Asymmetric responses of soil heterotrophic respiration to rising and decreasing temperatures

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ABSTRACT

Periodic changes in temperature commonly occur diurnally and seasonally. However, the response of soil heterotrophic respiration to rising and decreasing temperatures during these periods remains poorly understood; thus the feedback between climate change and carbon (C) cycling requires further investigation. In this study, soils from three grasslands in the Qinghai-Tibet Plateau were incubated separately at rising (from 5 °C to 31 °C) and decreasing (from 31 °C to 5 °C) temperatures modes over 161 days, to explore how soil heterotrophic respiration rates (Rs) respond to different temperature changes. The parameters of Rs and temperature sensitivity (Q10) were used for the analyses. In addition, microbial biomass C (MBC), microbial biomass nitrogen (N) (MBN), dissolved organic C (DOC), and other soil properties were measured. The results indicated a pronounced hysteresis of Rs for both rising and decreasing temperatures. Furthermore, the hysteresis loops differed in the different sites. Rs values were significantly higher for rising temperature (2.71 µg C g⁻¹ d⁻¹) versus decreasing temperature (1.75 µg C g⁻¹ d⁻¹) in all three alpine grasslands. The Q₁₀ values were significantly higher for decreasing temperature (2.42) versus increasing temperature (1.55), with these differences being observed over the 161-d incubation period. Furthermore, soil microbes (specifically, MBC and MBC/MBN) explained 46% of the total variation in Q₁₀, followed by substrate and other properties. Our results provide experimental evidence for the asymmetric responses of soil heterotrophic respiration to rising and decreasing temperatures. In addition, the microbial effect was primarily associated with soil heterotrophic respiration, suggesting strong asymmetric responses to rising and decreasing temperatures that require investigation in future studies.

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1. Introduction

In response to increasing CO₂ concentrations in the atmosphere, the global mean temperature is predicted to increase 2–7 °C by the end of the 21st century (Allison et al., 2011). Temperature modifies the amount of carbon (C) stored in soils by influencing soil heterotrophic respiration rates (Rs) and the global C balance (Davidson and Janssens, 2006). Most studies have investigated how temperature affects Rs in relation to rising temperature, with it being assumed the same effects occur with decreasing temperature (Xia et al., 2009; Peng et al., 2013). Thus, it is necessary to test this assumption by better characterizing the responses of Rs to changing temperature under both rising and decreasing temperatures.

In nature, periodic changes in temperature are common. For example, soil temperature typically rises during the daytime and decreases at night, representing a dynamic diurnal pattern (Fig. 1A). In addition, larger temperature changes occur over greater time scales such as over seasons and years (Fig. 1B). Temperature sensitivity (Q₁₀) is a useful index to describe the proportional change in Rs with a 10 °C increase in temperature (Lloyd and Taylor,
observed variability in Q2015). Usually, higher Q ranging from nearly 1 to over 12 (Hamdi et al., 2013; Liu et al., greatly at different temperatures and in different ecosystems, resulting in a higher Q10 values are detected under colder temperatures versus warmer temperatures (Kirschbaum, 1995). Multiple hypotheses have been developed to explain the observed variability in Q10. First, the C quality-temperature hypothesis predicts that Q10 is controlled by enzyme kinetics and should decrease as the quality of C in soil organic matter (SOM) increases (Bosatta and Ågren, 1999). C quality varies among different ecosystems (Craine et al., 2010a; Bosatta and Ågren, 1999), or even within the same ecosystems, depending on the season (Niu et al., 2011), soil depth (Rovira and Vallejo, 2002), and the different stages of SOM decomposition (early or late stages). Experimental evidence supports the C quality hypothesis by demonstrating that the decomposition of lower quality C substrates is more sensitive to changes in temperature than the decomposition of higher C quality substrates (Fierer et al., 2005). Alternatively, varying Q10 might be derived from the physiological adjustments of microbes (Bradford et al., 2008). Microbes inhabiting colder regions may be more sensitive to increasing temperature than those in warm regions, resulting in a higher Q10 (Karhu et al., 2014). Some studies have shown that the adaptation of soil microbes changes differently when temperature increases or decreases (Fenner et al., 2005; Bradford et al., 2008). Moreover, abiotic soil properties, such as oxidation-reduction potential (ORP), pH (Min et al., 2014), and the substrate used for microbial metabolism (Blagodatskaya et al., 2014), might influence Rs because these soil properties regulate microbial activity directly or indirectly. Microbial adaptation when

![Diurnal temperature variation](image)

![Season temperature variation](image)

**Fig. 1.** Diurnal (A) and seasonal (B) variation in temperature in natural ecosystems.

1994). Previous studies have demonstrated that Q10 values vary greatly at different temperatures and in different ecosystems, ranging from nearly 1 to over 12 (Hamdi et al., 2013; Liu et al., 2015). Usually, higher Q10 values are detected under colder temperatures versus warmer temperatures (Kirschbaum, 1995).

Temperature responses have been reported for Rsk; however, the mechanisms remain unclear (Vargas and Allen, 2008; Phillips et al., 2011). Traditional methods used to quantify Q10 are performed at a single constant incubation temperature (Wagai et al., 2013; Quan et al., 2014) or by placing multiple soil samples at different constant temperatures along a temperature gradient (Weedon et al., 2013; Xue et al., 2015). Unfortunately, these methods produce limited data to calculate Q10, and might influence the accuracy of Q10 to some extent. More importantly, soils incubated at a constant temperature might consume more substrate at higher temperatures compared to lower temperatures, leading to large differences in substrate supply (or C quality), which influence Q10 estimates, especially for long-term incubation experiments. These disadvantages of traditional incubation experiments must be overcome in future studies.

Here we designed a novel soil incubation experiment under rising (from 5 °C to 31 °C) and decreasing (from 31 °C to 5 °C) temperature regimes, to simulate periodic changes in temperature during daytime and night-time. Using an equipment with continuous measurement, we evaluated Rs (recorded approximately every 20-min) at intervals of 0, 7, 14, 21, 28, 49, 77, 105, 133, and 161 days, and calculated the corresponding Q10. We used three alpine soils from the Qinghai-Tibet grasslands to investigate hysteretic responses of Rs and Q10 under rising and decreasing temperatures, and to explore the influence of soil microbes and soil substrate quality.

### 2. Materials and methods

#### 2.1. Study sites

The experimental plots were selected from three main grassland types distributed widely across the Qinghai-Tibet Plateau. These grassland types were designated as alpine meadow, alpine steppe, and alpine desert (Fig. S1). The mean annual temperature at these sites ranged from −0.2 °C to 3.1 °C; and the mean annual precipitation ranged from 150.0 mm to 641.0 mm (Table 1). Detailed information on the three plots was primarily derived from a previous publication (Li et al., 2015) and is presented in Table 1.

#### 2.2. Field sampling

Field sampling was conducted in August 2013. In each plot, nine sampling quadrats (0.5 m × 0.5 m) were established at about 10 m intervals along three random transects in each selected grassland type. The community structure and aboveground biomass were investigated in each quadrat. Subsequently, surface litter was removed and soil samples (approximately 5 kg) were randomly collected using a soil sampler (10 cm in diameter) from the surface soil (0–20 cm) in each quadrat. The samples were passed through a 2-mm sieve and all visible plant material was manually removed. Homogenized soil samples from the same depth were mixed and divided into two subsamples. Then, approximately 100 g of each soil sample was air-dried to analyze soil properties [e.g., C, nitrogen (N), and pH]. Approximately 5 kg fresh soil was immediately packed in labeled polyethylene bags and stored in a portable refrigerator (4 °C). The soil was then transported to the laboratory for subsequent incubation experiments.
chemistry, microbial community, and substrate properties were incubated and from 31 °C.

Temperatures, as well as a more accurate calculation of improved exploration of the relationship between frequently (i.e., at intervals of several minutes) allows for an accurate measurement of the program of Wang et al. (2016). Measuring continuously measured during the model of varying temperature over 24-h based on the program of Wang et al. (2016). Measuring R₃ more frequently (i.e., at intervals of several minutes) allows for an improved exploration of the relationship between R₃ and changing temperatures, as well as a more accurate calculation of Q₁₀. Soil chemistry, microbial community, and substrate properties were measured by conducting destructive sampling at different incubation times to determine how they influence R₅ and Q₁₀.

### 2.3. Laboratory incubation and analysis

To overcome the disadvantages of traditional incubation experiments (see Introduction), we developed a new experimental design in this study, in which soil samples were incubated under continuously and periodically changing temperature conditions. The temperature ranged from 5 °C to 31 °C in the first phase of incubation and from 31 °C to 5 °C in the second phase of incubation (Fig. 2). R₅ and soil temperature were simultaneously and continuously measured during the model of varying temperature over 24-h based on the program of Wang et al. (2016). Measuring R₅ more frequently (i.e., at intervals of several minutes) allows for an improved exploration of the relationship between R₅ and changing temperatures, as well as a more accurate calculation of Q₁₀. Soil chemistry, microbial community, and substrate properties were measured by conducting destructive sampling at different incubation times to determine how they influence R₅ and Q₁₀.

#### 2.3.1. Incubation experiment

Forty-gams of fresh soil and 10 g of quartz sand (to prevent soil compaction) were placed in incubation bottles and adjusted to 55% soil water-holding capacity, which is commonly considered optimal for microbial activity. Soil sample for each grassland was divided into 15 replicates: three replicates for the repeated measurements of R₅ throughout the incubation period, and 12 replicates for five separate destructive sampling times to measure soil chemistry, microbial community, and substrate properties. All samples were pre-incubated for 7 days at 15 °C to minimize the mineralization pulse (Wagai et al., 2013) and to provide sufficient time for the stabilization of soil microbial populations (Sun et al., 2013). Subsequently, the samples were placed in an incubator with automatic temperature regulation. Considering the diurnal dynamics of air temperature and the limits of the incubator, four temperatures (6 °C, 14 °C, 22 °C, and 30 °C) were established, and each temperature was maintained for 6-h each day (Fig. 2A). We set the minimum temperature at 08:00 in the incubator, and, at the same time, R₅ was measured at 20-min intervals during both rising and decreasing temperature periods. As designed, R₅ was measured 10 times on days 0, 7, 14, 21, 28, 49, 77, 105, 133, and 161. The soil moisture of the incubated samples was adjusted at 3-d intervals on a weight basis. Soil substrate [dissolved organic C (DOC)], microbial characteristics [microbial biomass C (MBC) and microbial biomass N (MBN)], and chemical properties [pH, soil oxidation-reduction potential (ORP), and conductivity (COND)], were measured after 7, 21, 49, 105, and 161 days of incubation. To determine the actual temperature of the soil samples, button thermometers were buried in each soil sample (DS1922L, USA).

#### 2.3.2. Chemical analyses

Soil organic C (SOC) content (%) was determined by the Walkley-Black method (Nelson and Sommers, 1982). Soil total N (STN) concentration (%) was measured using a modified Kjeldahl wet-digestion procedure by Elemental Analyzer (Series II CHN/S/O Analyzer 2400; Perkin Elmer) (Gallaher et al., 1976). MBC (µg g⁻¹) and MBN (µg g⁻¹) were determined using the chloroform fumigation-extraction method of Vance et al. (1987). DOC was determined using suspensions extracted by 0.5 mol L⁻¹ K₂SO₄, and was analyzed by total organic C (TOC) analyzers (Elementar Liqui TOC, Elementar Co., Germany). Soil pH, ORP, and COND were measured in a soil-water slurry (1:2.5, w/v) by using an Ultrameter-2 pH meter (Myron L Company, USA). Soil WHC (%) and gravimetric moisture content (%) were measured in the laboratory according to the protocol of He et al. (2013).

### Table 1

Initial properties of plant and soil in the experimental plots.

| Grassland types | MAT (°C) | MAP (mm) | Altitude (m) | Vegetation coverage (%) | Aboveground biomass SOC (%) | STN (%) | MBC (µg g⁻¹) | MBN (µg g⁻¹) | ORP (mV) | pH |
|-----------------|---------|----------|--------------|-------------------------|---------------------------|---------|--------------|--------------|----------|-----|
| Alpine meadow   | 3.1     | 641.0    | 4618         | 74±8a<sup>b</sup>       | 162.91 ± 7.12a            | 2.50 ± 0.20a             | 0.28 ± 0.02a  | 204.80 ± 3.64a | 81.29 ± 2.54a | 250.31 ± 0.84a | 6.23 ± 0.06c |
| Alpine steppe   | -1.9    | 150.0    | 4527         | 22±4b                   | 90.04 ± 21.09b            | 0.95 ± 0.11b             | 0.13 ± 0.02b  | 120.37 ± 7.27b | 53.59 ± 1.35b | 194.67 ± 3.79b | 7.62 ± 0.02b |
| Alpine desert   | 0.2     | 189.6    | 4546         | 11±2c                   | 31.79 ± 11.30c            | 0.50 ± 0.03c             | 0.10 ± 0.01c  | 87.35 ± 0.55c  | 43.95 ± 1.62c | 164.67 ± 0.58b | 7.89 ± 0.02a |

<sup>a</sup> MAT, mean annual temperature; MAP, mean annual precipitation; SOC, mean soil organic carbon; STN, soil total nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; ORP, oxidation-reduction potential.

<sup>b</sup> The values in the table are presented as mean ± SD (n = 4); data with same letters with in the same column indicate no significant difference at P = 0.05.

**Fig. 2.** Changes in the incubation temperature in the incubator (A) and soil temperature during the measurement process (B) throughout the day.
2.3.3. Measurement of $RS$ and soil properties

Traditionally, 3–6 specific temperature gradients are established to determine $RS$, with $Q_{10}$ being calculated from this information (Craine et al., 2010a; Wetterstedt et al., 2010). In this study, a continuous measurement apparatus was used to measure (at 20-min intervals) the dynamics of $RS$ over 24-h at each incubation time point by using an automatic and continuous system. We assumed that all soil samples experience a temperature ranging from 6°C (in the middle of the night) to 30°C (in the afternoon) daily. To ensure the integrity of the data in the measurement process of $RS$, we expanded 1°C in the measurement of the incubator using an automatic temperature regulator (Julabo, Germany). For instance, the temperature in the incubator ranged from 6°C to 30°C during the first 12-h, and from 30°C to 6°C in the following 12-h, whereas the temperature measurements ranged from 5°C to 31°C during the first 12-h, and from 31°C to 5°C in the next 12-h. This method was modified from He et al. (2013) based on the designated program of Wang et al. (2016). Furthermore, a button thermometer (DS1922L) was used to measure the actual soil temperature when we measured $RS$, providing accurate paired data for $RS$ and soil temperature to calculate $Q_{10}$ (Wang et al., 2016). To reduce the error in button thermometer readings, we used five button thermometers to monitor soil temperature in the incubation bottles.

A new PRI-8800 Automatic Temperature Control Soil Flux System (PRI-8800, Pre-Eco, China) was newly developed and used to measure $RS$ as a modification of He et al. (2013). In brief, $RS$ was first calculated from the slope of the CO2 concentration using specific transformation factors:

$$RS = \frac{C \times V \times \alpha \times \beta}{m}$$  

where $Q_{10}$ is the average value of $Q_{10}$; $Q_{10-RT}$ is $Q_{10}$ for rising temperature; $Q_{10-DT}$ is $Q_{10}$ for decreasing temperature; $m$ is the number of times $Q_{10}$ is measured for rising temperature; and $n$ is the number of times.

$Q_{10}$ values were calculated using the following exponential equations (Lloyd and Taylor, 1994):

$$RS = a \times e^{bT}$$

$$Q_{10} = e^{10b}$$

where $RS$ is the soil heterotrophic respiration rate (µg C g$^{-1}$ d$^{-1}$); $Q_{10}$ is temperature sensitivity; $T$ is the soil temperature during measurement (the same as above); $a$ is the net soil heterotrophic respiration rate at 0°C; and $b$ is the temperature response factor.

The average $RS$ for rising temperature was calculated as follows:

$$RS - Ave(RT) = \frac{\sum_{i=1}^{m} RS - RT(i)}{m}$$  

where $RS_{-Ave} (RT)$ is the average value of $RS$ at rising temperature (µg C g$^{-1}$ d$^{-1}$); $RS_{RT}$ is $RS$ for rising temperature (µg C g$^{-1}$ d$^{-1}$); and $m$ is the number of time $RS$ is measured during rising temperature.

The average $RS$ for decreasing temperature was calculated as follows:

$$RS - Ave(DT) = \frac{\sum_{j=1}^{n} RS - DT(j)}{n}$$  

where $RS_{-Ave} (DT)$ is the average value of $RS$ for decreasing temperature (µg C g$^{-1}$ d$^{-1}$); $RS_{DT}$ is $RS$ for decreasing temperature (µg C g$^{-1}$ d$^{-1}$); and $n$ is the number of times $RS$ is measured for decreasing temperature. The average $Q_{10}$ [Q$_{10-\text{Ave} (RT)}$] for decreasing temperature and $Q_{10}$ [Q$_{10-\text{Ave} (DT)}$] for decreasing temperature were also calculated according to Eqs. 4 and 5.

The average $RS$ was calculated as follows:

$$RS - Ave = \frac{\sum_{i=1}^{m} RS - RT(i) + \sum_{j=1}^{n} RS - DT(j)}{(m+n)}$$  

where $RS_{-Ave}$ is the average $RS$ (µg C g$^{-1}$ d$^{-1}$); $RS_{RT}$ is $RS$ for rising temperature (µg C g$^{-1}$ d$^{-1}$); $RS_{DT}$ is $RS$ for decreasing temperature (µg C g$^{-1}$ d$^{-1}$); $m$ is the number of times $RS$ is measured for rising temperature; and $n$ is the number of times $RS$ is measured for decreasing temperature.

The average $Q_{10}$ was calculated as follows:

$$Q_{10} - Ave = \frac{\sum_{i=1}^{m} Q_{10} - RT(i) + \sum_{j=1}^{n} Q_{10} - DT(j)}{(m+n)}$$  

where $Q_{10-\text{Ave}}$ is the average value of $Q_{10}$; $Q_{10-\text{RT}}$ is $Q_{10}$ for rising temperature; $Q_{10-DT}$ is $Q_{10}$ for decreasing temperature; $m$ is the number of times $Q_{10}$ is measured for rising temperature; and $n$ is the number of times $Q_{10}$ is measured for decreasing temperature. $Q_{10}$ is measured for decreasing temperature.

MBC was calculated as follows:

$$MBC = (EC - EC_{ck}) \times 2.2$$

where MBC is soil microbial biomass C (µg g$^{-1}$); $EC$ is DOC after fumigation (µg g$^{-1}$); and $EC_{ck}$ is DOC before fumigation (µg g$^{-1}$). The correction factor was 2.2.

MBN was calculated as follows:

$$MBN = (EN - EN_{ck}) \times 2.2$$

where MBN is soil microbial biomass N (µg g$^{-1}$); $EN$ is dissolved organic N (DON) after fumigation (µg g$^{-1}$); and $EN_{ck}$ is DON before fumigation (µg g$^{-1}$). The correction factor was 2.2.

2.4. Statistical analysis

One-way analysis of variance (One-way ANOVA) was used to assess how grassland types affected vegetation coverage and aboveground biomass, soil water content, SOC, STN, MBC, MBN, pH, and ORP, as well as how incubation time affected $Q_{10}$. Repeated-ANOVA was used to explore how rising and decreasing temperatures affected $RS_{-Ave}$ and $Q_{10-\text{Ave}}$. Correlation analysis between the two variables was implemented to assess each factor associated with $RS$ and $Q_{10}$. Structural equation modeling (SEM) was used to evaluate the causal relationships among multiple interacting variables explicitly, and to determine the relative influence of microbial, substrate, and chemical properties on $Q_{10}$. SEM was conducted using the procedure of Amos 17 for Windows. All other statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA). Differences were considered statistically significant at $P=0.05$. All figures were prepared using SigmaPlot 10.0.

3. Results

3.1. Changes in $RS$ under rising and decreasing temperatures

Temperature significantly influenced $RS$ ($P<0.001$; Table 2). The average $RS$ over the whole 161-d period was higher for rising temperature (2.71 µg C g$^{-1}$ d$^{-1}$) compared to decreasing temperature (1.75 µg C g$^{-1}$ d$^{-1}$) for all three alpine grasslands (Fig. 3). This pattern was also observed for each of the nine individual daily measurement (Fig. S2). In addition, incubation time had a
significant effect on $R_S$ ($P < 0.001$; Table 3). Over the prolonged incubation times, $R_S$ decreased in all three grassland soils (Fig. S3). During the incubation process, increasing and decreasing temperatures also significantly influenced $R_S$ (rising temperature: $F = 202.124$, $P < 0.0001$; decreasing temperature: $F = 72.583$, $P < 0.0001$; Table 3). For rising temperature, $R_S$ increased and peaked at the highest temperature, whereas, for decreasing temperature, $R_S$ declined with decreasing temperature (Fig. S3). A pronounced hysteresis of $R_S$ was observed for diurnal temperature dynamics with rising and decreasing temperatures, with the hysteresis loops differing at different sites (Fig. S3).

### 3.2. Changes in $Q_{10}$ under rising and decreasing temperatures

The responses of $Q_{10}$ depended on whether the temperatures was rising or decreasing ($P < 0.001$; Table 2). The $Q_{10}$ values were lower at rising temperature (1.55) compared to decreasing temperature (2.42) in all three alpine grassland types (Fig. 4). Overall,

**Table 2**

Results of the t-test ($P$ values) showing how rising and decreasing temperatures affect soil heterotrophic respiration rate ($R_S$) and its temperature sensitivity ($Q_{10}$).

| Incubation time | $R_S$/C0 Ave | $Q_{10}$/C0 Ave |
|----------------|--------------|----------------|
| 7-d           | 0.062        | 0.036          |
| 14-d          | 0.008        | 0.015          |
| 21-d          | 0.085        | 0.030          |
| 28-d          | 0.043        | 0.153          |
| 49-d          | 0.084        | 0.051          |
| 77-d          | 0.020        | 0.029          |
| 105-d         | 0.094        | 0.026          |
| 133-d         | 0.129        | 0.020          |
| 161-d         | 0.199        | 0.015          |
| all days      | <0.001       | <0.001         |

* $R_S$/C0 Ave and $Q_{10}$/C0 Ave are the averages of $R_S$ and $Q_{10}$ under rising and decreasing temperature treatments, respectively.

**Table 3**

Results of the repeated ANOVA showing how grassland type and incubation time affect soil heterotrophic respiration rate ($R_S$) and its temperature sensitivity ($Q_{10}$).

| Source          | $R_S$/C0 RT $F$ | $P$       | $Q_{10}$/C0 RT $F$ | $P$       |
|-----------------|----------------|-----------|-------------------|-----------|
| Grassland type  | 793.637        | <0.0001   | 217.009           | <0.0001   |
| Incubation time | 202.124        | <0.0001   | 11.467            | <0.0001   |
| $G \times T$    | 4.020          | <0.0001   | 3.229             | 0.0001    |

| Source          | $R_S$/C0 DT $F$ | $P$       | $Q_{10}$/C0 DT $F$ | $P$       |
|-----------------|----------------|-----------|-------------------|-----------|
| Grassland type  | 1547.376       | <0.0001   | 54.415            | <0.0001   |
| Incubation time | 72.583         | <0.0001   | 3.229             | 0.022     |
| $G \times T$    | 11.102         | <0.0001   | 107.894           | <0.0001   |

* $R_S$/C0 RT and $Q_{10}$/C0 RT were $R_S$ and $Q_{10}$ under the rising temperature treatment, respectively.

b $R_S$/C0 DT and $Q_{10}$/C0 DT were $R_S$ and $Q_{10}$ under the decreasing temperature treatment, respectively.

**Fig. 3.** Changes in the soil heterotrophic respiration rate ($R_S$/C0 Ave) between rising and decreasing temperatures in different phases. 7-d, mean of the 7th day; 161-d, mean of the 161st day. Different lower case letters in the same column indicate significant differences at $P = 0.05$.

**Fig. 4.** Differences in the temperature sensitivity ($Q_{10}$) of soil heterotrophic respiration between rising and decreasing temperatures at day 7 (A) and day 161 (B) in three alpine grasslands. 7-d, mean of the 7th day; 161-d, mean of the 161st day. Different lower case letters in the same column indicate significant differences at $P = 0.05$. 

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the average of $Q_{10}$ was as follows: alpine meadow (1.25) < alpine steppe (2.06) < alpine desert (2.65). In addition, the difference in $Q_{10}$ across different grassland types became more apparent as incubation progressed (Fig. 4). Incubation time significantly influenced $Q_{10}$ (Table 3), irrespective of rising or decreasing temperatures. Over the prolonged incubation period, the values of $Q_{10}$ increased in all grassland types.

3.3. Influencing factors for $R_s$ and $Q_{10}$ under rising and decreasing temperatures

On average, $R_s$ was significantly correlated with DOC, MBC, MBN, MBC/MBN, pH, and COND (all $P < 0.001$) under both rising and decreasing temperatures (Table 4); however, $R_s$ was not significantly associated with ORP. Furthermore, $Q_{10}$ was significantly correlated with DOC, MBC, MBN, pH, COND ($P < 0.001$), and MBC/MBN ($P < 0.05$) under both rising and decreasing temperatures (Table 4).

Based on the SEM analyses, microbes (MBC, MBC/MBN), substrate (DOC), and soil chemical properties (pH, ORP) explained 76% of the variation in $Q_{10}$ at rising temperature (Fig. 5A). The most important factors were microbes ($R^2 = 0.77$), soil substrate ($R^2 = 0.15$), and chemical properties ($R^2 = 0.04$). Similarly, all three factors explained 75% of the variation in $Q_{10}$ at decreasing temperature (Fig. 5B). In this case, the important variables were soil microbes ($R^2 = 0.46$), soil substrate ($R^2 = 0.35$), and soil chemical characteristics ($R^2 = 0.10$).

4. Discussion

4.1. Asymmetric responses of soil heterotrophic respiration to rising and decreasing temperatures

$R_s$ exhibited asymmetric responses to rising and decreasing temperatures. Supporting previous studies (Buchmann, 2000; Li et al., 2015), exponential equations represent a good fit for the response of $R_s$ to changing temperatures in soils. One potential explanation for this phenomenon was that higher temperature increase microbial activity, which, in turn, enhances $R_s$ (the basis of most models) (Kirschbaum, 2010). Microbial groups in different micro-habitats responded differently to the same magnitude of temperature change in control experiments (Wu et al., 2010). The global warming trend makes it crucial to understand how asymmetric warming affects $R_s$. Liu et al. (2006) found that a distinct day/night temperature-independent pattern exists in soil respiration during the growing season. Thus, these researchers suggested that night-time measurements used to extrapolate soil respiration during the daytime might underestimate daytime soil respiration. Similarly, Niu et al. (2011) observed a parabolic-like pattern of net ecosystem exchange (NEE) in response to temperature change during both spring and autumn. However, at similar temperatures, NEE was considerably depressed during the decreasing temperature season compared to the increasing temperature season. Most previous studies have only considered soil respiration during the daytime, and tended to overlook night-time effects (Koskinen et al., 2014).

Different responses of $R_s$ to rising and decreasing temperatures result in an elliptical hysteresis loop. For rising temperatures, $R_s$ increased, but decreased for decreasing temperatures. However, $R_s$ at any given temperature was higher for rising temperature compared to decreasing temperature, supporting previous results (Niu et al., 2011; Phillips et al., 2011). Phillips et al. (2011) observed a semieliptical hysteresis loop when surface flux was plotted as a function of soil temperature. This phenomenon might be explained by C substrate supply and atmospheric CO$_2$ concentration, which also alter the lag times and hysteresis responses to varying degrees. Vargas and Allen (2008) reported that seasonal shifts influenced the diurnal hysteresis effects of $R_s$, suggesting that other biophysical mechanisms also regulate diurnal patterns of $R_s$ to some extent, not just soil temperature. The diurnal pattern of $R_s$ might depend on an interaction with the microbial physiological response. Moreover, some studies have demonstrated that ecosystem respiration also exhibits hysteresis, which might be attributed to diurnal changes in temperature (Pingintha et al., 2010). However, the mechanistic explanation for this pattern remains unclear, even though several physical and biological possibilities exist (Tang et al., 2005; Vargas and Allen, 2008). Further studies are required to explain the hysteresis effect and to emphasize its over- or underestimation for the emission of $R_s$.

In this study, temperature significantly influenced $R_s$ at different stages of incubation. In particular, $R_s$ was greater for rising temperatures compared to decreasing temperatures in all three alpine grasslands. A potential explanation for this trend is that, with rising temperature, the enzyme activity of soil microbes increases, resulting in the microbial community possibly adapting to a warming environment and, hence, increasing $R_s$. Conversely, during periods of decreasing temperature, soil enzyme activity decreases, which leads to a drop in $R_s$ (Koch et al., 2007). Furthermore, microbial adaptations to temperature might explain the asymmetric responses of soil heterotrophic respiration to diurnal, seasonal, and inter-annual asymmetric changes in temperature (Bradford et al., 2008).

4.2. $Q_{10}$ is higher for decreasing temperatures compared to rising temperatures

In this study, $Q_{10}$ was higher for decreasing temperatures (2.42) compared to rising temperatures (1.55) in all three alpine

| Property    | $R_{s-Ave}$ (RT) | $R_{s-Ave}$ (DT) | $Q_{10-Ave}$ (RT) | $Q_{10-Ave}$ (DT) |
|-------------|----------------|----------------|----------------|----------------|
| Substrate   |                |                |                |                |
| DOC         | 0.37           | <0.001         | 0.70           | <0.001         | 0.60           | <0.001         | 0.67           | <0.001         |
| Microbe     |                |                |                |                |
| MBC         | 0.46           | <0.001         | 0.44           | <0.001         | 0.64           | <0.001         | 0.50           | <0.001         |
| MBN         | 0.64           | <0.001         | 0.82           | <0.001         | 0.83           | <0.001         | 0.78           | <0.001         |
| MBC/MBN     | 0.21           | <0.001         | 0.23           | <0.001         | 0.19           | 0.002          | 0.37           | 0.002          |
| Chemistry   |                |                |                |                |
| pH          | 0.45           | <0.001         | 0.73           | <0.001         | 0.70           | <0.001         | 0.66           | <0.001         |
| COND        | 0.58           | <0.001         | 0.68           | <0.001         | 0.53           | <0.001         | 0.31           | <0.001         |
| ORP         | 0.02           | 0.342          | 0.10           | 0.038          | 0.14           | 0.011          | 0.13           | 0.038          |

* DOC, soil dissolved organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; ORP, oxidation-reduction potential; and COND, conductivity. $R_{s-Ave}$ and $Q_{10-Ave}$ were averages of $R_s$ and $Q_{10}$ under rising and decreasing temperature treatments, respectively. RT, rising temperature treatment; DT, decreasing temperature treatment.
We speculated that this difference is due to the temperature dependence in microbial C use efficiency. Thus, microbial C use efficiency might decrease from rising temperature to decreasing temperature. However, we did not measure microbial growth rates, exudation rates, or C use efficiency. Our study differs from previous studies because most previous reports that describe the response of C to climate warming did not consider the asymmetric effect of altering temperature on Q_{10} during daytime and nighttime periods. Sampson et al. (2007) observed that large seasonal changes in Q_{10} were associated with larger seasonal changes in photosynthesis. This fluctuation might be explained by the extreme changes in temperature and photosynthesis that occur across seasons, leading to changes in soil microbial properties and, therefore, variation in Q_{10} throughout the year. More specifically, the soil microbial community differed in its sensitivity and adaptability to fluctuating temperatures. Within a certain temperature range, rising temperature increases soil enzyme activity and accelerates Rs, whereas decreasing temperature reduces Rs. During times of increased temperature, microbes consume labile substrate as an energy source, which requires less activation energy, and results in a lower Q_{10} (Lefèvre et al., 2014). In contrast, for lower temperatures, microbes consume more recalcitrant substrate, which...
requires greater activation energy, and leads to higher $Q_{10}$ (Craine et al., 2010a). In addition, microbes have physiological self-regulation (an automatic instinctive reaction to regulate themselves) that affects their adaptation to temperature change. Under increasing temperature, microbes have lower temperature adaptability, resulting in a gradual increase in enzyme activity, which leads to less activation energy and lower $Q_{10}$. However, under decreasing temperature, microbes must adapt to high temperature by physiological self-regulation, which requires more activation energy and produces higher $Q_{10}$ (Craine et al., 2010b; Xu et al., 2012). In a field experiment in Canada, Gaumont-Guay et al. (2009) found significant differences in $Q_{10}$: specifically, $Q_{10}$ was lower during the daytime when temperature was increasing, temperature, and was higher at night when temperature was decreasing. This trend might be explained by the fact that daytime water limitation inhibits C absorption within the ecosystem, through an indirect negative effect that offsets the positive response of increased temperature. In contrast, water limitation is reduced at night, failing to offset the increasing temperature that promotes $R_S$; consequently, $Q_{10}$ is greater under lower night-time temperatures (Xia et al., 2008). This difference might also be due to differences in C quality. When incubating samples with changing temperature (low-high-low $T: \pm 5^\circ C$), Xu et al. (2012) found that soils with lower C quality had higher $Q_{10}$. These results have been confirmed by others (Hartley and Ineson, 2008). Thus, there might be differences in C quality between daytime and night-time periods, leading to differences in $Q_{10}$. The difference in $R_S$ between day and night generates hysteresis responses. Thus, $Q_{10}$ estimates are probably related to the orientation of the hysteresis loops, and could be described as lag-time functions.

4.3. Soil microbial properties control the responses of $Q_{10}$

Soil microbes played a more important role in determining $Q_{10}$ than substrate and soil chemical properties, even though $Q_{10}$ values were also significantly correlated with DOC, ORP, and pH (Table 4). In fact, soil microbes were the most important factor for $Q_{10}$ irrespective of the direction of temperature change (Fig. 5). Chemical properties (pH, ORP, substrate (DOC), and temperature primarily exerted an influence on $Q_{10}$ by regulating microbial activity (Wang et al., 2016). Similarly, Wei et al. (2014) confirmed that $Q_{10}$ depends on the structure of the soil microbial community. Variation in soil microbial communities has a significant effect on $Q_{10}$ during prolonged incubation times. Some studies demonstrated that $Q_{10}$ is closely associated with microbial decomposition efficiency (Thiessen et al., 2013; Karhu et al., 2014), which is related to soil substrate quality, the number of microbes, and environmental factors (Wagai et al., 2013; Leifeld and Lützow, 2014; He and Yu, 2016). Craine et al. (2010a) found that when substrate quality is high, reactants need less activation energy, and leading to lower $Q_{10}$. Fierer et al. (2003) reported that the $Q_{10}$ values of subsurface soil were significantly higher (3.9) than that of the surface soil (3.0), mainly because of lower soil substrate quality in the subsurface layer. $Q_{10}$ values were positively correlated with soil pH during both rising and decreasing temperature trends. Soil pH affects soil enzymatic activity through the structure of soil microbial community, which in turn indirectly influences $Q_{10}$ (Craine et al., 2010b; Min et al., 2014). Soil base saturation increases with decreasing pH, which leads to the increased heterogeneity of microbes and increased enzyme activity. Consequently, a positive feedback loop is formed for soil heterotrophic respiration (Sinsabaugh et al., 2008; Min et al., 2014). In this way, the pH might indirectly reflect variation in $Q_{10}$. The $Q_{10}$ values of the alpine grassland were positively and significantly correlated with soil pH in this study. Most likely, increasing or decreasing temperatures changed the rate of ion diffusion and disrupted the diffusion potential balance. Consequently, because these changes were affected by different temperatures, they led to variations in $Q_{10}$. Previous studies have demonstrated that soil pH correlates positively with $Q_{10}$, and that enzyme activity varies within a specific pH range (Craine et al., 2010b). Therefore, soil pH could be used to determine $Rs$ indirectly. Soil pH is crucial to enzyme functioning; thus, acidification might affect microbial composition and activity, and therefore, the decomposition of SOM (Pastorelli et al., 2013).

We also observed that $Q_{10}$ was negatively correlated with ORP under both increasing and decreasing temperature trends in this study. ORP is the result of a redox reaction of oxide and reduzate, and might reflect the macroscopic oxidation-reduction of all matter in the system. ORP characterizes the relative strength of oxidation and reduction (Zona et al., 2011) and is, therefore, an important indicator for the regulation of soil biochemical processes (Ascard et al., 2008). With increasing temperature, both soil microbial activity and soil oxygen consumption accelerate, and the redox potential decreases (Zona et al., 2011). During soil heterotrophic respiration, microbes break down longer C chains by oxidation (or open the C rings), followed by a complicated biochemical process where soil organic matter is oxidized to CO2, and high redox potential leads to the further degradation of the C chains, which release more CO2 (Luo and Zhou, 2006; Lipson et al., 2010).

Some studies have found that biophysical parameters regulate soil respiration under daily and seasonal patterns (Vargas and Allen, 2008). Similarly, we found that soil microbes (MBC, MBC/MBN) played more important roles than soil substrate (DOC) or soil chemical properties (pH, ORP) for regulating $Rs$. Therefore, our findings provide further experimental support that soil microbes are important in different temperature models.

5. Conclusions

The incubation experiment presented here was novel in that potentially avoided the effect of adaptation by soil microbes and, thus, large differences in substrate quality. Specifically, the experiment combined periodic changes in incubation temperature (to simulate daytime and night-time) with equipment that had continuous measurement function. This method improved our ability to explore the response of $Rs$ to temperature change, leading to greater accuracy. A pronounced hysteresis of $Rs$ was observed during rising and decreasing temperatures, with the hysteresis loops differing among sites. When comparing the processes of rising temperature and decreasing temperature, the $Rs$ values were higher during rising temperature, whereas $Q_{10}$ values were lower during rising temperatures. These findings indicate an asymmetric response of SOM decomposition to periodic changes in temperature at diurnal, seasonal, and inter-annual scales. In conclusion, for models to capture the response of microbial respiration to varying temperatures, hysteresis must be incorporated into microbial responses to rising and decreasing temperatures.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.12.002.

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