

# Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland

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## Abstract

Climate change can profoundly impact carbon (C) cycling of terrestrial ecosystems. A field experiment was conducted to examine responses of total soil and microbial respiration, and microbial biomass to experimental warming and increased precipitation in a semiarid temperate steppe in northern China since April 2005. We measured soil respiration twice a month over the growing seasons, soil microbial biomass C (MBC) and N (MBN), microbial respiration (MR) once a year in the middle growing season from 2005 to 2007. The results showed that interannual variations in soil respiration, MR, and microbial biomass were positively related to interannual fluctuations in precipitation. Laboratory incubation with a soil moisture gradient revealed a constraint of the temperature responses of MR by low soil moisture contents. Across the 3 years, experimental warming decreased soil moisture, and consequently caused significant reductions in total and microbial respiration, and microbial biomass, suggesting stronger negatively indirect effects through warming-induced water stress than the positively direct effects of elevated temperature. Increased evapotranspiration under experimental warming could have reduced soil water availability below a stress threshold, thus leading to suppression of plant growth, root and microbial activities. Increased precipitation significantly stimulated total soil and microbial respiration and all other microbial parameters and the positive precipitation effects increased over time. Our results suggest that soil water availability is more important than temperature in regulating soil and microbial respiratory processes, microbial biomass and their responses to climate change in the semiarid temperate steppe. Experimental warming caused greater reductions in soil respiration than in gross ecosystem productivity (GEP). In contrast, increased precipitation stimulated GEP more than soil respiration. Our observations suggest that climate warming may cause net C losses, whereas increased precipitation may lead to net C gains in the semiarid temperate steppe. Our findings highlight that unless there is concurrent increase in precipitation, the temperate steppe in the arid and semiarid regions of northern China may act as a net C source under climate warming.

*Keywords:* climate change, microbial biomass, precipitation, soil moisture, soil respiration, temperate steppe, temperature

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## Introduction

As consequences of the rising concentrations of atmospheric greenhouse gases, global mean temperature has increased by 0.76 °C since 1850 and will continue to increase by 1.8–4.0 °C at the end of this century (IPCC,

2007). Together with the rising temperature, global and regional precipitation regimes are predicted to change as well (Easterling *et al.*, 2000). Changes in both temperature and precipitation can profoundly influence the processes occurring at the ecosystem level, particularly ecosystem carbon (C) cycling and balance, thus exerting a feedback on climate (Cox *et al.*, 2000; Luo, 2007). Soil respiration, as the second largest C exchange pathway between the atmosphere and terrestrial ecosystems, is

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sensitive to alterations of temperature and precipitation/water availability. Changes in soil respiration largely determine responses and feedbacks of ecosystem, regional and global C cycling to climate change (Luo & Zhou, 2006). Therefore, to provide convincing projections of terrestrial C cycling, a better understanding of soil respiration components and driving factors is needed.

Temperature and water availability have been well documented to affect soil respiration and used as fundamental parameters in model simulations and predictions of soil respiration (Luo & Zhou, 2006). The empirical exponential functions between soil respiration and temperature predict increasing soil respiration with increasing temperature. However, total soil respiration has been reported to increase (Rustad *et al.*, 2001; Melillo *et al.*, 2002; Emmett *et al.*, 2004; Zhou *et al.*, 2007), decrease (Pajari, 1995; Saleska *et al.*, 1999) or not change (Wan *et al.*, 2007) across different ecosystems under experimental warming. Moreover, warming has been found to influence soil respiration differently during different time periods even in a single ecosystem (Verburg *et al.*, 2005; Wan *et al.*, 2007). Consistent with the empirical function that higher water availability stimulates soil respiration, the majority of manipulative experiments have reported positive responses of total soil respiration to increased precipitation/water availability (Schmidt *et al.*, 2004; Ford *et al.*, 2007; Patrick *et al.*, 2007; Wan *et al.*, 2007). Nevertheless, neutral response of soil respiration under altered precipitation/water availability (Hanson *et al.*, 2003) has also been detected. Failure of the empirical functions in explaining the above contradicting responses of soil respiration is largely caused by lack of consideration of the possible influence of initial conditions on the soil respiration responses (Shaver *et al.*, 2000).

By decomposing plant litter and soil organic matter, heterotrophic soil microorganisms can contribute substantially to total soil respiration (Lin *et al.*, 1999; Wan & Luo, 2003; Zhou *et al.*, 2007). Elevated temperature generally stimulates soil C turnover and consequent microbial C pool. However, inconsistent results of the effects of experimental warming on microbial biomass or respiration have been reported (Jonasson *et al.*, 1999; Ruess *et al.*, 1999; Zhang *et al.*, 2005; Zhou *et al.*, 2007). For example, elevated temperature has been reported to not affect microbial respiration (MR) (Zhang *et al.*, 2005), or to increase microbial biomass (Ruess *et al.*, 1999). In addition, the exposure time may influence the effects of elevated temperature. Microbial biomass may not respond to elevated temperature in the short term (5 years; Jonasson *et al.*, 1999), but show reductions in the long term (15 years) in the same experiment (Rinnan *et al.*, 2007b). Increased precipitation can stimulate soil microorganisms and activity (Davidson *et al.*, 2000b;

Hungate *et al.*, 2007). However, when water availability is above the optimum point, increased water availability can reduce oxygen concentration and suppress microbial activity (Linn & Doran, 1984; Horz *et al.*, 2004). Mechanistic understanding of how climate warming and changing precipitation regime influence soil and microbial respiration is essential for resolving the above-mentioned controversy.

Climate change-driving factors (temperature and precipitation) can not only directly impact the autotrophic (plant) and heterotrophic (microorganism) respiratory processes, but also indirectly affect these processes via changing soil temperature, soil water availability and supply of C substrate (Shaver *et al.*, 2000; Weltzin *et al.*, 2003; Wan *et al.*, 2007). Sensible heat (for air warming), latent heat (for evapotranspiration) and soil heat flux (for soil warming) constitute energy balance in ecosystems. Temperature and water availability may affect each other to influence soil respiration by energy balance (Wan *et al.*, 2007). Moreover, temperature and precipitation can change plant photosynthesis, growth, belowground C input, and thus the C substrate available for root and microbial respiratory activities. For example, improved soil water availability could indirectly affect soil microorganisms through (i) facilitating diffusion of soluble organic C substrates (Schmidt *et al.*, 2004; Hungate *et al.*, 2007) and (ii) enhancing plant growth, belowground C allocation and C substrate availability (Zak *et al.*, 1993, 1994). Therefore, distinguishing between the complex direct and indirect effects of climate warming and changing precipitation will facilitate the mechanistic understanding of soil respiration responses to climate change (Shaver *et al.*, 2000; Wan *et al.*, 2007).

A field manipulative experiment of warming and increased precipitation with four treatments (control, warming, increased precipitation, and warming plus increased precipitation) has been conducted for 3 years in a semiarid temperate steppe in northern China since 2005. Provided that water availability is the primary limiting factor in arid and semiarid regions, we hypothesize that increased precipitation may stimulate total soil and microbial respiration as well as microbial biomass in the temperate steppe. Experimental warming can stimulate evapotranspiration (Niu *et al.*, 2008), consequently reduce soil moisture (Harte *et al.*, 1995; Wan *et al.*, 2002), and aggravate soil water deficits in semiarid regions. Therefore, we hypothesize that reduced soil water availability under experimental warming could constrain root and microbial activities, offset the positive effects of elevated temperature, and lead to negative responses of soil and microbial respiration. Conceptual models of the effects of climate warming (Shaver *et al.*, 2000) and changing precipitation (Weltzin *et al.*, 2003) propose that the responses of terrestrial

ecosystems depend on the initial conditions of abiotic and biotic factors (temperature, water availability, plant and microbial communities; Shaw & Harte, 2001; Peñuelas *et al.*, 2004; Wan *et al.*, 2007). Because interannual variability in precipitation may result in discrepancy in soil microclimate and consequent decomposition and plant growth, we propose the third hypothesis that responses of soil and microbial respiration to climate change may vary with interannual fluctuations in precipitation.

## Materials and methods

### Study site

The experimental site (42°02'N, 116°17'E, 1324 m a.s.l.) is a typical temperate steppe located in Duolun County, Inner Mongolia, China. The mean annual precipitation is 385.5 mm, with approximately 86% occurring from May to September. The mean annual temperature is 2.1 °C, with the minimum and maximum monthly mean temperatures ranging from -17.5 °C in January to 18.9 °C in July. The soil type is chestnut soils (Chinese classification) or Calcic Luvisols according to the FAO classification with 62.75 ± 0.04% sand, 20.30 ± 0.01% silt, and 16.95 ± 0.01% clay, respectively. Soil bulk density and pH values are 1.31 ± 0.02 g cm<sup>-3</sup> and 6.84 ± 0.07, respectively. Soil organic C and total N contents are 16.10 ± 0.89 and 1.48 ± 0.10 g kg<sup>-1</sup>, respectively. The plant community at our experimental site is dominated by *Stipa krylovii* Roshev., *Artemisia frigida* Willd., *Potentilla acaulis* L., *Cleistogenes squarrosa* (Trin.) King, *Allium bidentatum* Fisc. ex prokh., and *Agropyron cristatum* (L.) Gaertn.

### Experimental design and soil sampling and measurements

The experiment used a nested design, with precipitation manipulated at the plot level and warming manipulated at the subplot level. Three blocks of 44 m × 28 m area were randomly selected. In each block, there were two 10 m × 15 m plots, with one assigned as ambient and the other one as increased precipitation treatment. Each 10 m × 15 m plot was divided into four 3 m × 4 m subplots with two warmed subplots and two unwarmed control subplots arranged randomly. The distance between any two subplots was 1 m. Thus, the experimental design consisted of 24 subplots and four treatments (control, warming, increased precipitation, and warming plus increased precipitation) with six replicates for each treatment.

In each of the precipitation treatment plots, six sprinklers were arranged in two rows to cover the 10 m × 15 m area evenly. In July and August, 15 mm of water was added weekly at once to the precipitation

treatment plots. Therefore, a total amount of 120 mm precipitation (approximately 30% of mean annual precipitation in the study site) was supplied in summer each year. In each warmed subplot, one infrared radiator (Kalglo Electronics Inc., Bethlehem, PA, USA) was suspended 2.5 m above the ground to heat the subplot continuously since April 28, 2005. The effect of infrared radiator on soil temperature has been proved to be spatially uniform within the warmed plots (Wan *et al.*, 2002). In the unwarmed control subplot, one 'dummy' heater with the same shape and size as the infrared radiator was suspended to simulate the shading effects of the heater.

Two PVC collars (11 cm in internal diameter and 5 cm in height) were permanently inserted 2–3 cm into the soil at two opposite corners of each subplot for soil respiration measurement. A LI-8100 portable soil CO<sub>2</sub> fluxes system (Li-Cor Inc., Lincoln, NE, USA) was used to measure the soil respiration twice a month in 2005 and 2006 and four times a month in 2007 between 10:00 AM and 12:00 AM (local time) during the growing seasons (May to October). Measurements were taken by setting the LI-8100 chamber on the PVC collars for 1–2 min. The values of two collars in each subplot were averaged as one replicate. Living plants inside the soil collars (if there is any) were removed by hand at least 1 day before the measurement to eliminate aboveground plant respiration. The clipped plant materials were left in the collars in order to include CO<sub>2</sub> release from decomposition of aboveground litter. Soil temperature (°C) at the depth of 10 cm was measured adjacent to each PVC collar using a thermocouple probe (LI-8100-201) connected to the LI-8100 at the same time of soil respiration measurement. Soil moisture (0–10 cm) was measured at two spots in each subplot adjacent to the PVC collars using a portable soil moisture device Diviner 2000 (Sentek Pty Ltd, Balmain, Australia) on the same day of soil respiration measurement.

Soil samples were collected from all the 24 subplots on August 1, 2005, August 5, 2006, and August 3, 2007, respectively. In each subplot, one soil core (15 cm in depth and 8 cm in diameter) was taken to get a fresh sample. After removing roots and stones by sieving with 2 mm mesh, the samples were stored in iceboxes, and subsequently transferred to the laboratory for microbial analysis. Subsamples for each soil sample were separated to measure the gravimetric water content and soil organic C and total N (air-dried, finely ground, and sieved with mesh <250 µm).

### Laboratory analysis for soil samples

The sand, silt, and clay contents of soil were determined by the hydrometer method in the laboratory. Soil organic C and total N were measured by the potassium

dichromate–vitriol oxidation method and Kjeldahl digestion, respectively.

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were estimated using the chloroform fumigation–extraction method (Vance *et al.*, 1987). Briefly, aliquots of the fresh soil (15 g dry weight equivalent for soil microbial biomass) were fumigated for 24 h with ethanol-free  $\text{CHCl}_3$ . Additional aliquots of fresh soil were used as unfumigated controls. Both the fumigated and unfumigated samples were then extracted with 60 mL of 0.5 M  $\text{K}_2\text{SO}_4$  for 30 min on a shaker.  $\text{K}_2\text{SO}_4$  extracts were filtered through 0.45  $\mu\text{m}$  filters and frozen at  $-20^\circ\text{C}$  before analyzing for extractable C and N by Liqui TOC $\alpha$  elemental analyzer (Elementar Liqui TOC, Elementar Co., Hanau, Germany). MBC and MBN were calculated from the differences between extractable C and N contents in the fumigated and the unfumigated samples using conversion factors ( $k_{\text{EC}}$  and  $k_{\text{EN}}$ ) of 0.45 both.

MR was measured by alkali absorption of  $\text{CO}_2$  evolved at  $25^\circ\text{C}$  for 4 days followed by titrating the residual  $\text{OH}^-$  with a standardized acid (Hu & Bruggen, 1997). Briefly, the fresh soil sample (20 g dry weight equivalent) was placed evenly in a 500 mL glass flask. The glass flask was connected to a glass tube (6 cm in diameter), in which 5 mL of 50 mM NaOH solution was injected to capture  $\text{CO}_2$  evolved by the soil in the flask. Then, the soil in the glass flask was incubated at  $25^\circ\text{C}$  in dark for 4 days. All results were expressed on an oven-dried ( $105^\circ\text{C}$ , 24 h) soil basis.

#### Laboratory incubation experiment

In order to further examine whether low water availability constrains the temperature responses of respiratory activities in the temperate steppe, a laboratory incubation experiment was designed to measure soil respiration along a soil moisture gradient under two incubation temperatures. Top soils (15 cm) in a  $1\text{ m} \times 1\text{ m}$  plot out of the fenced experimental site were taken, combined, and mixed completely to get a composite fresh sample. Roots and stones were removed by sieving (2 mm mesh) and then the soil sample was air-dried. The air-dried soil sample was divided into 60 subsamples (equivalent to 20 g oven-dried soil at  $105^\circ\text{C}$ , 24 h), and these subsamples were adjusted to 10 gravimetric-moisture levels ranging from 3% to 18%, respectively. Then, 30 subsamples with three replicates for each moisture level were incubated at  $18^\circ\text{C}$  and the other 30 subsamples were incubated at  $25^\circ\text{C}$  for 4 days to measure MR, respectively.

#### Vegetation sampling

In June 2005, one permanent  $1\text{ m} \times 1\text{ m}$  quadrat was established in each subplot. Plant species composition

in each quadrat was recorded in August at the peak plant biomass from 2005 to 2007 by visually estimating percent cover of each plant species. A  $1\text{ m} \times 1\text{ m}$  frame with 100 equally distributed grids (10 cm  $\times$  10 cm) was put above the canopy in each quadrat during the measurement. The percent cover of each species was recorded in all the grids and summed as the species cover in each quadrat. Total canopy cover was calculated by summarizing the percent cover of all species in the quadrat.

#### Statistical analysis

All data were tested for normal distribution before statistical analysis. Because the measuring frequency was not the same in different years, we first calculated the monthly mean values of soil respiration, and then used the monthly mean values to calculate seasonal mean soil respiration. Repeated-measure analysis of variance (RMANOVA) was used to examine the effects of warming, precipitation, and measuring time on soil respiration using monthly mean values (Table 1). Data of seasonal mean soil respiration (Table 1) and all microbial parameters were analyzed using four-way ANOVA for a blocked nested design to determine the main and interactive effects of year, warming, and increased precipitation (Table 2). Because of the interactions between year and manipulated treatments (warming or increased precipitation) on soil measurable variables, three-way ANOVA was applied to examine the effects of warming and increased precipitation in each year. In all the above analyses, effects of block were examined together with the treatment but not presented or discussed (Tables 1–3). In the laboratory incubation, two-way ANOVA was used to determine the main and interactive effects of temperature and moisture on MR.

**Table 1** *F*-ratios of the effects of increased precipitation (P), warming (W), and measuring time (T) on soil respiration ( $n = 6$ )

	2005	2006	2007	2005–2007
Block (B)	12.59**	45.98***	8.34**	59.48***
Time (T)	593.0***	362.0***	333.6***	218.3***
Precipitation (P)	42.86***	91.91***	241.0***	332.5***
Warming (W)	5.37*	1.20	5.06*	10.27**
T $\times$ P	2.67**	61.23***	48.27***	21.17***
T $\times$ W	1.04	1.27	1.50	0.25
P $\times$ W	0.00	2.64	0.58	2.80
T $\times$ P $\times$ W	1.01	1.53	2.41	0.69

\* $P < 0.05$ . \*\* $P < 0.01$ . \*\*\* $P < 0.001$ .

Repeated-measure ANOVA was used for each of the 3 years and four-way factorial ANOVA was used for the seasonal mean values across all the 3 years.

**Table 2** *F*-ratios of ANOVA of the effects of year (Y), increased precipitation (P), and warming (W) on the measured microbial variables ( $n = 6$ )

	MBC	MBN	MBC/C	MBN/N	MR
Block (B)	10.63***	17.04***	3.83*	2.98	10.92***
Year (Y)	43.76***	48.46***	23.30***	8.72***	4.85*
Precipitation (P)	13.58***	50.15***	14.01***	28.91***	61.18***
Warming (W)	11.58**	43.30***	1.23	26.13***	41.78***
Y × P	0.34	5.30**	0.79	7.37**	6.20**
Y × W	2.82	10.39***	0.42	4.44*	1.19
P × W	0.01	6.54**	0.00	1.84	6.03*
Y × P × W	0.04	1.49	0.40	0.64	4.94*

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

MBC, microbial biomass C; MBN, microbial biomass N; MBC/C, proportion of microbial biomass C in total C; MBN/N, proportion of microbial biomass N in total N; MR, microbial respiration.

**Table 3** Changes (%) in soil respiration (SR) and microbial variables induced by warming and precipitation treatment over time

Parameters	Precipitation-induced changes			Warming-induced changes		
	2005	2006	2007	2005	2006	2007
SR	13.7***	29.3***	62.5***	4.7*	2.9	6.7*
MBC	10.7	14.5*	30.8**	-0.17	-16.6*	-25.4**
MBN	10.5	25.5**	70.8***	-0.48	-25.5***	-39.0***
MBC/C	23.4	10.4	15.7**	0.92	-7.11	-13.8
MBN/N	7.61	13.4*	76.1***	-5.5	-22.1**	-36.4**
MR	12.5*	35.4***	47.3***	-15.0*	-22.1**	-23.0***

See the footnote in Table 2 for the explanations for abbreviations. \*\*\*,and\*\*\* represent  $P < 0.05$ , 0.01, and 0.001, respectively (three-way ANOVA).

Because of the interactions between temperature and moisture, we then used one-way ANOVA to test the effects of temperature on MR at different moisture levels.

To further investigate which variable has the greatest effect on soil microbial biomass and MR, stepwise multiple regressions were conducted, with microbial biomass and MR as the dependent variables, and soil temperature, moisture, soil organic C, total N, and plant cover as the independent variables. All statistical analyses were performed using SAS V.8.1 (SAS Institute Inc., Cary, NC, USA).

## Results

### *Changes in soil temperature and moisture*

There were strong interannual fluctuations in the growing-season precipitation. In comparison with the long-

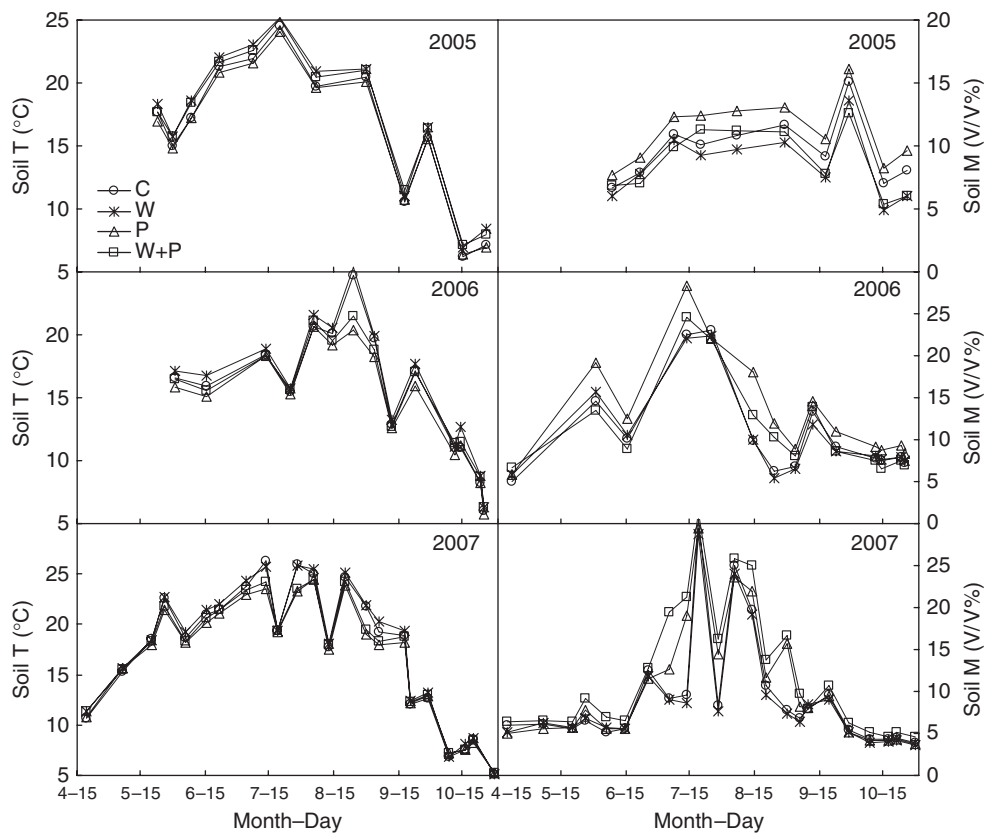
term (1953–2006) mean precipitation from April to October (362.8 mm), April–October precipitation was 13.5% and 44.5% lower in 2005 (313.7 mm) and 2007 (201.4 mm), but 12.1% higher in 2006 (406.7 mm), respectively.

Experimental warming significantly increased soil temperature at the depth of 10 cm by 1.83 (RMANOVA,  $P < 0.001$ ), 1.08 ( $P < 0.001$ ), and 0.58 °C ( $P < 0.05$ ) in 2005, 2006, and 2007, respectively (Fig. 1). Increased precipitation reduced seasonal mean soil temperature by 1.31 ( $P < 0.001$ ) and 1.34 °C ( $P < 0.001$ ) in 2006 and 2007, respectively, while seasonal mean soil temperature was not affected by increased precipitation in 2005 ( $P > 0.05$ ). No interactions of warming × increased precipitation or warming × time were found to affect soil temperature in any of the 3 years ( $P > 0.05$ ). In contrast, there were significant interactive effects of increased precipitation × time on soil temperature in 2006 ( $P < 0.001$ ) and 2007 ( $P < 0.001$ ), but not in 2005 ( $P > 0.05$ ).

Experimental warming reduced soil moisture (0–10 cm), on average, by 3.43 (absolute difference,  $P < 0.01$ ) and 2.22% v/v ( $P < 0.01$ ) in 2005 and 2006, respectively, but had no effects in 2007 ( $P > 0.05$ ). Increased precipitation did not affect soil moisture in 2005 ( $P > 0.05$ ), but significantly increased it by 3.13 ( $P < 0.001$ ) and 4.33% v/v ( $P < 0.001$ ) in 2006 and 2007, respectively. There were interactions of warming × increased precipitation in affecting soil moisture in 2006 ( $P < 0.001$ ) and 2007 ( $P < 0.01$ ). For example, soil moisture in 2006 was 2.60% v/v greater ( $P < 0.01$ ) in the increased precipitation than in the control plots, whereas no differences were found between the warming and the warming plus increased precipitation treatments. Experimental warming did not affect soil moisture under natural precipitation, but decreased it by 2.14% v/v ( $P < 0.05$ ) under increased precipitation. Significant interactive effects of warming × time on soil moisture were observed in 2005 only ( $P < 0.05$ , warming significantly decreased soil moisture in the middle growing season). However, there were interactions of increased precipitation × time in affecting soil moisture in all the 3 years (all  $P < 0.001$ ).

### *Soil respiration*

There was strong intra- and interannual variability in soil respiration, which could be inferred from the significant time (RMANOVA) and year effects (four-way ANOVA; Table 1). Soil respiration was usually greater in the middle growing season and lower in the early and late growing season (Fig. 2). However, extra precipitation (212 mm relative to the long-term mean of 105 mm) and high soil moisture (Fig. 1, middle left panel) in July 2006 not only led to lower soil respiration than expected in late July 2006, but also reduction in respiration under



**Fig. 1** Seasonal dynamics of soil temperature ( $^{\circ}\text{C}$ ) and moisture (% v/v) in 2005, 2006, and 2007. C, control; W, warming; P, increased precipitation; W + P, warming plus increased precipitation. Each data point represents the mean value of the six subplots in each of the four treatments.

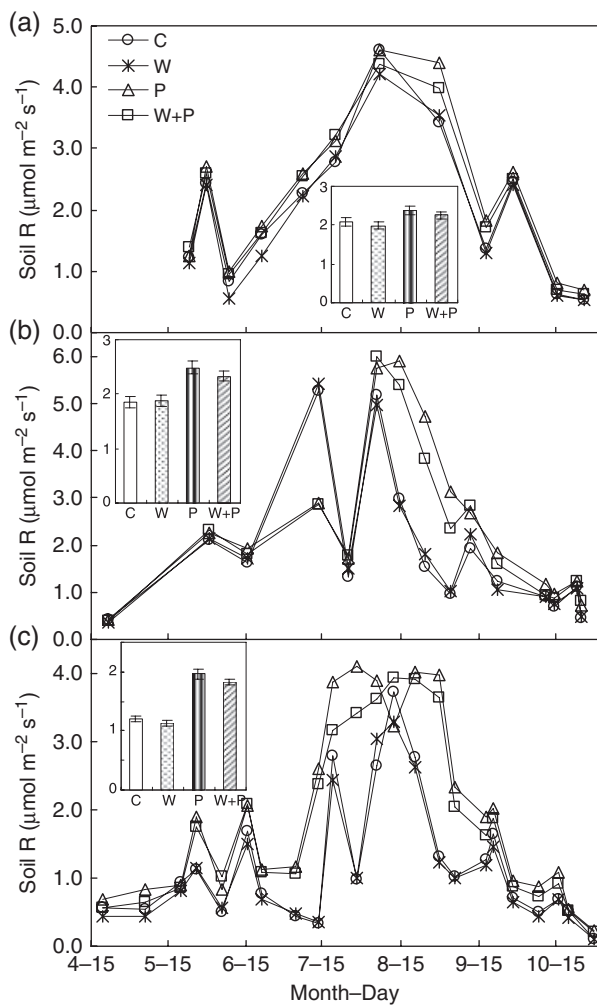
increased precipitation (Fig. 2b). Nevertheless, year-to-year variability in seasonal mean soil respiration was positively related to interannual fluctuations in April–October precipitation. The greatest April–October precipitation in 2006 led to the highest seasonal mean soil respiration in this year ( $2.26 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). In response to the severe drought in the early growing season (April–late July) and the lowest April–October precipitation in 2007, seasonal mean soil respiration was lowest in this year ( $1.53 \mu\text{mol m}^{-2} \text{s}^{-1}$ ).

Across the 3 years, exponential function described well the relationship between soil respiration and soil temperature (all  $P < 0.001$ ), whereas soil moisture content accounted for 34.0–41.4% (all  $P < 0.001$ ) of the seasonal variations in soil respiration with positive linear functions across the four treatments. Soil temperature and moisture together contributed to 65.0–96.1% of the seasonal variations in soil respiration.

Increased precipitation significantly impacted soil respiration in 2005 (RMANOVA,  $P < 0.001$ ), 2006 ( $P < 0.001$ ), and 2007 ( $P < 0.001$ ), respectively. Experimental warming also had significant effects on soil respiration in 2005 ( $P < 0.05$ ) and 2007 ( $P < 0.05$ ), but

not in 2006 ( $P > 0.10$ ). When analyzed across the 3 years using seasonal mean soil respiration (four-way ANOVA), precipitation treatment stimulated soil respiration by 31.6% ( $P < 0.001$ ), whereas experimental warming significantly reduced it by 4.5% ( $P < 0.01$ ). In addition, the effects of increased precipitation on soil respiration varied with time ( $P < 0.001$ ), whereas no other two-way or three-way interactions were found (Table 1). There was an increasing trend of the precipitation-induced stimulation of mean soil respiration in terms of both relative (Table 3) and absolute changes ( $0.56$ ,  $1.09$ , and  $1.46 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The negative effects of warming on soil respiration (Table 3) tended to increase with decreasing precipitation in the growing season.

Across the 24 subplots (all  $n = 24$ ), seasonal mean soil respiration was positively dependent on total plant cover in the middle growing season in 2005 ( $r^2 = 0.26$ ,  $P < 0.05$ ), 2006 ( $r^2 = 0.36$ ,  $P < 0.01$ ), and 2007 ( $r^2 = 0.14$ ,  $P < 0.10$ ). Furthermore, changes in soil respiration induced by increased precipitation ( $r^2 = 0.24$ ,  $P < 0.001$ ) and warming plus increased precipitation ( $r^2 = 0.31$ ,  $P < 0.001$ ) were positively correlated with the alterations in soil moisture.



**Fig. 2** Seasonal dynamics and mean values (inserts, mean SE,  $n = 6$ ) of soil respiration in 2005 (a), 2006 (b), and 2007 (c). C, control; W, warming; P, increased precipitation; W + P, warming plus increased precipitation. Each data point represents the mean value of the six subplots in each of the four treatments.

#### Soil microbial biomass and respiration

There was substantial interannual variability in all the microbial parameters (Table 2). Experimental warming significantly impacted MBC, MBN, and the proportion of microbial biomass N in total N (MBN/N). However, the proportion of microbial biomass C in total C (MBC/C) was not affected by experimental warming (Table 2). When averaged across the 3 years, warming decreased MBC, MBN, and MBN/N by 13.4%, 21.2%, and 21.2%, respectively (Fig. 3). In contrast, increased precipitation significantly stimulated MBC, MBN, MBC/C, and MBN/N by 16.8%, 29.3%, 23.6%, and 26.0%, respectively. In addition, both the negative effects of warming and the positive effects of increased precipitation on these microbial variables increased with time (Table 3).

All the effects of warming, increased precipitation, and their interactions on MR were statistically significant (Table 2). Warming decreased MR, on average, by 20.3%, whereas increased precipitation stimulated it by 31.6% across the 3 years (Fig. 4). Similar to those of MBC and MBN, both the negative responses of MR to warming and the positive responses to increased precipitation displayed a gradual increase over time (Table 3). Warming decreased MR by 15.0% and 24.1% under the ambient and increased precipitation treatments, respectively, whereas increased precipitation enhanced MR by 38.5% and 23.6% in the unwarmed and warmed subplots, respectively. Moreover, three-way interactions of year  $\times$  warming  $\times$  increased precipitation were also observed (Table 2).

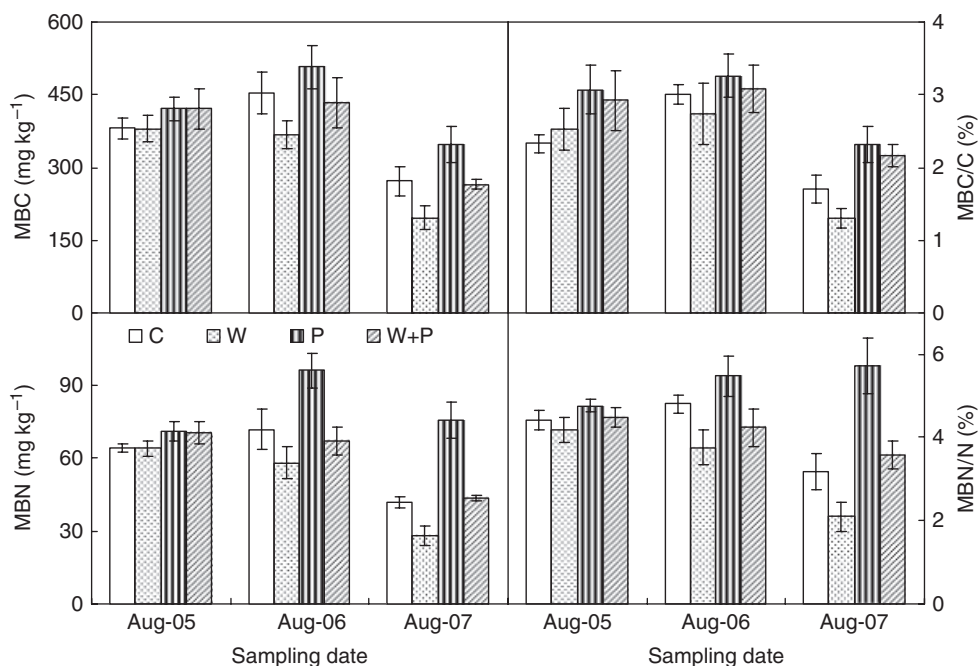
#### Laboratory incubation experiment

The results of laboratory incubation showed significant effects of both temperature and moisture (both  $P < 0.001$ ) on MR. In addition, the two factors strongly interacted with each other to influence MR ( $P < 0.001$ ) (Fig. 5). There was no effect of temperature on MR at the four lower soil moisture levels ( $P > 0.05$ , one-way ANOVA). In contrast, MR was 6.2–19.9% (all  $P < 0.01$ ) greater at 25 °C than at 18 °C at the six higher soil moisture levels. MR generally increased with the increasing soil moisture at both incubation temperatures. The positive effects of soil moisture on MR were stronger under the lower (slope: 3.99 at 25 °C; slope: 3.92 at 18 °C) than under the higher (slope: 2.19 at 25 °C; slope: 2.24 at 18 °C) soil moisture range, suggesting greater water sensitivity of soil MR when soil moisture is limiting.

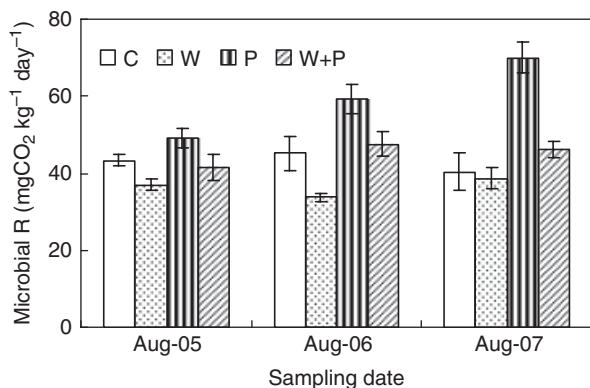
#### Dependency of soil microbial parameters on abiotic factors

Stepwise multiple regression analyses (all  $n = 24$ ) revealed that soil moisture content alone explained 32% of the spatial variation in MR in 2005 across the 24 subplots. Combinations of soil moisture (partial  $r^2 = 0.54$  and 0.47) and temperature in July (partial  $r^2 = 0.12$  and 0.11) accounted for 66% ( $P < 0.001$ ) and 58% ( $P < 0.001$ ) of the MR variations in 2006 and 2007, respectively. MBC exhibited positive linear relationships only with soil moisture content in 2005 (partial  $r^2 = 0.29$ ,  $P < 0.01$ ), 2006 (partial  $r^2 = 0.44$ ,  $P < 0.001$ ), and 2007 (partial  $r^2 = 0.32$ ,  $P < 0.01$ ). Variability in MBN could be ascribed to soil moisture alone in 2005 (partial  $r^2 = 0.43$ ,  $P < 0.001$ ) and 2006 (partial  $r^2 = 0.58$ ,  $P < 0.001$ ) and to soil moisture (partial  $r^2 = 0.46$ ), temperature (partial  $r^2 = 0.20$ ), and total N (partial  $r^2 = 0.07$ ) in 2007 ( $P < 0.001$ ).

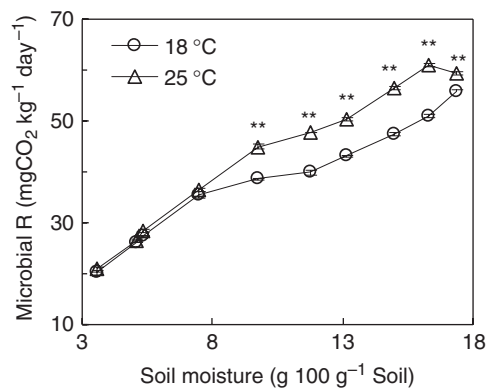




**Fig. 3** Effects of warming and precipitation on soil microbial biomass C (MBC), microbial biomass N (MBN), proportion of microbial biomass C in total C (MBC/C), proportion of microbial biomass N in total N (MBN/N) in 2005, 2006, and 2007, respectively (mean  $\pm$  SE,  $n = 6$ ). C, control; W, warming; P, increased precipitation; W + P, warming plus increased precipitation.



**Fig. 4** Effects of warming and precipitation on soil microbial respiration (MR) in 2005, 2006, and 2007, respectively (mean SE,  $n = 6$ ). C, control; W, warming; P, increased precipitation; W + P, warming plus increased precipitation.



**Fig. 5** Effects of soil moisture on soil microbial respiration at two incubation temperatures (18 and 25 °C) in the laboratory incubation (mean  $\pm$  SE,  $n = 3$ ). Double asterisk (\*\*) denotes statistically significant difference between the two temperature levels under the same soil moisture condition at  $P < 0.01$ .

## Discussion

### *Interannual variability in soil respiration and microbial properties caused by interannual fluctuations in precipitation*

In the present study, we demonstrated strong interannual variations among the measured variables (Tables 1 and 2). The temperate steppe in our study site is located in semiarid region, and water availability is the primary

limiting factor for plant growth and ecosystem productivity (Niu *et al.*, 2008). Therefore, interannual variability in the measured parameters could have been largely attributable to the interannual fluctuations in precipitation. Total April–October precipitation in 2007 was only 64.2% and 49.5% of that in 2005 and 2006, respectively. Consequently, seasonal mean soil respiration in 2007 was 17.3–43.2% and 21.1–39.9% lower than that in 2005 and 2006, respectively, across the four treatments



(Fig. 2). In addition, the microbial parameters also showed substantial interannual variations in response to the year-to-year changes in precipitation (Table 2, Figs 3 and 4). The finding that increasing soil MR along the soil moisture gradient in the laboratory incubation is consistent with the regulation of water availability on these variables observed in the field. Our observations are also in line with previous studies in which annual ecosystem productivity was tightly coupled to annual precipitation and was greater in wet years than in dry years (Hayes & Seastedt, 1987; Sala *et al.*, 1988). Actually in our study, total plant cover in 2007 was 27.0–39.0% and 35.2–46.5% lower than that in 2005 and 2006, respectively, across the four treatments.

#### *Positive effects of increased precipitation on soil respiration and microbial properties*

In our experimental site, significant contributions of soil moisture to the seasonal variability in soil respiration and the spatial variations in microbial parameters strongly suggest controlling of soil water availability over plant and microbial activities in the temperate steppe ecosystem (Zak *et al.*, 1993; Williams *et al.*, 2000). Therefore, as expected and in concordance with the previous studies, increased precipitation stimulated soil respiration (Fig. 2), microbial biomass and respiration (Figs 3 and 4). Thus, these findings support our first hypothesis.

Improved water availability under increased precipitation could have directly stimulated activities of plant roots and soil microbes including extracellular enzymes (Schimel *et al.*, 1999; Davidson & Janssens, 2006), thus enhancing total soil and microbial respiration (Patrick *et al.*, 2007). The direct effect of increased soil moisture on MR can also be accounted for by the increasing MR with soil moisture content in the laboratory incubation (Fig. 5). Moreover, increased precipitation/water availability can also impact soil and microbial respiration through stimulating plant growth and consequent belowground C allocation and C substrate (Zak *et al.*, 1993, 1994). In supporting this speculation, we found that, across the 3 years in this experiment, total plant cover was stimulated, on average, by 19.1% under increased precipitation.

Contrary to the above positive direct and indirect effects of increased precipitation/water availability, one indirect effect of increased precipitation/soil water availability could negatively influence soil and microbial respiration via reducing soil temperature. Higher soil moisture may change the energy balance, leading to more energy dissipated as latent heat (for evapotranspiration) and less as soil heat flux (for soil warming), and thus lower soil temperature (Wan *et al.*, 2007).

Therefore, lower soil temperature may suppress soil and microbial activities and respiration, given that soil moisture is not limiting. Further, our observation that there were negative relationships between the precipitation-induced changes in soil respiration and the changes in soil temperature (data not shown) is consistent with this proposition.

In this study, the positive effects of increased precipitation/water availability were much stronger than the negative indirect effects via reducing soil temperature, and thus led to enhanced total and microbial respiration in the temperate steppe (Figs 2 and 4). In addition, at the ecosystem level, the stimulation of gross ecosystem productivity (GEP) (9.5%, 43.0%, and 75.8% in 2005, 2006, and 2007, respectively; Niu *et al.*, 2008) was greater than the enhancement of soil respiration under increased precipitation. The results imply that increased precipitation has resulted in greater photosynthetic C gains than respiratory C losses, leading to the positive effects of increased precipitation on net ecosystem CO<sub>2</sub> exchanges (Niu *et al.*, 2008).

#### *Effects of long-term increased precipitation*

In the laboratory incubation, we found greater water sensitivity of MR at the lower than at the higher moisture contents (Fig. 5), i.e., given the same magnitudes of changes in water availability, a greater increase in MR when the initial moisture content was low. Therefore, it is expected that the enhancement of total soil and microbial respiration under the increased precipitation is greatest in the driest year 2007 and least in the wettest year 2006. The findings that the positive responses of both soil respiration and microbial variables were greatest in 2007 are in accordance with this expectation. However, we found unexpected results that the smallest increases in total soil and microbial respiration were in 2005 rather than 2006. During the experimental period, the magnitudes of the precipitation-induced stimulation of soil and microbial respiration and microbial biomass increased with year (Table 3). The increasing trend of the changes in soil respiration is consistent with that of soil moisture changes, whereas opposite to that of the changes in soil temperature under increased precipitation. The findings indicate that water-mediated responses of soil respiratory processes to changing precipitation occur in the semi-arid temperate steppe.

#### *Negative effects of warming on soil respiration and microbial properties*

The findings that there were significant reductions in all soil respiration, microbial biomass and respiration

induced by experimental warming irrespective of the precipitation regimes (Figs 2–4) verify our second hypothesis. Our results are different from those in various terrestrial ecosystems (Rustad *et al.*, 2001; Melillo *et al.*, 2002; Emmett *et al.*, 2004; Wan *et al.*, 2005; Zhou *et al.*, 2007) where soil respiration was stimulated by warming. Nevertheless, reduced soil and microbial respiration have been reported in an alpine meadow (Saleska *et al.*, 1999), in some season in a tallgrass prairie (Verburg *et al.*, 2005) and in some season in an old-field grassland (Wan *et al.*, 2007).

The direct effect of elevated temperature is usually expected to be a stimulation of ecological processes. However, variations in the initial conditions of other factors may confound the temperature effects on terrestrial ecosystems (Shaver *et al.*, 2000; Peñuelas *et al.*, 2004; Wan *et al.*, 2007). The positive responses to elevated temperature of soil respiration, litter and soil organic matter decomposition, microbial biomass and respiration (MacDonald *et al.*, 1995, 1999; Parton *et al.*, 1995; Rustad *et al.*, 2001; Melillo *et al.*, 2002; Rinnan *et al.*, 2007a), and plant biomass growth, root deposition and activity (Fitter *et al.*, 1999; Rustad *et al.*, 2001; Peñuelas *et al.*, 2004) have been reported in ecosystems where water is not limiting. In contrast, when soil moisture is low, and thus constitutes the primary limiting factor, increased temperature may not result in a stimulation of microbial processes (Davidson *et al.*, 2000a; Peñuelas *et al.*, 2004; Sowerby *et al.*, 2005). The insignificant changes in MR under elevated temperature at lower soil moisture contents in our laboratory incubation (Fig. 5) can be accounted for by the above speculation.

Elevated temperature also influences soil and microbial respiration indirectly (Shaver *et al.*, 2000; Norby & Luo, 2004; Wan *et al.*, 2005, 2007). For example, increased evapotranspiration under warming leads to reduced soil water availability (Harte *et al.*, 1995; Wan *et al.*, 2002; Niu *et al.*, 2008). Lower soil moisture and exacerbated water limitation (Fig. 1), on the one hand, can suppress plant root and microbial activities and respiration, especially in arid and semiarid regions. On the other hand, reduced soil moisture can also constrain plant growth and belowground C allocation as well as decomposition of plant litter and soil organic matter (Davidson & Janssens, 2006). As a consequence, this would result in reduced supply of C substrate for roots and microorganisms, leading to decreased respiration. In the same experiment, our previous study revealed that plant photosynthesis and GEP were reduced by experimental warming (Niu *et al.*, 2008). In addition, soil respiration showed positive linear dependence on GEP ( $r^2 = 0.53, 0.71, \text{ and } 0.72$  in 2005, 2006, and 2007, all  $P < 0.001$ ) across the 24 subplots. Consistent with our observations, a slight decrease in

plant biomass by warming in Spain has also been attributed to increased water loss in the water-stress site (Peñuelas *et al.*, 2004). However, when comparing with the warming effects on GEP and soil respiration, the reductions in GEP (8.4%, 5.1%, and 15.3% in 2005, 2006, and 2007, respectively) were greater than those in soil respiration (Table 3). This implies that warming has caused greater respiratory C losses relative to photosynthetic C gains, thus leading to a negative impact on net ecosystem exchange in the semiarid temperate steppe (Niu *et al.*, 2008).

In this semiarid temperate steppe ecosystem, the indirect warming effects via reducing water availability and plant growth were stronger than the direct stimulating effects of elevated temperature. As a consequence, negative responses of soil and microbial respiration to warming occurred (Saleska *et al.*, 1999; Shaver *et al.*, 2000). These results are in agreement with our second hypothesis. The exacerbating negative impacts of experimental warming on microbial parameters over time (Table 3) suggest an accumulative effect of experimental warming on soil microorganisms.

## Conclusions

In the semiarid temperature steppe in northern China, strong interannual variations in total soil respiration and microbial biomass and respiration with annual precipitation and their positive responses to increased precipitation indicate that water availability is involved in regulation of soil respiratory processes (Davidson *et al.*, 2000b). Negative responses of total soil respiration, MR, and microbial biomass to experimental warming could have been attributed to the offset or reverse of the direct positive effects by the indirect negative effects via aggravating water stress and suppressing plant growth. Increasing trends of the positive effects of precipitation treatment and the negative effects of warming could be accounted for by the year-to-year changes in soil temperature and moisture induced by the respective treatment, but not by the interannual variability in growing-season precipitation amount. Our observations indicate that increased precipitation is relatively a much stronger driving factor than warming in arid and semiarid regions. Unless there is concurrent increase in precipitation, the temperate steppe in the arid and semiarid regions of northern China may act as a net C source under climate warming.

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