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Responses of soil microbial respiration to thermal stress in alpine steppe on the Tibetan plateau

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Summary

Extensive research has focused on the temperature sensitivity of microbial respiration in tundra and alpine meadows. However, the response of microbial respiration to thermal stress in alpine steppe soils, which have less organic matter and greater aeration, has received less attention. We investigated spatial and temporal variations in microbial respiration using an incubation experiment under different temperature (0, 15 and 30°C) and different percentages of water-holding capacity (WHC) (50 and 100%) conditions in the alpine steppe, and using a subsequent cooling experiment determined the 'thermal stress' of soil microorganisms in response to increased temperature in the alpine steppe ecosystem. Microbial respiration rates decreased with increasing temperature at both 50 and 100% WHC for three sampling locations. Thermal stress of soil microorganisms under increased temperatures was found in the alpine steppe because subsequent cooling led to an increase in microbial respiration rates. Soil moisture did not affect microbial respiration, but the temperature sensitivity (Q_{10}) of microbial respiration varied with the interactive effect between soil moisture and sampling location. Our findings suggest that the response of microbial respiration to high temperature may not be always positive as bacteria may experience thermal stress in the alpine steppe. Therefore, it is necessary to include thermal stress responses in alpine steppe models, as they may represent an important negative feedback effect on microbial respiration.

Introduction

Interest in the factors that control microbial respiration is growing because of its importance in the global carbon (C) cycle and because of potential feedbacks to climate change (Fang *et al*., 2005). Current models predict that global warming will increase net CO_2 emissions from terrestrial ecosystems because of the strong sensitivity of microbial respiration to temperature (Cox *et al*., 2000; Friedlingstein *et al*., 2003). Although increased rates of respiration have been observed in many warming experiments (Schindlbacher *et al*., 2008), the magnitude of the initial positive response to temperature often declines over time (Eliasson *et al*., 2005). Furthermore, many studies have found that the temperature sensitivity of respiration declines with increasing temperature (Mikan *et al*., 2002; Bradford *et al*., 2008). However, the response of 'thermal stress' of soil microorganisms to high temperature has seldom been considered in alpine ecosystems (Hartley *et al*., 2008) although it has the potential to reduce the projected soil C losses associated with global warming.

Extensive research has focused on the temperature sensitivity of microbial respiration in tundra and alpine meadows (Hartley *et al*., 2008; Suh *et al*., 2009). Microbial respiration in water-limited ecosystems has received less attention (Liu *et al*., 2009). Compared with alpine mesic/hydric ecosystems such as wetlands, peatlands and permafrost meadows, alpine steppe soils have reasonably good drainage and aeration, allowing roots and soil fauna to penetrate into mineral soil layers, thus mixing soil organic matter (SOM) with mineral particles (Gao *et al*., 2006). The favourable conditions for SOM decomposition (Davidson & Janssens, 2006) and water limitation of net primary productivity (NPP) result in relatively small C densities. Therefore, in alpine steppe ecosystems with small SOM contents and good aeration (Gao *et al*., 2006), microbial respiration may have different responses to global warming than in alpine mesic/hydric ecosystems. Regardless of the experimental and modelling

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Table 1 Summary information on sampling locations in the alpine steppe

Location	Latitude	Longitude	Elevation / m above sea level	MAT / °C	MAP / mm	TOC / %	TN / %
1	34°58′N	98°07′E	4240	−3.7	322	1.57 (0.16)	0.16 (0.02)
2	34°07′N	95°50′E	4270	−1.6	417	1.28 (0.12)	0.12 (0.01)
3	33°45′N	92°10′E	4654	−3.8	300	0.76 (0.16)	0.10 (0.01)

MAT and MAP are mean annual air temperature and mean annual precipitation. TOC = total soil organic carbon. TN = total soil nitrogen. Mean ($n = 5$) with standard errors indicated in parentheses.

approaches used, the debate over the temperature sensitivity of decomposition should be broadened beyond upland mineral soils to include alpine steppe soils (Davidson & Janssens, 2006).

The Tibetan plateau, the world's largest high-altitude region, has merited considerable attention because of its large stocks of soil organic C and high temperature sensitivity (Zhao, 2009). After alpine meadow, alpine steppe is the second most widespread vegetation type on the Tibetan plateau (Zhao, 2009) and recent studies have demonstrated climate warming effects here (Holmes *et al*., 2009). Expected climate changes in the region are likely to affect C cycling and balance strongly, thus exerting a feedback effect on climate (Zhao, 2009). The objectives of the present study were to (i) investigate spatial and temporal variations in microbial respiration at different soil temperatures and moistures in the alpine steppe, (ii) evaluate the temperature sensitivity of microbial respiration and (iii) determine through a cooling experiment whether increased temperature results in thermal stress of soil microorganisms in this alpine steppe ecosystem.

Materials and methods

Study area and soil sampling

The study area was located in the Three Rivers Headwater region of the Tibetan plateau in China. The alpine steppe consists of hardy perennial xeric herbs such as *Stipa purpurea* Grisebach, *Carex moorcroftii* Falc. and *Dalea racemosa* with dwarf shrubs such as *Artemisia wellbyi*. The soil type is a cryic calcic aridisol (Chinese Soil Taxonomy) or Cambisol (FAO/UNESCO system) (Wang *et al*., 2007) with a large sand content, and a pH between 8.3 and 8.9 (Gao *et al*., 2006). Samples were obtained from three locations in the same alpine steppe dominated by *Stipa purpurea* along a >500 km transect from the southeast to the northwest of the region. The sampling locations provided a typical representation of this ecosystem and ranged from 33°45′ to 34°58′N, 92°10′ to 98°07′E, and from 4240 to 4650 m above sea level in elevation. Mean annual temperature (MAT) and mean annual precipitation (MAP) ranged from −1.6 to −3.8°C and 300–417 mm, respectively. Soil samples were collected in July to August 2008, with three replicated sites located about 50 m apart at each location. At each sampling site, five subsample soil cores were taken by an auger (10 cm diameter) at 0–10 cm soil depth. Subsamples were air-dried and sieved to 2 mm. Roots were removed in the field to provide homogeneous samples and to reduce microbial respiration during the sampling period. These soil samples were stored separately and taken back to the laboratory. The total organic carbon (TOC) and total nitrogen (TN) in the soils ranged from 0.76 to 1.57% and 0.10–0.16%, respectively (Table 1).

Incubation experiment

Soil gravimetric moisture content was determined after oven-drying samples at 105°C for 24 hours. Subsamples of each soil were used for determination of TOC and TN (measured with a Shimadzu 5000 SOC analyser; Shimadzu, Kyoto, Japan). We determined total water-holding capacity (WHC) by placing 20 g of fresh soil into 10-cm funnels and soaking them in water to saturate the soil to provide eight replicates. Soils were covered with a plastic sheet to minimize evaporation losses and left for 4–8 hours to drain before being reweighed. This operation was repeated several times to ensure that a constant mass was reached. The soil was then oven-dried at 105°C to a constant mass. WHC was determined as the percentage of water retained after several hours of drainage (equal to 100% WHC, about 20% of the soil dry weight). Subsamples of surface soil (0–10 cm) at each location were bulked and homogenized.

Soil samples of 10 g (based on dry weight at 105°C) were incubated using air-dried soil in 1000-ml glass bottles for 74 days at three temperatures (0, 15 and 30°C) (±0.4°C) and two moisture contents (50 and 100% WHC), with six treatments in a full-factorial design and three replicates of each treatment. Each bottle at each temperature was randomly assigned to an incubator after each measurement of microbial respiration or at 7-day intervals during the incubation experiment in order to eliminate the potential effect of different incubators on microbial respiration. The 0°C treatment reflected a temperature slightly greater than the mean annual soil temperature, while the 30°C treatment represented the extreme maximum soil temperature recorded; 15°C approximately represents the mean soil temperature during summer in the region. Soil temperatures were not decreased below 0°C to avoid changing substrate availability caused by alterations in the proportion of liquid water present (Mikan *et al*., 2002). Generally, 50–60% WHC is considered to be the optimum moisture content or unstressed range (Howard & Howard, 1993; Rey *et al*., 2005) or 50–70% for laboratory incubations of soils (Paul *et al*., 2001),

while 100% WHC sometimes occurs in the region after rainstorms. Sample moisture was monitored gravimetrically and was adjusted with distilled water on a daily basis.

Cooling experiment

Hartley *et al*. (2008) suggested that if thermal acclimation is responsible for the down-regulation of microbial activity observed at increased temperatures, then microbial activity must be gradually up-regulated when temperatures are reduced. On the basis of this, and in order to determine the hypothesis that thermal stress occurs in the response of microbial respiration to raised temperature in the alpine steppe, after the 74-day incubation, we then incubated the soils for 14 days in a cooling experiment, in which temperatures were reduced to 5 and 10°C for samples incubated at 15 and 30°C, respectively, without altering soil moisture. The temperature of samples pre-incubated at 0°C was kept constant.

Microbial respiration measurements and Q_{10} coefficients

Samples were incubated for 1 day before measurements were started to allow short-term equilibration after rewetting the soil. Microbial respiration rates were measured during the incubation period on days 2, 4, 7, 10, 13, 16, 23, 30, 37, 44, 59, 74, 81 and 88. The first gas samples were taken from the headspace of the bottles using a 60-ml gas-tight syringe by drawing and plunging the syringe three times for homogeneous gas sampling when the bottles were opened to assure exchange with free air outside each time. Then all bottles were sealed with rubber stoppers for 40 minutes before measurements and gas samples were taken again. The microbial respiration rates were measured as the difference between accumulated CO_2 concentrations during the 40-minute incubation in the headspace of the sample glass bottles. For all samples, CO_2 concentration was measured by gas chromatography (HP Series 4890D, Hewlett Packard, Santa Clara, CA, USA) within 24 hours following sampling. The minimum detectable difference was less than 5×10^{-6} μg C s^{-1} for the instrument. Microbial respiration rates were expressed as μg CO_2 g $soil^{-1}$ $hour^{-1}$.

Normally, Q_{10} expresses the temperature sensitivity of microbial respiration (Davidson & Janssens, 2006). Using the microbial respiration rates at different locations and at three different soil temperatures with two soil moistures, the Q_{10} values were calculated as Q_{10}L (low; using the microbial respiration rates measured at 0 and 15°C), Q_{10}M (medium; using the microbial respiration rates measured at 0 and 30°C) and Q_{10}H (high; using the microbial respiration rates measured at 15 and 30°C), with each moisture content using the average respiration rates (R) at two interval temperatures (T) (Howard & Howard, 1993):

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}, \quad (1)$$

where R_1 and R_2 indicate the mean microbial respiration rate at T_1 and T_2 temperature levels, respectively.

Statistical analysis

The repeated measures method of the General Linear Model (SPSS 13.0, SPSS Inc. Chicago, IL, USA) was used to assess the significance of the impacts of incubation temperature, moisture, location and incubation day and their interactions on microbial respiration rates, in which temperature, moisture and location were treated as between-subject variables and incubation day was treated as a within-subject variable. Multi-comparisons were measured for microbial respiration rates of different treatments at different soil locations. Because the cooling treatment was carried out after 74 days, cooling experiment data (the differences in microbial respiration rates between incubation days 81 and 74 and between incubation days 88 and 81) were analysed using two-way ANOVA procedures based on the sampling date with incubation temperature and moisture as the two main factors in the model. For temperature coefficients (Q_{10}), a three-way ANOVA was used to test differences between location, moisture, temperature range with initial temperature and their interactions using a General Linear Model-Univariate procedure. Stepwise multiple linear regression analysis was performed to test the possible dependence of microbial respiration rates on soil TOC, TN, soil temperature, soil moisture, latitude, longitude, altitude, MAT and MAP of the sampling location. All significances mentioned in the text are at the $P < 0.05$ level.

Results

Response of microbial respiration rates to soil temperature and moisture

Generally, there were significant responses of microbial respiration to soil temperature, and interactive effects were observed between soil temperature and sampling location, and between soil temperature and sampling location and incubation day on microbial respiration (Table 2). However, soil moisture did not significantly affect microbial respiration (Table 2). The microbial respiration in soils from locations 1 and 3 with different treatments peaked on incubation day 4 (Figure 1a,c), but the day of peak value occurrence varied with different treatments in location 2 (Figure 1b). After incubation day 10 the variation in microbial respiration was relatively small for the 0°C treatment, whereas microbial respiration for the 15 and 30°C treatments decreased slowly through incubation day 74 in location 1 and 3 soils (Figure 1a,c). The differences in microbial respiration between different treatments almost disappeared after incubation day 10 in location 2 soil (Figure 1b).

When data for all locations were pooled, regardless of soil moisture, mean microbial respiration for the 0°C treatment (1.71 μg CO_2 g $soil^{-1}$ $hour^{-1}$) was significantly greater than that for the 15 and 30°C treatments, and there was no significant difference between the 15 and 30°C treatments (1.25 and 1.36 μg CO_2 g $soil^{-1}$ $hour^{-1}$ for the treatments at 15 and 30°C, respectively) during the incubation (Figure 1). An interactive effect between temperature and location on mean

Table 2 Microbial respiration from repeated-measure ANOVAs using soil temperature, soil moisture and location as between-subject variables and incubation day as a within-subject variable

Model	d.f.	F	*P*
Location (L)	2	1286.728	0.368
Temperature (T)	2	1.029	**0.000**
Moisture (M)	1	29.513	0.622
L × T	4	0.247	**0.008**
L × M	2	4.053	0.297
T × M	2	1.256	0.141
L × T × M	4	2.071	0.081
Day (D)	11	2.265	**0.000**
D × L	22	27.559	**0.003**
D × T	22	2.069	**0.000**
D × M	11	3.264	0.930
D × L × T	44	0.455	**0.002**
D × L × M	22	1.799	0.505
D × T × M	22	0.969	0.067
D × L × T × M	44	1.507	0.581

Bold numbers indicate significant difference.

microbial respiration was found (Table 2). For example, the mean microbial respiration for the 0°C treatment in location 1 soil was significantly greater (Figure 1a) and that for the 30°C treatment in location 3 soil was significantly smaller (Figure 1c) than those for the other two treatments in soils from each site, whereas there were no significant differences among all temperature treatments in location 2 soil (Figure 1b).

Stepwise regression analysis indicated only significant negative correlation between microbial respiration and soil temperature. Soil temperature explained 57% of the variation of the mean microbial respiration during incubation to day 74 (Figure 2).

Temperature sensitivity of microbial respiration rates (Q_{10})

The mean Q_{10} values under different temperature ranges (Q_{10}L, Q_{10}M and Q_{10}H) are shown in Figure 3. Temperature range did not significantly affect the Q_{10} value ($P > 0.05$). However, the Q_{10} value was significantly affected by location ($P = 0.007$) and by interactions between location and soil moisture ($P = 0.044$). The Q_{10} value was greater in location 2 soil (0.96) than in soils from location 1 (0.79) and location 3 (0.86). There were no significant differences in Q_{10} values among locations under 50% WHC, while the Q_{10} value was greater in location 2 soil than in soil from locations 1 and 3 under 100% WHC (Figure 3). In all cases the calculated Q_{10} values were less than or close to 1.

Thermal stress of microbial respiration in response to temperature

Statistical analysis showed that only soil temperature significantly affected the microbial respiration differences between incubation days 74 and 81 for all treatments ($P < 0.001$). For the 0°C treatment, the microbial respiration continued to decline

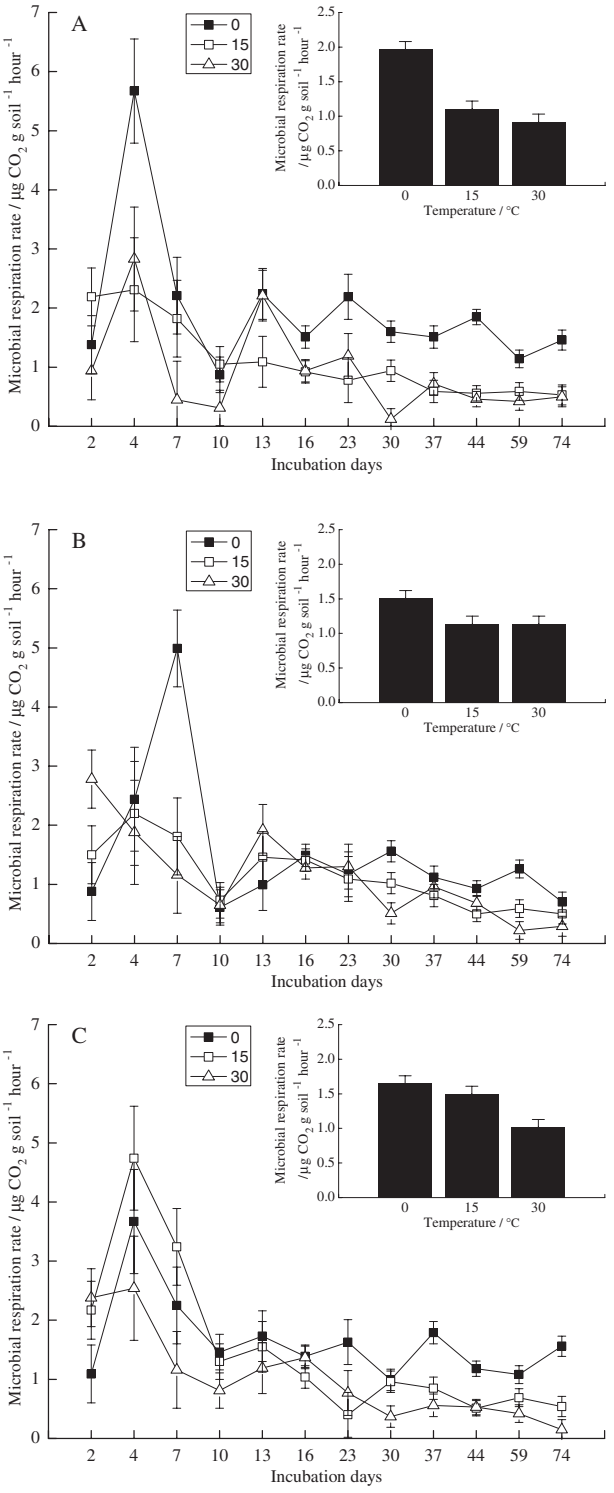

Figure 1 Dynamics of mean microbial respiration rates during the incubation period under different temperature treatments at each location in the alpine steppe. Location sequence is as for Table 1. (A) Location 1; (B) location 2; (C) location 3. Bar graphs represent the microbial respiration (mean + SE) according to temperatures. Data for different soil moistures were pooled because of the insignificance of its effects on microbial respiration rates.

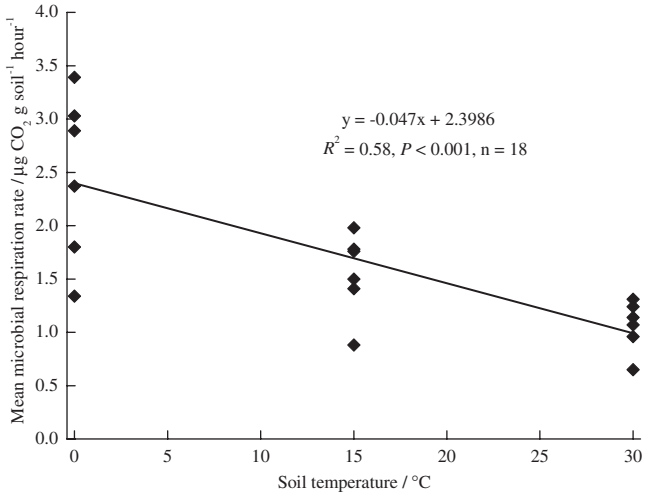

Figure 2 Stepwise regression analysis using microbial respiration rate as the dependent variable, and total organic carbon concentration and total nitrogen concentration in soils and latitude, longitude and altitude of sampling locations, soil temperature and soil moisture as variables under different treatments. Only soil temperature was retained.

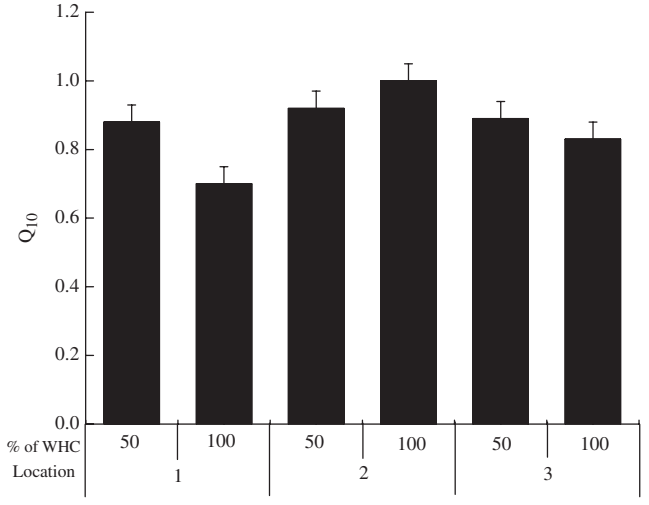

Figure 3 The temperature sensitivity of microbial respiration rates (Q_{10}) at different locations with different soil moistures. The mean Q_{10} value (for Q_{10}L, Q_{10}M and Q_{10}H) under different temperature ranges (0–15, 0–30 and 15–30°C) is shown because temperature range did not significantly affect Q_{10} value ($P > 0.05$). WHC = water holding capacity. Location sequence is as in Table 1. Means with standard errors are shown.

during incubation between days 74 and 81, whereas the microbial respiration on incubation day 81 significantly increased when soil temperatures were cooled from 15 to 5°C or from 30 to 10°C compared with temperatures at incubation day 74 (Figure 4). There were no significant differences in microbial respiration for treatments during incubation between days 81 and 88 ($P > 0.05$).

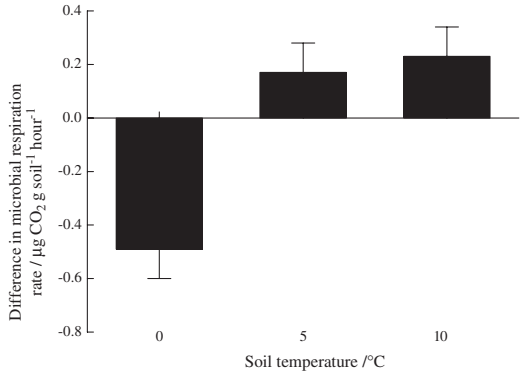

Figure 4 Difference in mean microbial respiration rates between incubation day 81 and day 74 during the cooling experiment. Treatment 0°C was kept unchanged, but soil temperatures of 15 and 30°C were cooled to 5 and 10°C during the cooling experiment. Means with standard errors are shown.

Discussion

Responses of microbial respiration and thermal stress to raised temperature

In our incubation study we found that raising temperature to 30°C significantly reduced microbial respiration throughout the incubation period regardless of soil moisture (Figure 2), although this response varied with soil sample location within the alpine steppe ecosystem (Figure 1). A previous study in a semiarid grassland found a reduction of microbial respiration at high temperatures (Liu *et al*., 2009). Allison & Treseder (2008) also found similar results with increasing temperature but with concurrent reduced soil moisture. Although a pulse of CO_2 production following the rewetting of dry soils was observed for all of our soils between incubation days 4 and 7 (Figure 1), excluding and including the first 7 days of the incubation data produced similar results (Table S1). The mean microbial respiration values were 1.37, 0.85 and 0.77 μg CO_2 g $soil^{-1}$ $hour^{-1}$ for both percentages of WHCs at 0, 15 and 30°C, respectively, when excluding data for the first 7 days of incubation. These results suggested that the pulse of microbial respiration did not change the pattern of response of microbial respiration to temperature during the experimental period regardless of whether the data for the first 7 days of incubation were included or excluded.

Greater microbial respiration was observed at 0 ± 0.4°C in all soil locations: this result may be attributed to two reasons. The first is that increasing temperature to 30°C may cause thermal stress of soil microorganisms in the alpine steppe at high (>4000 m) elevation. In support of this logic, our cooling experiment showed that microbial respiration rates increased significantly when incubation temperatures were cooled from 15 to 5°C and from 30 to 10°C (Figure 3). This may suggest that there is a potential for up-regulation of activity following extended exposure to cold. This response was probably driven by physiological adaptations of microorganisms to low temperature regimes (Rinnan *et al*., 2009). It is well known that microbes may experience physiological

changes and become tolerant to cold conditions, and maintain membrane fluidity or synthesize cold-tolerant enzymes (Margesin *et al*., 2007). Increased temperature may reduce the activity of the cold-tolerant enzymes, which decreases microbial respiration (Margesin *et al*., 2007). Under long-term cold conditions in the alpine steppe in our study, where labile C depletion occurs more slowly, microbially-mediated soil respiration could adjust its optimal temperature to colder temperatures through acclimatization. In our experiment, soil C content was between 0.76 and 1.57% on a mass basis, which is relatively small in comparison with other experimental warming sites (Hartley *et al*., 2008). Thus, microbes in substrate-limited conditions are likely to adopt defensive strategies (such as reducing their respiration) at increased temperatures. The second reason for greater microbial respiration at low temperatures could be that, in our study, the temperature control of the incubations was $0 \pm 0.4^{\circ}$C, which may have induced a freeze-thaw effect. Freeze-thaw fluctuations in soil temperature are common in some temperate and in most high-latitude and high-altitude ecosystems, even in summer (Grogan *et al*., 2004). Some studies show that freezing and subsequent thawing of soils often results in an initial flush of microbial respiration (Schimel & Clein, 1996) because freeze-thaw cycles can lyse a substantial proportion of microbial cells, resulting in C and nitrogen releases into the surrounding soil (Ivarson & Sowden, 1970; DeLuca *et al*., 1992), which may in turn enhance activity and/or cause shifts in microbial community competition.

Effects of moisture on microbial respiration

Extensive laboratory and field studies show that water deficit impedes the diffusion of substrate in water films and in soil microbial activity, causing a decline in respiration rates, while a large water content can inhibit CO_2 emissions (Rey *et al*., 2005; Davidson & Janssens, 2006). Soil temperature and soil moisture often co-vary in the field. Usually, raised temperatures occur when soil is dry, and low temperatures coincide with wetter soil water conditions (Rustad *et al*., 2001). The response of microbial respiration to temperature varies with soil moisture (Liu *et al*., 2009). Some studies have shown that the optimum soil water content is usually somewhere near WHC (Luo & Zhou, 2006) or in an unstressed range at 50–60% of WHC (Howard & Howard, 1993; Rey *et al*., 2005). Therefore, in our study, it is not surprising that soil moisture did not affect microbial respiration because soil water content did not limit microbial respiration. This suggests that a rainstorm in the region would not induce a pulse increase in microbial respiration if soil water content is more than 10% (about 50% of WHC). Hence, our study suggested that the differences in microbial respiration among treatments were mainly controlled by temperature.

Temperature sensitivity of microbial respiration (Q_{10})

The Q_{10} depends upon soil temperature, soil moisture (Yuste *et al*., 2007) and the geographical location (Vanhala *et al*., 2008). Several earlier studies reported that the Q_{10} values usually decreased with increasing temperature (Mikan *et al*., 2002; Bradford *et al*., 2008). However, we found that temperature range did not significantly affect the Q_{10} values and these values were less than 1 in the alpine steppe in our study, probably because of thermal stress of microorganisms at temperatures $>15^{\circ}$C. Additionally, we did not find the difference noted by others in Q_{10} values at different soil moisture levels. Liu *et al*. (2009) observed that no effect of temperature on microbial respiration occurred under water stress. Similarly, Schindlbacher *et al*. (2008) did not find this effect of soil moisture on Q_{10}. However, the interactive effect between soil moisture and sampling location significantly influenced the Q_{10} value. This result indicated that Q_{10} may be affected by other confounding factors such as substrate availability (Conant *et al*., 2011).

Conclusions and implications

Our results, obtained for soils from alpine steppes with small soil organic C contents and dry conditions, indicate that rising temperatures in alpine ecosystems may not always cause a positive feedback to the soil C cycle. To our knowledge, our study is the first to determine the thermal stress of microbial respiration in reaction to increased temperature in an incubation experiment with a cooling treatment. Soil moisture either at 50 or 100% of WHC did not affect microbial respiration, but the interactions between soil moisture and soil location significantly influenced the temperature sensitivity of microbial respiration. Alpine steppe locations with a greater MAT were more temperature-sensitive to warming when soil moisture was 100% of WHC. Overall, our study suggests that thermal stress of soil microorganisms in response to increased temperature may counteract the positive feedback between the C cycle and climatic warming, and may act as a short-term mechanism of homeostasis in the earth system because of the reduced temperature sensitivity of microbial respiration in the alpine steppe region. Therefore, this response should be considered explicitly in models when predicting climate change using carbon dynamics in alpine steppe ecosystems. Given the cause of this 'thermal stress' response is not well known, further research to understand the 'thermal stress' of microbes better is still needed.

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Supporting Information

The following supporting information is available in the online version of this article:

Table S1. Microbial respiration from repeated-measure ANOVAS using soil temperature, soil moisture and location as between-subject variables and incubation day after 7 days of incubation as a within-subject variable.

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