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ORIGINAL PAPER

Precipitation regime drives warming responses of microbial biomass and activity in temperate steppe soils

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Abstract Although numerous warming experiments have examined the impacts of elevated temperature on soil microbial activities, most are based on responses from a single site. To investigate how precipitation regime regulates warming effects on the carbon cycle, we conducted manipulative warming experiments in desert steppe, typical steppe, and meadow steppe along a precipitation gradient in northern China. Soil temperature, moisture, microbial biomass C (MBC), N (MBN), and microbial respiration were measured from 2006 to 2009. Soil moisture was significantly reduced by warming in the typical steppe but not affected in the desert and meadow steppe. Across the 4 years, warming decreased MBC and microbial respiration in the desert and typical steppe but not in the meadow steppe. The magnitude of warming-induced reductions in MBC and microbial respiration declined as site precipitation increased. Across the three sites, the changes in soil MBC, MBN, and microbial respiration were all positively correlated with annual precipitation and changes in belowground net primary productivity. Our results suggest that precipitation regime controls the response of soil microbial activity and biomass to warming, possibly by regulating soil moisture and substrate availability. With increasing precipitation, the stimulatory effects of warming on soil microbial activity and biomass outweigh the inhibitory effects due to declining soil moisture.

Keywords Drought · Elevated temperature · Grassland · Microbial activities · Precipitation · Soil moisture · Substrate availability

Introduction

Soil microbes exert a strong influence on carbon cycling in terrestrial ecosystems via decomposition of plant litter and soil organic matter (Bardgett et al. 2008; Wardle et al. 2004; Zhou et al. 2012). Consequently, the responses of soil microbes to elevated temperature have impacts on the responses of soil C inputs and feedbacks to warming (Allison et al. 2010). A better understanding of how soil microbes respond to elevated temperature would therefore facilitate predictions of soil C cycling under climate warming.

Elevated temperature directly influences soil C turnover (Melillo et al. 2002) and soil microbial growth and activity (Blagodatskaya et al. 2014; Natali et al. 2012) in both lab incubations and field experiments. Warming can affect soil microbial activities by altering both soil temperature and moisture (Carlyle et al. 2011) as well as biotic processes such as plant production, litter input, root growth, and root exudates (Hobbie and Chapin 1998; Natali et al. 2012; Yin et al. 2013).

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Soil microbial activity and biomass responses to warming have been investigated in numerous field manipulations across various terrestrial ecosystems with both increases and declines observed. For example, elevated temperature increased soil microbial biomass or respiration in an old field (Bell et al. 2010), Arctic tundra (Sistla and Schimel 2013), temperate heathland (Haugwitz et al. 2014), and in some incubation experiments (Blagodatskaya et al. 2014; Menichetti et al. 2015) by enhancing microbial metabolic rates or by extending the plant growing season. Conversely, warming reduced soil microbial activities by amplifying soil drying in a semiarid temperate steppe (Liu et al. 2009). In a tallgrass prairie, soil microbial activity showed no response to warming despite an increased fungal to bacterial ratio (Zhang et al. 2005). Given these different responses across sites, coordinated manipulative warming experiments along environmental gradients could help reveal the mechanisms driving soil microbial responses to warming at a regional scale (Fraser et al. 2013).

In the temperate steppe of northern China, soil moisture is a primary factor controlling plant growth and soil microbial activities (Xia et al. 2009). Warming-induced changes in soil moisture negatively affected soil microbial activities in a typical steppe ecosystem (Liu et al. 2009). To investigate warming effects on activity and biomass of soil microbial communities and the underlying mechanisms at a broader regional scale, warming manipulations have been conducted at three sites along a precipitation gradient in this region since 2006 (Zhang et al. 2015). Given that soil microbial activities are often driven by soil moisture rather than temperature as moisture declines (Liu et al. 2009; Shaver et al. 2000), we hypothesized that warming would inhibit microbial activity by increasing soil drought stress in the gradient sites with lower precipitation (Xia et al. 2009). In contrast, we expected warming to increase microbial activity in the meadow steppe site with the highest rainfall. We also hypothesized that warming effects on microbial activity would depend on interannual variation in precipitation, with greater magnitude of warming effects in wetter years due to the moisture limitation being removed. We further examined the mechanisms underlying these hypotheses by correlating microbial responses with metrics of net primary production at the gradient sites.

Materials and methods

Study sites and experimental design

The manipulative warming experiment was conducted in three steppe ecosystems spanning a natural precipitation gradient in Northern China. Two sites are located in Siziwang Banner (desert steppe) and Duolun County (typical steppe) in Inner Mongolia. The third (meadow steppe) site is located in Changling County in Jilin Province. Site characteristics are listed in Table 1, and annual precipitation is shown in Fig. 1a.

The warming experiment was established in the desert, typical, and meadow steppe sites in April 2006. This study was a part of large experiment that included warming and N manipulation in a randomized block design. We used the experiment to test the effect of warming treatments on microbial biomass and activity at a regional scale. At each site, we analyzed 12 plots, with 6 randomly assigned to the warming treatment and the other 6 randomly assigned as controls. In each warming plot, one MSR-2420 infrared radiator (Kalglo Electronics Inc., Bethlehem, PA, USA) was suspended 2.25 m above the ground starting in April 2006. To stimulate the shading effects of the infrared radiator, a "dummy" heater with the same shape and size as the infrared heater was also suspended 2.25 m high in each warming control plot. Plots were 2×3 m and located within a 667-m^2 area at each site.

Soil samples (12 total per site) were collected from warming and control plots once per year in August from 2006 to 2009 (samples were not collected from meadow steppe in 2007). Sampling was timed to coincide with maximum aboveground biomass in each site. In each plot, three soil cores (15 cm depth and 5 cm diameter) were taken randomly and combined into a composite sample. After removing roots and stones by sieving (2 mm mesh), the samples were stored on ice and transferred to the lab for dissolved organic carbon (DOC), inorganic N concentration, and microbial analyses. Subsamples were taken to measure gravimetric water content and soil chemical properties (air-dried, finely ground, and sieved to <250 μm).

Measurements

Soil temperature (^{o}C) at 10 cm depth was measured using a thermocouple probe (Li-8100-201) once per week over the growing season. Gravimetric soil moisture was measured using fresh soil samples once each year in August. Soil inorganic N (NH_4^+-N and NO_3^--N) was extracted with 2 M KCl solution, and concentrations of NH_4^+-N and NO_3^--N in the extracts were measured using a flow injection analyzer (SAN-System, Netherlands). Soil pH values were determined with a combination glass electrode (soil to water *w*/*v* ratio 1:2.5).

Soil microbial biomass C (MBC) and microbial biomass N (MBN) were estimated using the chloroform fumigation-extraction method (Vance et al. 1987). Briefly, fresh soil samples were transferred to the lab, and field moist samples (15 g dry weight equivalent) were fumigated for 24 h with ethanol-free $CHCl_3$. Fumigated and unfumigated samples were extracted with 60 ml 0.5 M K_2SO_4 for 30 min on a shaker. K_2SO_4 extracts were filtered through 0.45-μm filters and frozen at −20 °C prior to analyzing extractable C and N by an elemental analyzer (liqui TOC, Analysensysteme, Germany).

Table 1 Mean annual temperature (MAT), precipitation (MAP), soil properties and the dominant plant species in the three sites, respectively

Sites	Coordinates	MAT (°C)	MAP (mm)	Soil type	Sand content (%)	Soil C (g kg^{-1})	Soil N (g kg^{-1})	C:N	Soil pH	DOC (mg kg^{-1})	Inorganic N (mg kg^{-1})	Dominant plant species
Desert steppe	41° 47′ N 111° 53′ E 1456 m a.s.l.	3.4	183	Chestnet (China) Kastanozem (FAO)	71.6 ± 0.07	11 ± 0.7	1.13 ± 0.07	9.3 ± 0.4	8 ± 0.04	56.6 ± 4.1	14.4 ± 1.8	*Stipa breviflora* Griseb. *Artemisia frigida* Willd. *Cleistogenes songorica* (Roshev.) Ohwi.
Typical steppe	42° 02′ N 116° 17′ E 1324 m a.s.l.	2.1	385	Chestnet (China) Haplic calcisols (FAO)	62.9 ± 0.04	14.1 ± 0.8	1.45 ± 0.05	9.7 ± 0.5	7.2 ± 0.08	54.7 ± 4.3	18.3 ± 1.8	*Stipa krylovii* Roshev. *Artemisia frigida* Willd. *Potentilla acaulis* L.
Meadow steppe	44° 45′ N 123° 45′ E 160 m a.s.l.	6.4	471	Chernozem (China) Chernozem (FAO)	57.3 ± 0.06	9.1 ± 0.8	0.95 ± 0.09	10 ± 0.6	9.3 ± 0.18	44.2 ± 3.5	9.7 ± 0.5	*Leymus chinensis* (Trin.) Tzvel. *Chloris virgate* Sw. *Carex duriuscula* C. A. Mey.

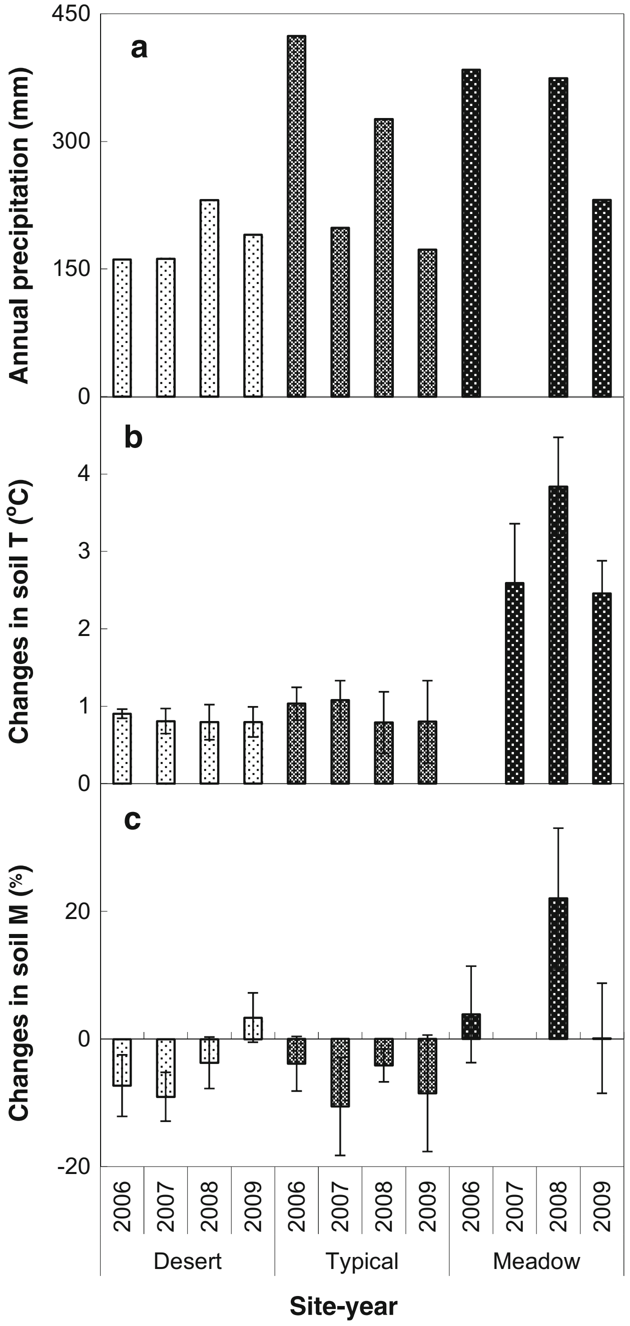


Fig. 1 Annual precipitation (**a**), warming-induced changes in soil temperature (T) (**b**) and soil moisture (M) (**c**) in the desert, typical, and meadow steppes in 2006, 2007, 2008, and 2009. Each column represents the mean (±SE, *n* = 6) difference in soil temperature and moisture between the control and warming treatments

MBC and MBN were calculated from the difference between extractable C or N content in the fumigated versus the unfumigated samples using a conversion factor of 0.45. DOC was measured as C in the unfumigated soil extracts.

Microbial respiration was measured by alkali absorption of CO_2 evolved at 25 °C for 14 days, followed by titration of the residual OH^- with a standardized acid (Hu and vanBruggen 1997). Soil samples (20 g oven-dried equivalent,) were placed in a 500-ml glass flask connected to a glass tube (6 cm in diameter) in which 5 ml 0.05 M NaOH solutions were injected to capture evolved CO_2. All results are expressed on an oven-dried (105 °C, 24 h) soil basis.

Aboveground net primary production (ANPP) was estimated by clipping living biomass at the end of August each year from 2006 to 2009. All living plant tissues were harvested from a 1 × 1-m quadrat in each plot, and surface litter in the same quadrat was also collected. All plant samples, including litter, were oven-dried at 70 °C for 48 h and weighed to determine biomass.

Belowground net primary productivity (BNPP) was estimated with the root in-growth method. In early May of each year, we excavated two 40-cm-deep cylindrical holes using an 8-cm diameter soil core sampler in each plot. The soils were replaced in the same hole after removing roots via 2-mm sieves. We collected root in-growth samples in late October by using a 5-cm diameter soil core sampler at the center of the original root ingrowth holes. The dry mass of roots was determined by oven-drying at 70 °C to constant weight.

Statistical analysis

Soil temperature was averaged to calculate a mean value for each year. To improve data normality (based on the Shapiro-Wilk test), soil M, MBN, and microbial respiration were log-transformed. A three-way analysis of variance (ANOVA) was used to examine the effects of site, year, and warming treatment on soil temperature, moisture, and microbial parameters (Table 2). Likelihood ratio tests showed that adding block as a random factor did not improve the ANOVA model, so we omitted it. Due to the presence of significant interactions between site and warming treatment, we also used two-way ANOVAs within sites to determine the effects of year, warming, and their interaction on the response variables (Table 3). We used analysis of covariance with soil moisture as the covariate and site and warming treatment as main effects to test for soil moisture effects on microbial parameters.

Table 2 Results of three-way analysis of variance on the effects of warming (W), site (S), year (Y), and their interactions on soil microclimate and microbial parameters

Source	*df*	Soil T	Soil M	MBC	MBN	MR
Y	3, 110	<0.001	<0.001	<0.001	<0.001	<0.001
S	2, 110	<0.001	<0.001	<0.001	<0.001	<0.001
W	1, 110	<0.001	<0.001	0.118	<0.001	0.026
Y × S	5, 110	<0.001	<0.001	1	0.066	1
Y × W	3, 110	0.162	0.259	0.596	0.061	0.157
S × W	2, 110	<0.001	<0.001	0.006	0.003	0.009
Y × S × W	5, 110	0.833	0.635	0.694	1	1

Data are *P* values

Soil T soil temperature, *Soil M* soil moisture, *MBC* microbial biomass C, *MBN* microbial biomass N, *MR* microbial respiration

Table 3 Results of two-way analysis of variance on the effects of warming (W), year (Y), and their interaction on soil microclimate and microbial parameters

Sites	Source	*df*	Soil T	Soil M	MBC	MBN	MR
Desert	Y	3, 40	<0.001	<0.001	<0.001	<0.001	<0.001
	W	1, 40	<0.001	0.161	<0.001	<0.001	0.023
	Y × W	3, 40	0.959	0.284	0.521	0.187	0.895
Typical	Y	3, 40	<0.001	<0.001	<0.001	<0.001	0.045
	W	1, 40	<0.001	0.064	0.024	0.015	0.018
	Y × W	3, 40	0.918	0.855	0.97	0.746	0.384
Meadow	Y	2, 30	0.017	<0.001	<0.001	<0.001	<0.001
	W	1, 30	<0.001	0.242	0.18	0.488	0.091
	Y × W	2, 30	0.246	0.128	0.404	0.718	0.760

Data are *P* values

Soil T soil temperature, *Soil M* soil moisture, *MBC* microbial biomass C, *MBN* microbial biomass N, *MR* microbial respiration

Stepwise multiple regressions and simple linear regressions were used to test for the relationships between warming-induced changes in soil microbial properties, warming-induced changes in BNPP, and annual precipitation. These regressions were conducted on annual mean differences calculated for each of the sites. Data on changes in belowground net primary productivity were provided by the three field experimental stations where the manipulative experiments were set up. We used a similar regression approach to test for relationships among the raw microbial data (rather than warming-induced changes), precipitation, and ANPP. Significance was accepted at the $P < 0.05$ level of probability. All statistical analyses were performed with SAS software (SAS Institute Inc., Cary, NC, USA).

Results

Treatment-induced changes in soil temperature and moisture differed among the three sites (Table 2, significant site × warming interactions). Averaged across the 4 years, experimental warming significantly increased soil temperature by 0.83, 0.92, and 2.96 °C (all $P < 0.001$, *t* test) in the desert, typical, and meadow steppes, respectively (Table 3; Fig. 1b). In response to warming, soil moisture declined by 4.32 % in the desert steppe and 6.43 % in the typical steppe but increased by 9.39 % in the meadow steppe (Fig. 1c), although only the change in the typical steppe was significant ($P = 0.02$, *t* test). No interactions between warming and year were found to affect either soil temperature or soil moisture in any of the sites.

There was significant variability in soil MBC, MBN, and microbial respiration across the three sites (Table 2, $P < 0.001$ for site effects). Warming effects on these variables also varied significantly by site (Table 2, $P < 0.01$ for site × warming interactions). Averaged over the 4 years in the desert steppe (Table 3), warming reduced MBC (Fig. 2a), MBN (Fig. 2b), and microbial respiration (Fig. 3a) by 17.3 ($P < 0.001$), 20.1 ($P < 0.001$), and 13.5 % ($P < 0.05$), respectively. These values also declined under warming in the typical steppe by 10.3, 12.6, and 9.3 % (all $P < 0.05$, Figs. 2c, d, 3b), respectively. There was no warming effect on soil MBC (Fig. 2e), MBN (Fig. 2f), or microbial respiration (Fig. 3c) in the meadow steppe. All microbial parameters differed significantly by year ($P < 0.001$, Table 2), likely resulting from interannual differences in precipitation (Fig. 1) and their effects on soil moisture (Fig. 4). Averaged across sites and years, all microbial parameters increased with increasing soil moisture ($P < 0.001$, ANCOVA).

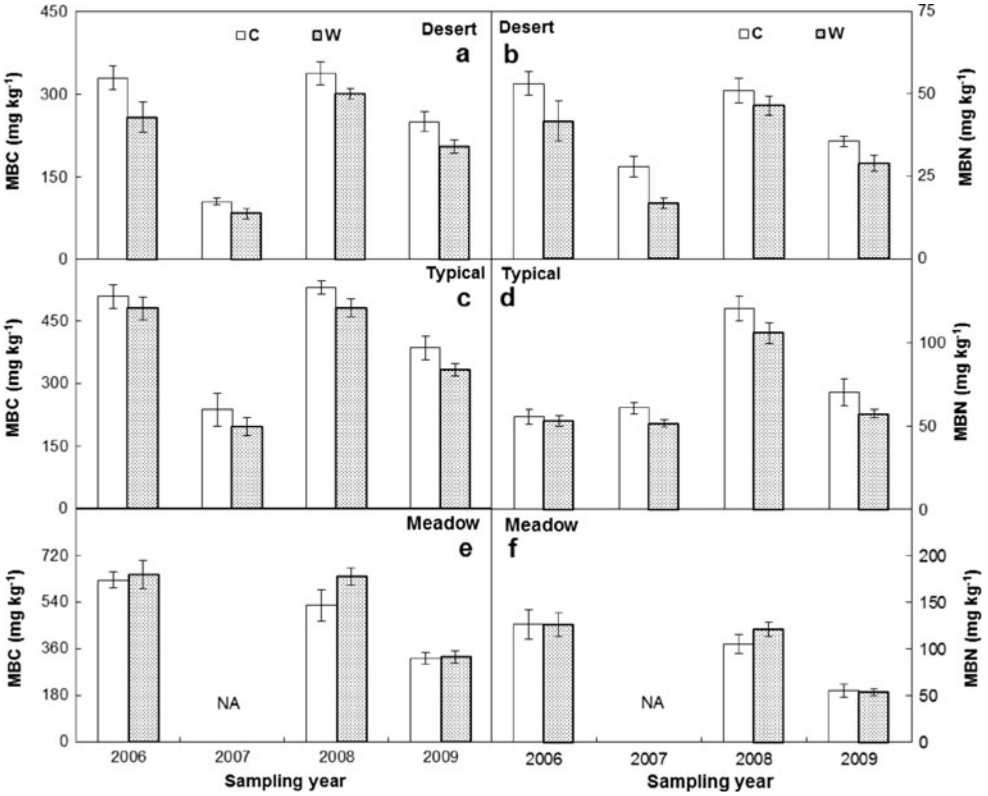
Simple regression analyses showed that precipitation explained 53 % of the variation in mean MBC change with warming ($n = 11$, Fig. 5a); only precipitation was retained in a stepwise multiple regression that also included change in belowground net primary productivity ($n = 8$). For mean MBN and microbial respiration change with warming, only change in belowground net primary productivity was retained in the multiple regressions, and it explained 69 and 68 % of the variation, respectively ($n = 8$, Fig. 5d, f). For the absolute amount of MBC, precipitation explained 65 % of the variance and was the only variable retained in a multiple regression that also included ANPP ($n = 132$, Fig. S1a). For the absolute amount of MBN and microbial respiration, ANPP explained 52 and 53 % of the variance, respectively, and was the only variable retained in the multiple regressions ($n = 132$, Fig. S1d, S1f).

Discussion

Warming effects on soil microbial activity depend on site characteristics

In partial agreement with our first hypothesis, we found that reductions in both MBC and MBN with warming were evident at lower precipitation but attenuated with increasing site precipitation across the three steppes (Figs. 1 and 2). We had originally hypothesized that warming would stimulate microbial activity at our wettest site, but instead, there was no significant effect. It is possible that high sand content in our steppe soils limits the effects of higher moisture and the potential for respiration response to warming (Moyano et al. 2012). Differences in microbial community composition (i.e., fungal to bacterial ratios) across the sites could also cause

Fig. 2 The effects of warming on (**a**, **c**, **e**) soil microbial C (MBC), and (**b**, **d**, **f**) N (MBN) in the desert, typical, and meadow steppes in 2006, 2007, 2008, and 2009. Each column represents the means (±SE, $n = 6$) under the control and warming treatments



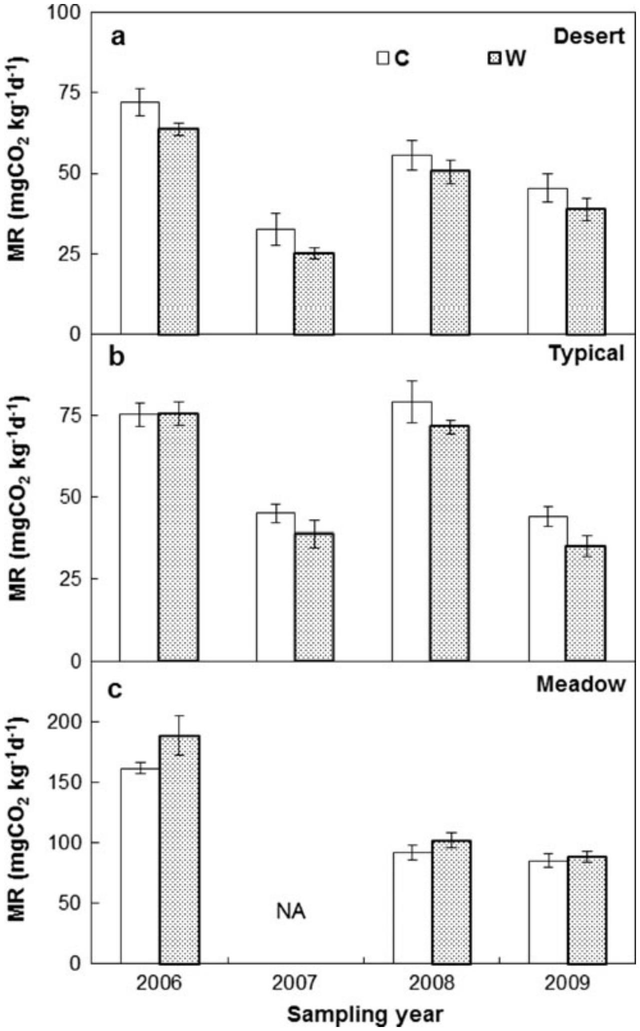


Fig. 3 The effects of warming on soil microbial respiration (MR) in the desert (**a**), typical (**b**), and meadow (**c**) steppes in 2006, 2007, 2008, and 2009. Each column represents the means (±SE, $n = 6$) under the control and warming treatments

differences in warming responses of microbial activity, although the microbial differences are not consistent from year to year (Zhang et al. 2015).

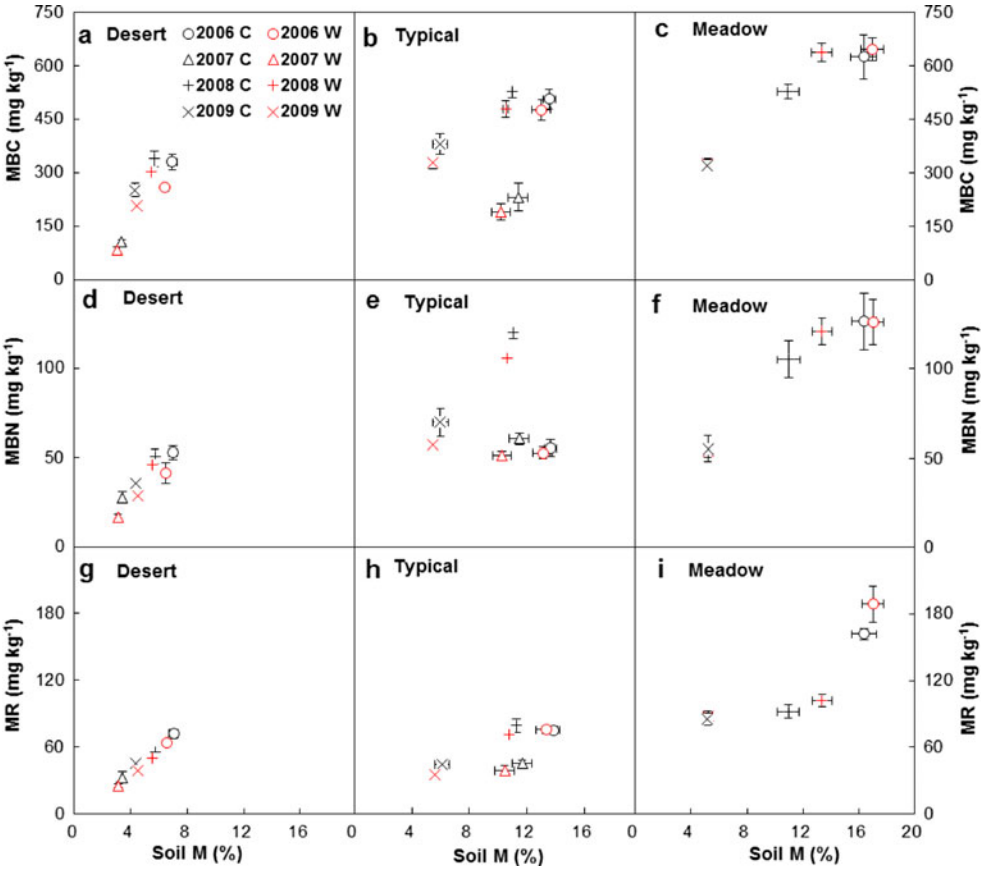
Still, our results are consistent with soil moisture as a controlling factor for soil microbial responses to elevated temperature (Garten et al. 2009). Warming has been found to decrease soil microbial activities due to declines in soil moisture in semiarid (Liu et al. 2009) and semidesert ecosystems (Sharp et al. 2013). A previous study at our sites found that most microbial groups (defined by phospholipid fatty acids) responded negatively to warming in a drier year (2007) but positively in a wetter year (2006) (Zhang et al. 2015). The desert and typical temperate steppe ecosystems regularly experience drought because of the low annual precipitation levels of 180 and 385 mm, respectively. Also, the relationship between soil moisture and microbial parameters is stronger in the desert steppe than in the meadow and especially in the typical steppe (Fig. 4). The slight but significant decreases in soil moisture indicate that warming can exacerbate drought and induce substantial negative effects on microbial physiology (Allison et al. 2010; Schimel et al. 2007).

Warming-induced drought can also influence soil microbial activities through indirect abiotic or biotic factors. For example, drought reduces water-filled pore space in soil, facilitates physical and chemical protection of organic matter, and decreases substrate diffusivity, thereby dampening positive microbial responses to warming (Davidson and Janssens 2006; Moyano et al. 2013). Moreover, the changes in plant growth resulting from warming have impacts on soil microbial activities via substrate availability. In contrast to the stimulation of warming on plant growth and belowground C inputs under warming in tundra ecosystems (Natali et al. 2012), warming-induced drought decreased belowground net primary productivity by 18 % (Fig. S2, $P < 0.05$) in the desert steppe and thus reduced substrate availability. These results support our first hypothesis that soil microbial activity would be suppressed due to exacerbation of drought in the desert and typical steppe.

Long-term drought could select for increased drought tolerance of soil microbial communities (Schimel et al. 2007). Hence, the inhibitory effects of elevated temperature-induced drought on soil microbial biomass and activity should be weaker in the desert steppe than in the typical steppe. However, we found evidence for greater negative effects of increasing soil temperature on soil MBN in the desert steppe ($P < 0.001$ for site × temperature interaction), potentially due to increased competition for N between plants and soil microbes under these conditions. This result suggests that desert steppe microbes are not more tolerant of warming and drought (Fig. S3). One possible explanation is that changes in soil water repellency may have contributed to greater declines in soil microbial variables in the desert steppe as in other semiarid regions (Doerr et al. 2000; Goebel et al. 2011). Initial reductions in surface soil moisture under warming may have increased soil water repellency and reduced infiltration capacity with negative consequences for microbial respiration (Goebel et al. 2011). This effect may have been more pronounced in the desert soils because they contain more sand (71.6 ± 0.07 versus 62.9 ± 0.04 % in the typical steppe and 57.3 ± 0.06 % in the meadow steppe), and sandy soils are more susceptible to increasing water repellency with drying (Doerr et al. 2000).

Our data show that the warming treatment was more effective in heating the soils in the meadow steppe than in the other two sites. Greater soil moisture in the meadow steppe might have conducted surface heat to depth more efficiently compared with the drier soils in the desert and typical steppes. Still, this larger soil warming effect was not associated with significant changes in microbial activity in the meadow steppe, despite ample moisture availability.

Fig. 4 Soil microbial variables in response to soil moisture in the desert, typical, and meadow steppe



Dependence of microbial warming responses on interannual precipitation variability

Consistent with our second hypothesis, we found that responses of microbial activity and biomass to warming depended on annual precipitation within sites (Fig. 5). Not only were warming effects on microbial activity greater in wetter sites, but they were also greater in wetter years. In addition to affecting the warming response, interannual variation in precipitation affects absolute microbial activity (Fig. S1) (Yan and Marschner 2014) and ANPP, with greater values in wetter years.

The interannual variation in precipitation may have affected microbial responses to warming directly via interannual variation in soil moisture (Fig. 4) (Moyano et al. 2013) or indirectly via increasing belowground C resource availability (Kaiser et al. 2010; Koranda et al. 2013). This interannual variability of warming effects, to some degree, is similar to a study in California annual grassland where soil microbial responses to climate manipulation were affected by the level of stress imposed by interannual variation in climate (Gutknecht et al. 2012). Similarly, other ecosystems have also shown distinct seasonal responses of soil microbial activities to warming because of soil moisture variation (Rinnan et al. 2009; Suseela and Dukes 2013).

Mechanisms of microbial response to warming manipulation

Warming-induced changes in soil moisture may also affect primary production with indirect consequences for microbial activity. Our multiple regression results showed a positive dependence of soil MBN and microbial respiration on ANPP (Fig. S1), implying that greater moisture availability may stimulate microbial activity through greater plant growth and associated soil inputs of carbon and nutrients (Kaiser et al. 2010). However, greater inputs in the more productive meadow steppe did not increase the DOC pool there (Table 1) probably because the greater microbial respiration in the meadow steppe resulted in a greater consumption of labile C (Sistla et al. 2013). Since root exudates and litter are the most important source of easily assimilable C for soil microbes (Brant et al. 2006), changes in belowground carbon inputs under warming could also impact soil microbial activity (Bardgett et al. 2008; Scott-Denton et al. 2006).

Consistent with this idea, we observed positive correlations between changes in soil microbial respiration and MBN and changes in belowground net primary productivity (Fig. 5). Although the change in BNPP was

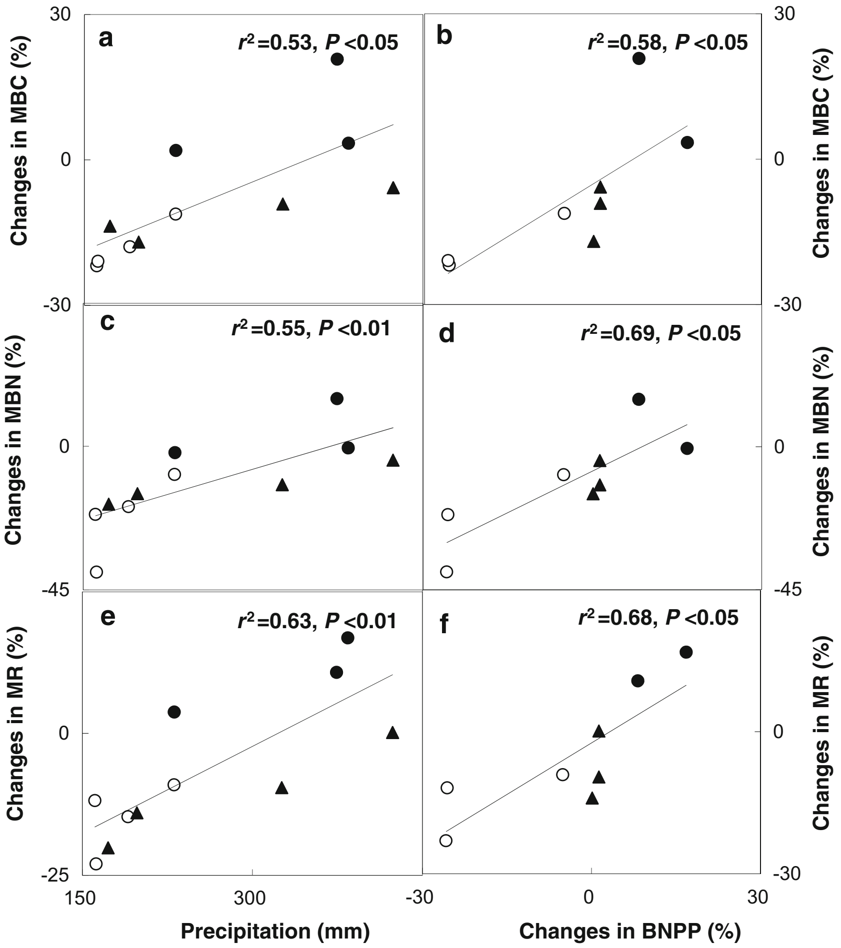


Fig. 5 Simple linear regressions for mean warming-induced changes in soil microbial biomass C (MBC), changes in soil microbial biomass N (MBN), and changes in soil microbial respiration (MR) with annual precipitation (**a**, **c**, and **e**, $n = 11$) and with mean changes in belowground net primary productivity (BNPP) (**b**, **d**, and **f**, $n = 8$) across the three sites from 2006 to 2009. Belowground net primary productivity data were available in the desert and typical steppes from 2006 to 2008 and in the meadow steppe in 2006 and 2008. The *open circles*, *solid triangles*, and *solid circles* represent desert, typical, and meadow steppe, respectively

not a statistically significant driver of the change in MBC according to stepwise multiple regression, there was a clear positive relationship between the change in MBC and the change in BNPP. With our small sample sizes, it is difficult to disentangle the drivers of microbial biomass through multiple regressions, leading us to conclude that both precipitation and changes in BNPP may be important. Overall, our study suggests the potential for warming to affect microbial biomass and activity directly through temperature change and indirectly through changes in plant productivity.

Conclusions

Warming has direct and indirect effects on soil microbial biomass and respiration with implications for C cycling in terrestrial ecosystems. Here, warming effects on soil microbial biomass and respiration were regulated by variation in precipitation across sites and years. In drier sites and years, warming reduced soil microbial activity by reducing soil moisture. With increasing precipitation along our gradient, the inhibitory effects of warming diminished or reversed as soil moisture increased. Our study indicates that predictions of ecosystem C response to warming should account for spatial and temporal variation in precipitation and resulting effects on soil moisture. Temperature and precipitation constraints should be combined to evaluate warming effects on ecosystems in future C cycle models.

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