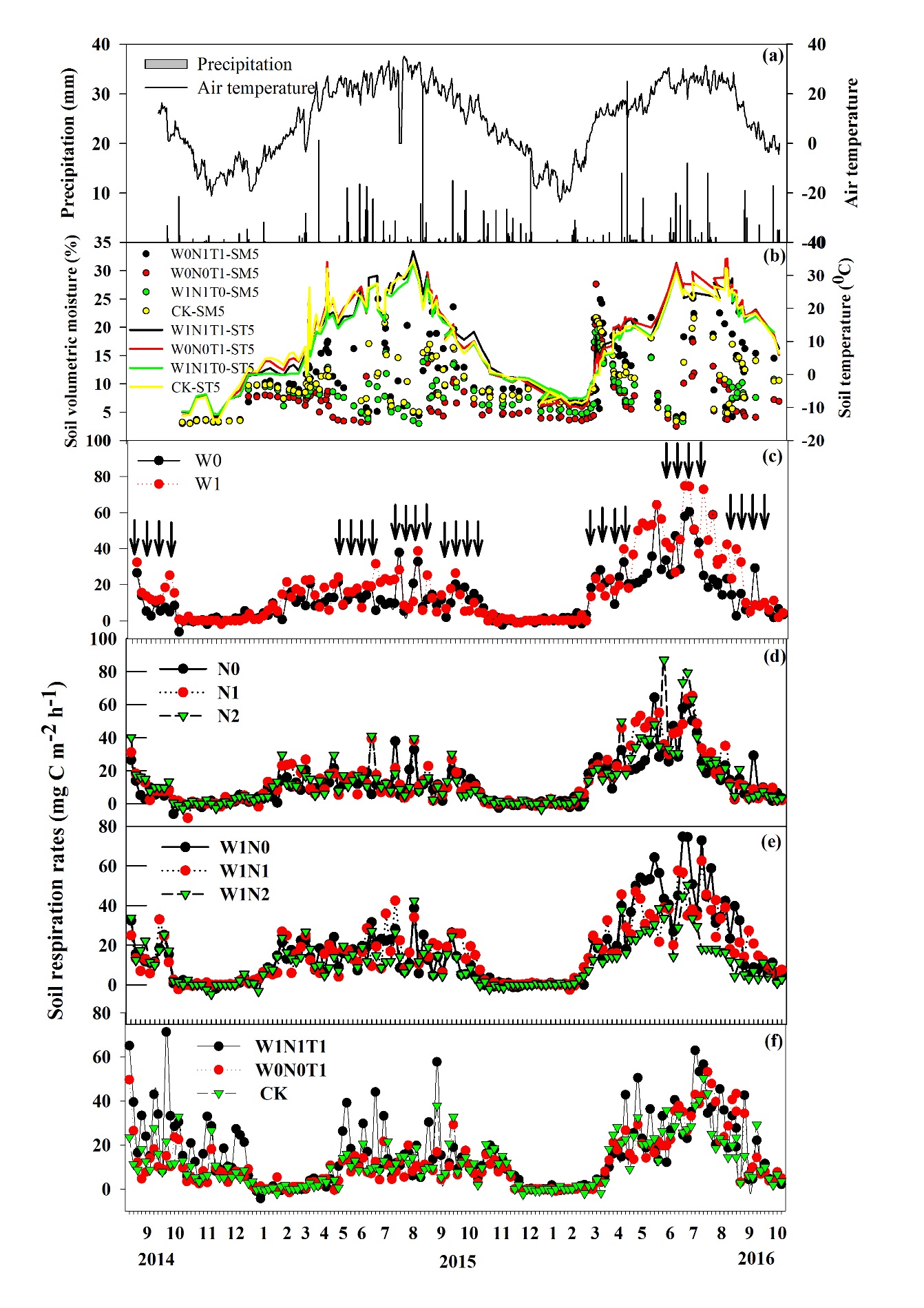
**Fig. 4** The relationship of soil respiration with microbial biomass carbon (MBC, a); microbial biomass carbon (MBN, b); soil NO3-N (c); NH4+-N content (d), x represents soil NH4+-N content; soil dissolved organic carbon (DOC, e); pH (f); soil moisture (g) and soil temperature (h).

**Fig. 5** Structure equation modeling (SEM) test the multivariate (soil moisture, soil temperature, soil NH4+-N content, DOC and pH) effects on Rs (n=34). Single-headed arrows show that the effect of different key controls on Rs were analyzed. The green arrows indicated positive effects, and red arrows showed negative effects. And the width of the arrows indicate the strength of the relationship. The numbers are standardized path coefficients, which can show the importance of the variables in the model. Goodness-of-fit statistics for the model are shown below the model. \* indicate significant effect at P < 0.05.

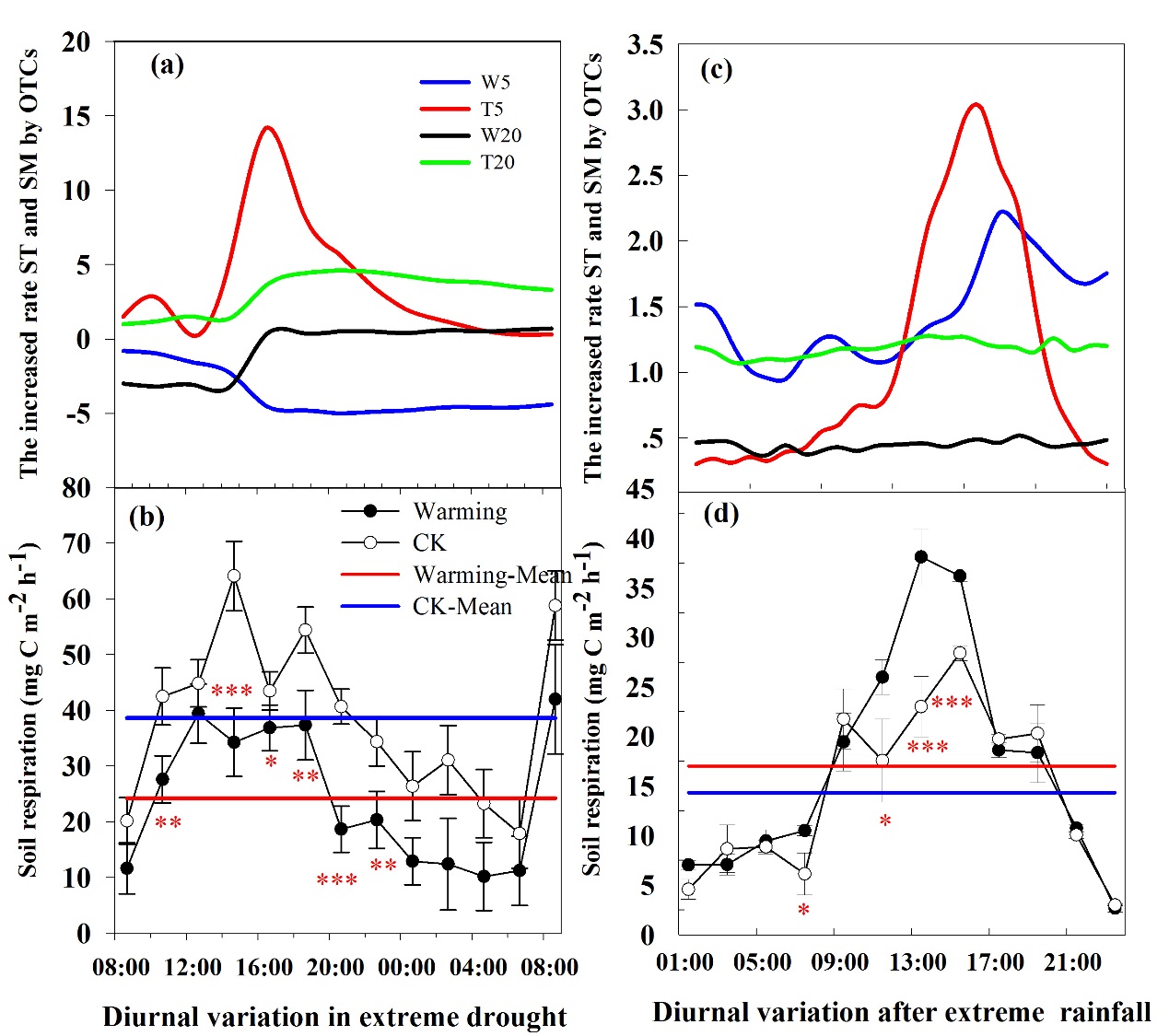
**Fig. 1**



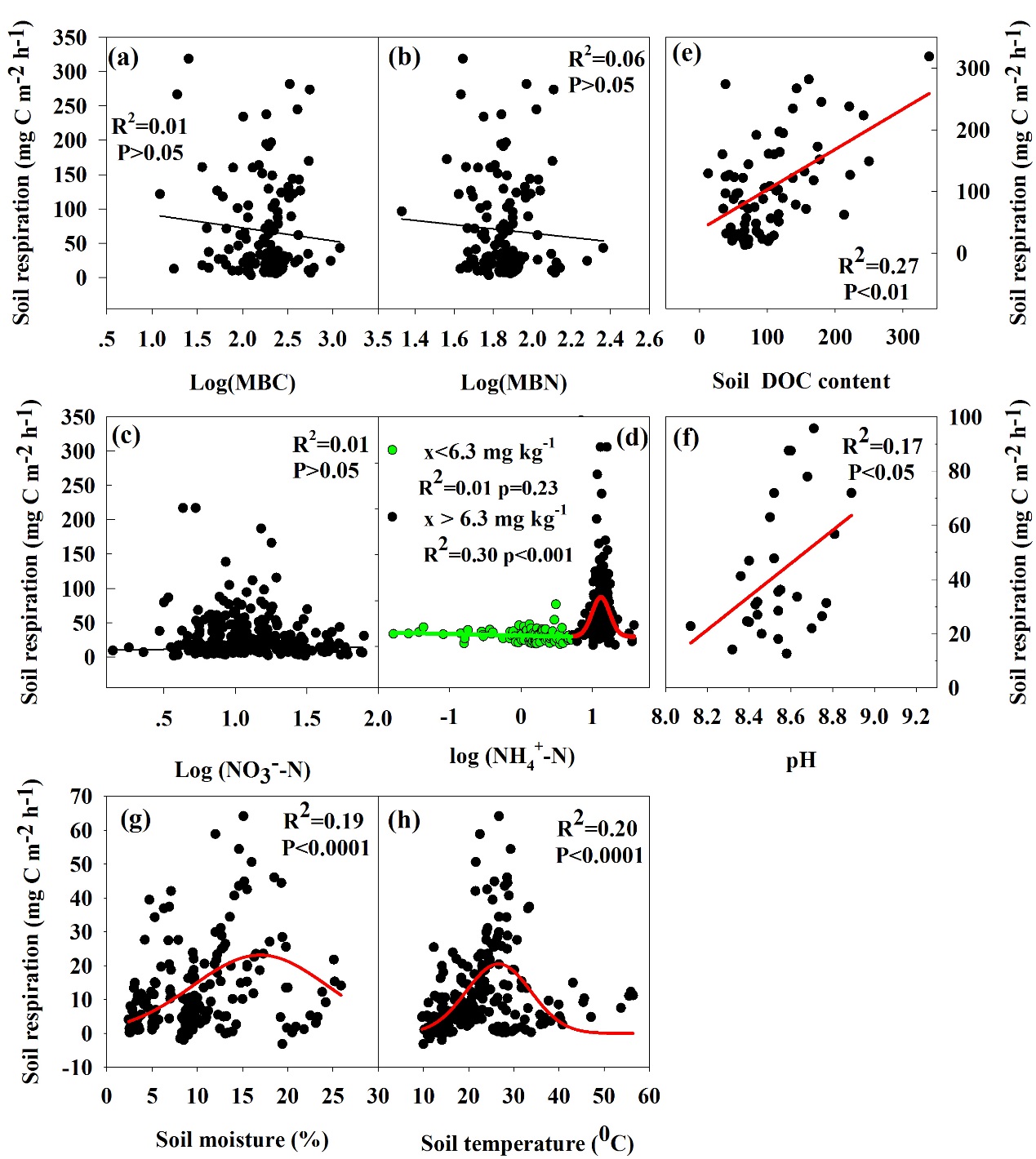
**Fig. 2**



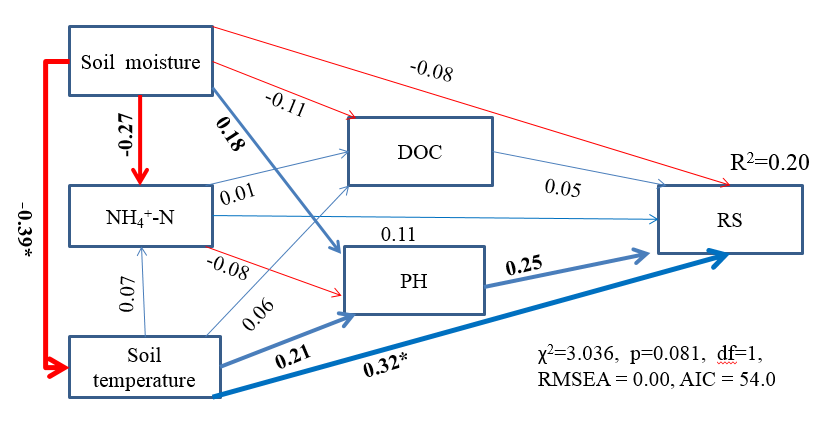
**Fig. 3**

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**Fig. 4**

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**Fig. 5**



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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Prec. | | temp. | | Rs rate (kg C ha-1) | | | | | | | | | | | | | | | |
| Treatments | mm | | 0C | | W0N0 | | W0N1 | | W0N2 | | W1N0 | | W1N1 | | W1N2 | | W1N1T1 | | T1 | |
| Annual | 175.75  ±7.75 | | 4.63  ±0.36 | | 1090.11  ±450.78 | | 1338.26  ±599.12 | | 1299.41  ±537 | | 1450.78  ±543.70 | | 1304.77  ±383.29 | | 1043.77  ±233.23 | | 1196.84  ±334.31 | | 981.19  ±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 | |

**Table 2** Tests of significance of year (Y), warming (T), precipitation (W) and nitrogen addition (N) on soil respiration (*Rs*) by multivariate ANOVA (F and P values). The accumulated effect of precipitation, N deposition and warming on *Rs* in 2015 and 2016 (F and P values) as assessed by repeated measures ANOVA. \*, \*\* and \*\*\* indicate significant effects at P < 0.05, 0.01, and 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\*\*\* |