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Warming Changed Soil Respiration Dynamics of Alpine Meadow Ecosystem on the Tibetan Plateau

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ABSTRACT

Alpine meadow system underlain by permafrost on the Tibetan Plateau contains vast soil organic carbon and is sensitive to global warming. However, the dynamics of annual soil respiration (R_s) under long-term warming and the determined factors are still not very clear. Using open-top chambers (OTC), we assessed the effects of two-year experimental warming on the soil CO_2 emission and the Q_{10} value (temperature sensitivity coefficient) under different warming magnitudes. Our study showed that the soil CO_2 efflux rate in the warmed plots were 1.22 and 2.32 times higher compared to that of controlled plots. However, the Q_{10} value decreased by 45.06% and 50.34% respectively as the warming magnitude increased. These results suggested that soil moisture decreasing under global warming would enhance soil CO_2 emission and lower the temperature sensitivity of soil respiration rate of the alpine meadow ecosystem in the permafrost region on the Tibetan Plateau. Thus, it is necessary to take into account the combined effect of ground surface warming and soil moisture decrease on the R_s in order to comprehensively evaluate the carbon emissions of the alpine meadow ecosystem, especially in short and medium terms.

1. Introduction

Alpine zones are usually characterized with a long seasonal surface soil freezing and a short vegetation period. Soil organic carbon stored in the alpine soils is huge due to low decomposition

rates^[22,23]. However, these regions would be undergone severe impact due to a higher rate of temperature increase under global warming^[17]. For the Tibetan Plateau, the alpine meadow ecosystem with $6.37 \times 10^5 \text{ km}^2$ area, (~50 % of total alpine grassland area) holds 11.3 Pg of carbon (C)^[35]. The C loads in the alpine

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soils are significantly higher compared to those in the warmer soils^[10]. Therefore, any small change in this soil C pool can possibly trigger the atmospheric CO₂ concentration surging and thus the dynamics of global climate^[2,7,9,12,13,18,44].

Since 1850, the world mean temperature has been rising and will increase by a further 1.8 – 4.0 °C by the end of this century^[29,42,17]. The temperature of the ecological alpine zone of the Tibetan Plateau has also been rising at a rate of 0.32 °C/10a in the recent 30 years^[26,39]. Moreover, study predicted that this region would experience greater warming in the future^[17]. So future climatic warming will trigger a sharp release of this C reservoir by R_s , thereby altering the alpine meadow ecosystem from a net carbon sink to a net source of atmospheric CO₂^[3,55]. Studies also have shown that climate warming would reduce species richness in alpine meadows and alter the above- and belowground productivity of the Tibetan Plateau^[20,21].

To understand the mechanisms controlling soil respiration of the alpine meadow ecosystem, many experimental studies have been conducted in-situ^[5,8,33,22,53]. However, the results are significantly different from site to site. Most of the investigations focus on the temperature dependency of C mineralization and evaluate Q_{10} ^[1,5,41]. A little information on the role of soil moisture in controlling R_s also exists^[7,9], which is not enough to derive any specific relationship, especially when combined with near surface warming in high altitude regions (underlain by permafrost). In addition, the R_s during the growing seasons is well studied^[8,53], but the temporal patterns of R_s in non-growing season and the determined factors are not very clear.

Therefore, in this research, we conducted a comprehensive experimental study to better understand the response of soil C under different warming magnitudes. We increased the near-surface air temperature with different amplitudes of the alpine meadow ecosystem on the Tibetan Plateau in situ for two years. The temporal variations of soil CO₂ efflux rate, soil temperature and moisture at different depths, and correlations among them were examined carefully. Our objectives were to determine (1) how the R_s responded to warming with different temperature magnitudes (2) how the Q_{10} of R_s in the alpine meadow ecosystem changed as the amplitude of warming increased and (3) how the surface temperature and moisture of soil regulated R_s in the alpine meadow ecosystem underlain by permafrost.

2. Materials and methods

2.1 Study Site

The experiment was carried out in the Beiluhe region (34° 49' 25.8" N, 92° 55' 45.1" E), distributed with alpine meadow ecosystem, on the Tibetan Plateau (Figure 1). The study site is representative with an area about 151.6 km² and the altitude ranges from 4600m to 4800 m. The climate is frigid and dry with the frozen duration from October to April of the next year. The mean annual temperature is -3.60 °C and the annual precipitation is about 423.79 mm^[46]. The dominant species are *Kobresia pygmaea*, *K. humilis*, *Sergievskaja*, *K. capillifolia*, and *C. scabriostris*^[40,54].

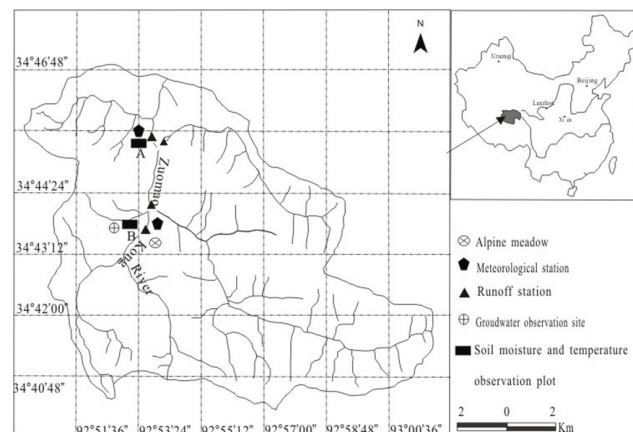


Figure 1. Location of the experimental site at the Beiluhe region on the Tibetan Plateau

2.2 Experimental Setup

The experiment was carried out in a selected alpine meadow area, where the vegetation coverage was above 70%. We used a passive warming device, open-top chamber (OTCs), to create an artificially warmed environment^[32,30]. In October 2010, five OTCs with height of 40 cm and coverage area of 0.98 m² and another five ones with height of 80 cm and coverage area of 2.01 m² were installed. Five control plots with area of 1 m² (100 cm × 100 cm) were also set up near the OTCs.

The air relative humidity and temperature at height of 20 cm above the near surface were determined (Vaisala HMP45AC, Finland) in both OTCs and control plots. Soil temperatures were measured at 5, 20, and 40 cm depths using thermistor sensors. The accuracy and resolution of these sensors were ± 0.05 °C and 0.01 °C respectively after calibrated. Soil moistures in the OTCs and control plots were also measured at the depths of 5, 20, and 40 cm using calibrated sensors (EC-5, Decagon USA). These measurements were automatically recorded with

a CR1000 datalogger (Campbell Scientific, Logan, UT, USA) at 1 h intervals. The distance between the OTCs and the control plots ranged from 8m to 10 m. Thus three group plots with different temperature increments were established in the alpine meadow site: (1) alpine meadow plots with no warming treatment (Control), (2) alpine meadow plots with 40 cm-high OTCs treatment (OTC1), (3) alpine meadow plots with 80 cm-high OTCs treatment (OTC2). In each plot, a collar of 20cm internal diameter and 10cm height made with polyvinyl chloride (PVC) was inserted into the soil with 2cm offset.

Soil CO₂ efflux rate was determined using a LI-8100A automated soil gas flux system (LI-COR, Lincoln, NE, USA). The live aboveground vegetation within the soil collars was pruned away 24h prior to each measurement [24]. Soil CO₂ emission rate was measured with five replicate collars in each treatment plot. Measurement was carried out once every five days in the growing months (May- September) and every ten days in the non-growing months (January- April and October - December). For each measurement, the period between 09:00 and 12:00 h Beijing standard time (BST) was chosen to minimize daily variations in R_s . The R_s was represented by the average of five replicates of each treatment and represented the daily mean soil CO₂ efflux.

2.3 Soil and Biomass Sampling and Analysis

At the start of the experiment in late October 2010, soil samples (100 cm³) were collected randomly at the 0 – 10cm and 10 – 20cm depths beside each plot and stored in a refrigerator for further analysis. To determine the aboveground biomass, all the plants in a 0.25 m² area were clipped from the plot selected randomly and were stored with paper bags, and then were air-dried and weighted. To determine the belowground biomass, the soil and root were dugged out above the 40 cm depth and wrapped with gauze and completely rinsed using tap water, and then the roots were air-dried and weighted. All the vegetation samples were oven dried (48 h, 80 °C) before weighing. Soil bulk density was determined using cutting rings with 5.3cm in diameter. Soil organic matter content was measured by calculating loss on ignition (550 °C, 8 h) [16]. Total nitrogen (N) content was analyzed using the Kjeldahl method [4].

2.4 Statistical Analysis

The differences among the plots with different warming treatments were evaluated using one-way analysis of variance (ANOVA) and least significance difference (LSD). Two-way ANOVA was applied to examine the

impacts of different warming increments on R_s , soil temperature and moisture during the whole experimental period. To examine correlations among the air temperature, soil temperature and moisture, and the R_s , the Pearson correlation was carried out. In addition, to test the dependency of R_s on soil temperature and moisture, exponential regression was implemented. According to the regression analysis results, an exponential curve of the form $y = \beta_0 e^{\beta_1 x}$ was applied, where y was $t R_s$, β_0 and β_1 were fitted constants and T was the 5 cm soil temperature. To compare dependency of R_s on soil temperature in each warming treatment, Q_{10} values were calculated, where $Q_{10} = e^{10 \beta_1}$. All tests were done at the 5 % level of significance and all statistical analyses were performed with Origin software (Origin 8.0, OriginLab Corporation, USA).

3. Results

3.1 Property Difference between Treatment Plots

Statistical analysis found that the biomass and soil properties sampled beside the control, OTC1 and OTCs plots were similar (variance analysis, $F < 5$, $P = 0.05$) when the experiment was carried out (Table 1). In all plots, the soil bulk density, soil organic carbon, and total N content at the 10-20cm depth were higher than those at the 0-10cm depth. The belowground biomasses were much greater than those of aboveground.

Table 1. Biomass and soil properties at the different warming treatment plots

Variable	Depth (cm)	Control plots	OTC1 plots	OTC2 plots	Significance
Bulk density (g cm ⁻³)	0–10	0.89 ± 0.2	0.87 ± 0.3	0.85 ± 0.4	n.s.
	10–20	0.98 ± 0.1	1.01 ± 0.1	0.97 ± 0.2	n.s.
Soil organic C (kg m ⁻²)	0–10	0.48 ± 0.06	0.47 ± 0.04	0.51 ± 0.05	n.s.
	10–20	1.32 ± 0.04	1.35 ± 0.02	1.29 ± 0.05	n.s.
Soil total N (g m ⁻²)	0–10	41.3 ± 7.2	40.1 ± 6.7	40.8 ± 5.9	n.s.
	10–20	117.6 ± 12.8	119.4 ± 9.4	115.3 ± 10.4	n.s.
Above-ground biomass (kg m ⁻²)		0.33 ± 0.04	0.35 ± 0.02	0.32 ± 0.03	n.s.
Below-ground biomass (kg m ⁻²)		2.41 ± 0.4	2.37 ± 0.2	2.43 ± 0.3	n.s.

Note: n.s.: no statistical significance; Values are means (n = 5) ± standard deviation (SD)

3.2 Warming Effects on Air Temperature and Soil Hydrothermal Properties

The air temperatures between the OTC2, OTC1, and Control plots showed an obvious difference from each other at a 0.05 level (ANOVA, $F = 57$, $P = 0.001$). The warming magnitudes at the OTCs plots varied largely during the study period (Figure 2). Daily mean air temperature in the OTC2 and OTC1 plots were always higher than that in the Control plots. However, the near-surface air temperature in the OTCs and Control plots had a similar change trend. In general, the lowest of air temperature in all plots appeared in December and the highest occurred in July.

Influenced by the air temperature rising in the OTCs plots, the soil temperatures at different measuring depths changed greatly (Figure 3). The larger magnitude of the temperature increased in the OTCs plots, the higher the soil temperature at different depths. The soil moistures in the OTCs plots also changed greatly due to the near-surface air temperature increase (ANOVA, $F > 23.08$, $P = 0.0001$). The higher the near-surface air temperature, the more decrease of the soil moisture content in the OTCs plots (Figure 4). The detailed impacts of experimental warming on the air temperature, soil temperature and soil moisture in this alpine meadow ecosystem can be referred to the authors' published work^[47].

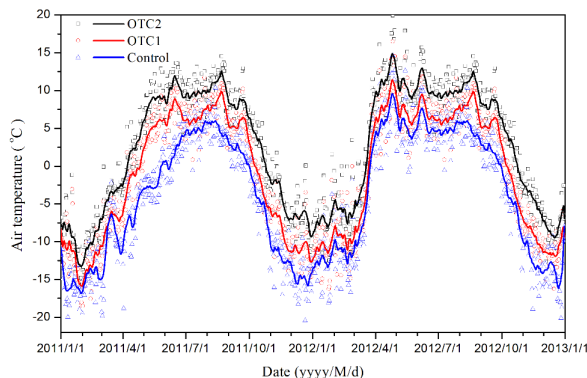


Figure 2. Near-surface air temperature variations in the different warming treatment plots of an alpine meadow ecosystem for two years

Note: Air temperature values are means of five plots every day and the lines are plotted with the adjacent-averaging method. Black open squares, red open circles, and blue open triangles are near-surface air temperatures in plots with different warming treatments. The corresponding solid lines with the same colors represent the mean variations in air temperatures.

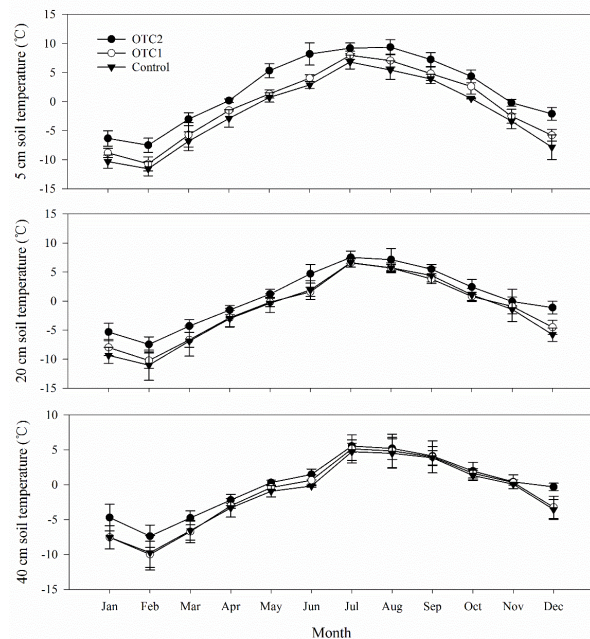


Figure 3. Monthly variations of soil temperatures at 5, 20 and 40 cm depths in an alpine meadow ecosystem with different warming treatments.

Note: Soil temperature values are means of each month of two years in five plots. Bars represent the corresponding standard errors of the means.

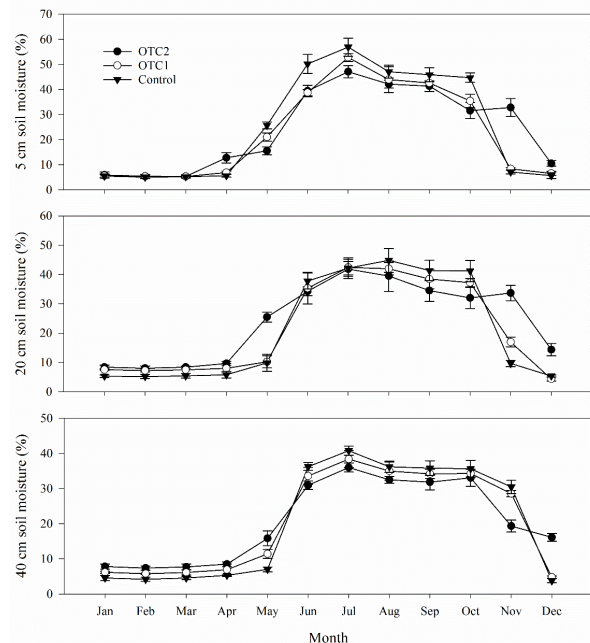


Figure 4. Monthly variations of soil moistures at 5, 20 and 40 cm depths in an alpine meadow ecosystem with different warming treatments

Note: Soil moisture values are means of each month of two years in five plots. Bars represent the corresponding standard errors of the means.

3.3 Dynamics of R_s in Different Warming Treatment Plot

The dynamics of soil CO₂ efflux rate at different warming treatment plots are shown in Figure 5. The warming effects on R_s appeared to be most pronounced during the warm seasons (May to September), where significant differences existed at a 99 % confidence level ($p \leq 0.005$). In the cold seasons (January to April and October to December), the R_s differed marginally among the OTC2, OTC1 and Control plots ($0.05 \leq p \leq 0.07$).

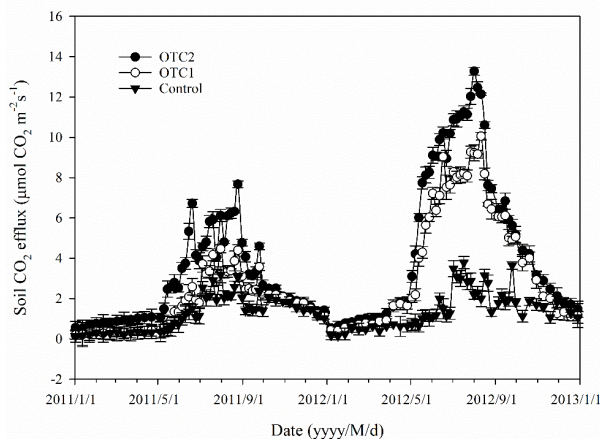


Figure 5. Variations of soil CO₂ efflux rate in an alpine meadow ecosystem with different warming treatments in two years

Note: Symbols are means ($n = 5$), and bars represent the corresponding standard errors of the means.

All the R_s in the different warming treatment plots started to rise from April and reached to the maximum in August. Thereafter it began to decrease gradually and came to the minimum in December (Figure 5). In 2011, the monthly mean R_s in the OTC2, OTC1 and Control plots attained their maximums of 5.9, 3.7 and 2.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, in August. Then they began to decrease gradually and came to the minimums of 0.6, 0.2 and 0.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, in December (Table 2). The dynamics of R_s in 2012 was similar with that in 2011 but the maximums of R_s in 2012 were much greater.

The difference of inter-annual variation in R_s in different warming treatment plots was significant ($p \leq 0.05$). As the duration of experimental warming extended, the maximums of the R_s showed an increase trend. For example, in 2011 the maximums of the monthly mean CO₂ efflux rate were 5.9 in the OTC2 plots and 3.7 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the OTC1 plots, but the corresponding maximums of R_s in the OTC2 and OTC1 plots reached 11.2 and 8.3 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in 2012 (Table 2). For the Control plots, the maximums of R_s in 2011 and 2012 were 2.33 and 2.17 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. The R_s also showed an obvious difference among different months for the same warming treatment during the experimental period (Table 2). In 2011, the annual amplitudes of R_s were 5.38, 3.45 and 2.28 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, in the OTC2, OTC1 and Control plots. While in 2012, the corresponding annual amplitudes of R_s in the OTC2, OTC1, and Control plots were 10.57, 7.87 and 2.99 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. The coefficients of temporal variation (CV) of R_s in the OTC2, OTC1 and Control plots in 2011 and 2012 were 72.4, 76.7, 71.5% and 84.5, 83.2, 64.7%, respectively.

3.4 Impacts of Soil Temperature on R_s

For the alpine meadow ecosystem with different warming treatments, the dynamics of R_s was regulated by the soil temperatures greatly and the R_s showed a significant positive relation to the 5 cm soil temperature (Figure 6, $P < 0.05$). Soil temperature was an important explanatory variable in controlling the R_s for the different warming treatments. By comparatively analyzing the R_s among the different warming treatments (OTC2, OTC1, and Control), the dynamics of R_s were significantly different among the different warming treatments ($p < 0.05$).

Table 2. Monthly soil respiration flux, the corresponding amplitude of variation and coefficients of temporal variation in an alpine meadow with different warming treatments in two years

Year	Treatment	Soil CO ₂ flux (μmol CO ₂ m ⁻² s ⁻¹)													
		Jan.	Feb.	March	April	May	June	July	August	Sep.	Oct.	Nov.	Dec.	Amplitude	CV(%)
2011	OTC2	0.6±0.3	0.8±0.3	0.9±0.2	1.1±0.3	2.2±0.7	4.5±0.5	5.2±0.1	5.9±0.1	3.2±0.2	2.4±0.1	1.9±0.2	1.5±0.1	5.38	72.4
	OTC1	0.2±0.4	0.3±0.3	0.3±0.2	0.4±0.3	0.9±0.2	2.0±0.3	3.7±0.5	3.5±0.2	2.6±0.2	2.2±0.1	1.8±0.4	1.3±0.1	3.45	76.7
	Control	0.2±0.3	0.3±0.3	0.3±0.4	0.3±0.2	0.5±0.16	1.2±0.32	2.5±0.3	2.3±0.4	1.6±0.2	1.9±0.2	1.6±0.4	1.2±0.2	2.28	71.5
2012	OTC2	0.6±0.2	0.9±0.1	1.1±0.2	1.7±0.2	6.2±0.3	9.6±0.4	11.2±0.6	10.6±0.2	6.1±0.3	3.9±0.3	2.5±0.2	1.7±0.4	10.57	84.5
	OTC1	0.5±0.1	0.7±0.1	0.8±0.1	1.7±0.3	4.0±0.3	7.5±0.4	8.3±0.3	8.3±0.3	5.5±0.7	3.6±0.4	1.8±0.3	1.2±0.3	7.87	83.2
	Control	0.2±0.2	0.5±0.2	0.5±0.1	0.7±0.3	0.9±0.4	1.4±0.5	3.2±0.4	2.9±0.3	2.2±0.1	1.6±0.4	1.5±0.1	1.4±0.2	2.99	64.7

Note: Values are means ($n = 5$) \pm SD

ontrol), it showed that the R_s and the 5cm soil temperature had the same changing trend, and the higher the 5cm soil temperature increased, the greater the soil CO_2 efflux rate. At the OTC2 plots, the R_s increased from 0.53 to 13.28 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as the 5cm soil temperature rose from -7.35 to 11.81 $^{\circ}\text{C}$; while at the OTC1 plots, when the 5cm soil temperature increased from -9.29 to 10.96 $^{\circ}\text{C}$, the corresponding R_s increased from 0.19 to 10.06 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The CO_2 efflux rates at the OTC2 and OTC1 plots were 0.93 to 9.39 and 0.69 to 6.66 times greater than that at the Control plots, respectively.

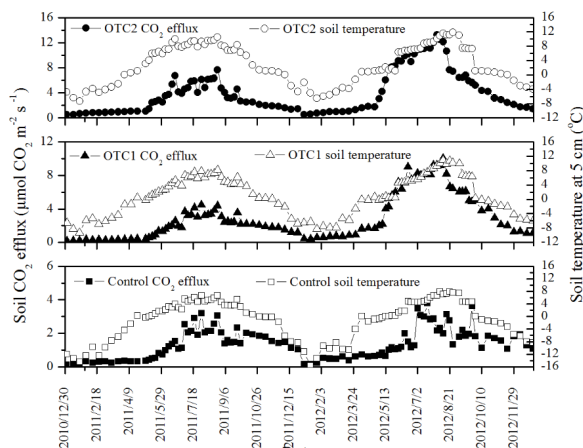


Figure 6. Dynamics of R_s and the 5cm soil temperature during the experimental period at the different warming treatment plots. Symbols are means ($n = 5$)

However, the inter-annual variations of R_s at the experimental warming plots (OTC2 and OTC1) were significantly different between 2011 and 2012 although the 5cm soil temperatures changed gently inter-annually. As the experimental warming period extended, the soil CO_2 efflux rates showed an apparent increasing trend especially in the growing seasons (May to September) ($P < 0.05$). Experimental warming increased daily mean R_s by 0.96 ~ 3.26 times in 2012 than that in 2011 at the OTC2 plots; at the OTC1 plots, the increase multiples of R_s varied between 0.71 and 5.55. Whereas at the Control plots, the R_s showed no significant interannual variation ($P > 0.05$).

The correlation of the 5cm soil temperature and the R_s was fitted with an exponential model, which could explain 58 % – 65% of variations in soil respiration (Figure 7). As soil depth increased, the dependency of soil respiration on temperature declined ($P > 0.05$). The Q_{10} values were 3.67, 4.06 and 7.39, respectively, at the OTC2, OTC1 and Control plots, which tended to decline as the amplitude of near-surface air temperature increased (Figure 7).

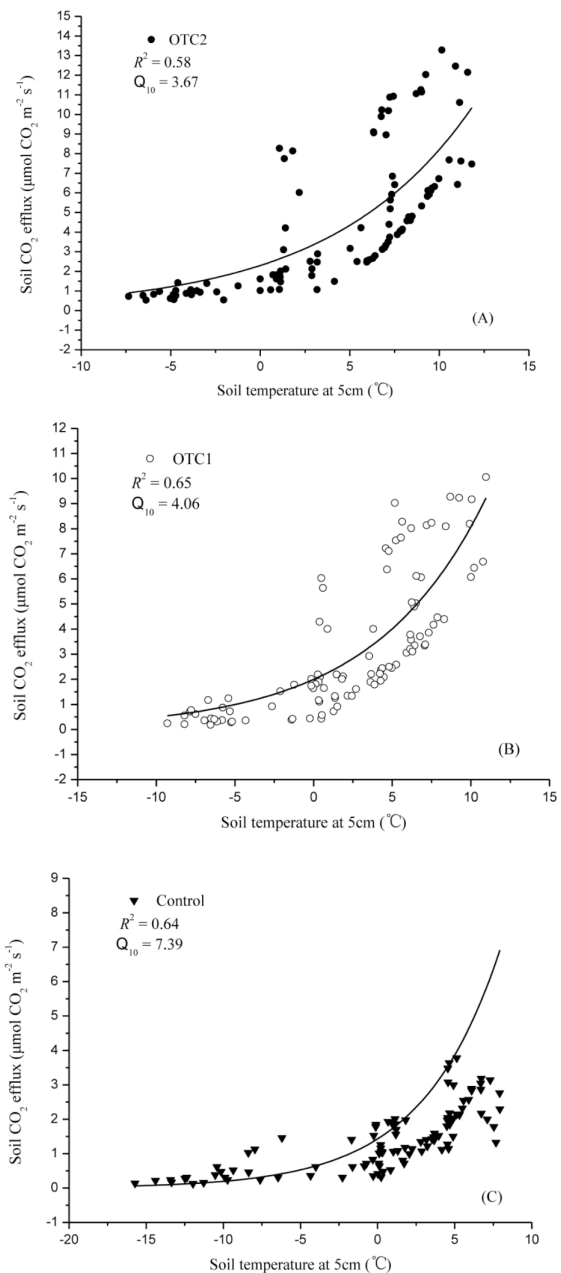


Figure 7. Correlation of the R_s and the 5cm soil temperature during the experimental period at the different warming treatment plots (A-OTC2, B-OTC1, and C-Control)

3.5 Impacts of soil moisture on R_s

The soil moisture also affected R_s significantly in the alpine meadow ecosystem with different warming treatments ($P < 0.05$, Figure 8). In the Control, OTC1 and OTC2 plots, the dynamics of R_s was with that of soil moisture at the 5cm depth during 2011 and 2012. By comparatively analyzing the soil moisture variations in the

Control, OTC1 and OTC2 plots, it showed that the more the soil moisture at 5 cm declined, the greater the R_s increased. However, compared the variations of R_s in 2011 with that in 2012, the soil CO_2 emission rate showed an obvious increasing trend under the condition that the 5cm soil moisture had no significant changes in the Control, OTC1 and OTC2 plots.

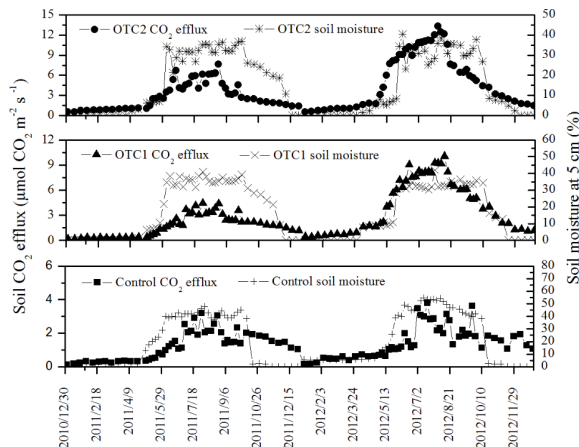


Figure 8. Dynamics of R_s and the 5cm soil moisture during the experimental period in the different warming treatment plots. Symbols are means ($n = 5$).

Although the soil moisture decreased as the amplitude of the near-surface air temperature increased, the intensity and variation amplitude of R_s improved. Correlation analysis indicated that the relationship between the R_s and the 5cm soil moisture accorded with an exponential model ($y = a_0 e^{a_1 M}$) in the different warming treatment plots, where y was the R_s , a_0 and a_1 were fitted constants and M was the 5cm soil moisture. The model explained 64 % – 68% of variations in soil respiration (Figure 9). As the soil deepened, the correlation of the R_s and the soil moisture content weakened ($R^2 < 0.4$).

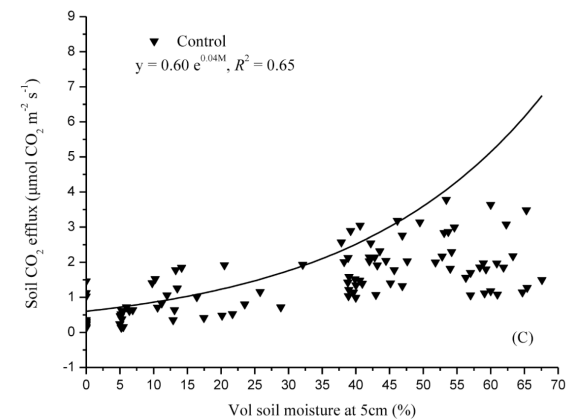
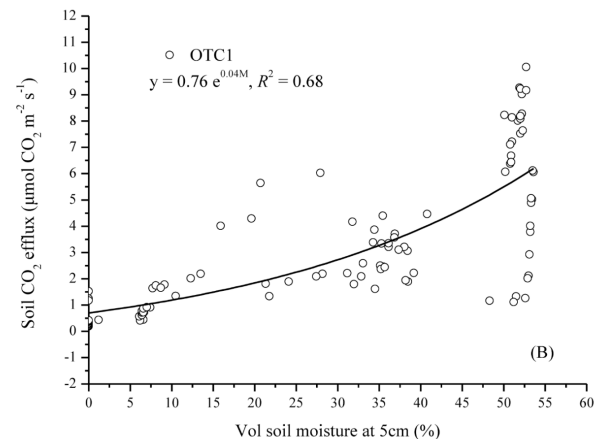
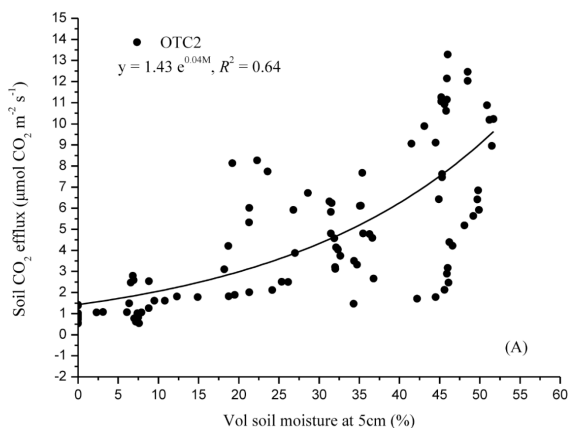


Figure 9. Correlation of the R_s and the 5cm soil moisture during the experimental period in the different warming treatment plots (A-OTC2, B-OTC1, and C-Control).

4. Discussion

The ground surface was passively warmed by the OTCs via trapping solar radiation^[45]. Although the OTCs had a warming effect both in the day and night, it primarily happened in the daytime due to the higher levels of solar radiation^[30]. The amplitudes of the experimental warming treatments in this study were 6.33 (OTC2) and 2.96 °C (OTC1), respectively. Meanwhile, the temperature increments via the OTCs in our study were greater than that reported by the studies in the arctic region^[31,36,48]. This may be attributed to the strong solar radiation on the Tibetan Plateau. In the OTCs plots, the greater air temperature increment resulted that the surface soil temperature increased much higher and the soil moisture content declined much greater in the experimental period. During our experimental warming, the increment of soil temperature at the 5cm depth was 0.61 – 5.72 °C, being similar with the study result in Northern Tibet by Lu et al^[27]. The decrease in soil moisture of 11.8 – 20.5 % at the depth 5 cm was comparable with the reported studies^[15,31,36,48].

This study showed quite a homogenous warming of the soil down to a depth of at least 40 cm, where more than 95 % of the roots and most of the labile C in the organic layer were located. As a consequence, the increases of shallow soil temperature and a decrease of soil moisture would cause great impact on the R_s of the alpine meadow ecosystem on the Tibetan Plateau.

Global warming was predicted to firstly cause effect on the C reservoir of the alpine and tundra ecosystems distributed in the high latitude and the high altitude regions^[25]. It was reported that warming caused the R_s to increase by 9.2 to 80 % on the Tibetan Plateau^[25,51]. Whereas, the increments of the R_s in our study were by 232 and 122 % in average at the condition of the near-surface warming by 6.91 and 3.59 °C, respectively. The effect of R_s promotion stimulated by warming in our study was more obvious compared with that reported elsewhere. This may be because the alpine meadow ecosystem on the Tibetan Plateau has adapted to the cold environment for a long time and is very sensitive to warming. Although the R_s was very low ($0.2 \mu\text{molm}^{-2} \text{s}^{-1}$) in the control plots, the warming of the near-surface air temperature stimulated and accelerated the root respiration and the soil microbial respiration, which resulted the soil CO_2 emitted intensely in the warming months. However, the mechanisms of how the experimental warming regulated the dynamics of the R_s in the alpine meadow ecosystem still remains unclear due to the soil microbial respiration and the root respiration are difficult to be distinguish presently. So, much more detailed researches should be conducted to segregate the R_s into microbial and root respiration. In addition, prolonged study periods are needed to elucidate the physiological responses of the components of R_s to warming for the alpine meadow ecosystem on the Tibetan Plateau.

Most studies have shown that the R_s was mainly controlled by the soil temperature and the soil moisture content, which were the two most important impact factors on the R_s dynamics^[6,27,52]. Our results also demonstrated that the R_s under the different warming treatments correlated significantly to the soil temperature and the moisture content on the Tibetan Plateau ($p < 0.05$, $R^2 > 0.5$). The temperature sensitivity coefficient of R_s was considered one of the most important parameters in evaluating the extent to which R_s was affected by temperature^[14]. The Q_{10} values of R_s in the different warming treatment plots at our experimental site were 3.67, 4.06 and 7.39 for the OTC2, OTC1, and Control, respectively. Our study demonstrated that the response of R_s of the alpine meadow ecosystem on the Tibetan Plateau to nearsurface warming was sensitive and rapid. The percentage of 58% – 65% variation in R_s could be explained by the change of temperature.

Whereas, the warming of the near-surface air temperature led to the decline in the Q_{10} , which suggested that the dependency of the R_s on temperature decreased as the amplitudes of experimental warming rose in the alpine meadow ecosystem on the Tibetan Plateau. The decrease in Q_{10} of R_s as the warming amplitude rose may be attributed to the following mechanisms: (1) the soil inside the OTCs plots was dried by warming, which reduced the root and microbial activity, and (2) the substrate was limited and the temperature sensitivity of the soil enzyme decreased when it was exposed to a warm environment in a short period of time^[19,28,43].

What's more, the R_s was low in the dry conditions because the soil drying could decrease the activity of the root and the soil microorganism and could inhibit its respiration via blocking the microbial to utilize the available substrate^[50]. However, the soil CO_2 emission increased to a maximum at the intermediate moisture levels until it began to decrease when moisture content excluded oxygen^[34,37]. A study showed that the soil moisture content together with the belowground biomass could account for 82 % of the R_s in an alpine grassland on the Tibetan Plateau^[11]. Whereas, although the soil moisture content decreased by 11.8% – 20.5 % at the 5cm depth and declined in different degree at the 20cm and 40cm depths in the warming treatment plots at our study site, the R_s was much higher in the OTCs plots than that in the control plots during the study period (Figure 5). This was probably because the experimental warming caused the soil moisture content to reach the optimum water content^[50] for the R_s and as a result more oxygen was diffused into the soil, which accelerated the aerobic respiration of the soil microorganisms at the OTCs plots.

In addition, the experimental warming changed the freezing and thawing process of the active layer, prolonged the thawing period, and increased the thawed depth at our alpine meadow ecosystem site^[47]. As a result, the microbial activities in the deep soil was activated^[38,49] and the C assimilated by the canopy was consumed remarkably as the lower frozen soil thawed, which then increased the supply of carbohydrates to the below-ground microorganisms. So the R_s of the alpine meadow ecosystem on the Tibetan Plateau would be accelerated by the long-term warming. The soil moisture decrease due to warming would be an important factor regulating the R_s process in the alpine meadow ecosystem on the Tibetan Plateau. Nevertheless, much effort should be paid on revealing the mechanism of R_s increasing as the soil moisture declined and on the threshold of the soil moisture regulating the R_s of the alpine meadow ecosystem on the Tibetan Plateau. In addition, how to distinguish the root respiration and the

soil microbial respiration under different controlled soil moisture condition of the alpine meadow ecosystem on the Tibetan Plateau is still a difficult issue.

5. Conclusions

In our study, the daily mean nearsurface temperature increased by 6.33°C and 2.96°C, respectively, at the OTC2 and the OTC1 plots compared with that at the Control plots. The temperature increase amplitude at the OTC2 plots was higher than the warming increment (3.8°C) predicted by the IPCC on the Tibetan Plateau by the end of 21st century^[17]. Our study clearly showed that the R_s of the alpine meadow ecosystem on the Tibetan Plateau was rapidly responsive to the nearsurface warming. Experimental warming resulted in the increase of soil CO₂ emission rate by approximately 232% and 122 % but the reduction of Q_{10} by 3.72 and 3.33, respectively, under the condition of the two different warming magnitudes in the alpine meadow ecosystem. This result suggests that the sensitivity of response of the soil CO₂ emission to temperature increase would weaken as global warming continues.

However, it is still uncertain whether the carbon losses by R_s would be offset by an increase of vegetation biomass for the alpine meadow ecosystem on the Tibetan Plateau. Thus, comprehensive studies, regarding photosynthetic carbon fixation, plant respiration, vegetation biomass dynamic and the distinction between root and microbial respiration, are needed to clarify the mechanism of carbon budget of the alpine meadow ecosystem in the scenario of global warming.

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References

- [1] Bekkua, Y.S., Nakatsubob, T., and Kume, A.. Effect of warming on the temperature dependence of soil respiration rate in arctic, temperate and tropical soils. *Applied Soil Ecology*, 2003, 22 (3): 205–210.
- [2] Bellamy, P.H., Loveland, P.J., Bradley, R.I., Lark, R.M., and Kirk, G.J.D.. Carbon losses from all soils across England and Wales 1978–2003. *Nature*, 2005, 437: 245–248.
- [3] Biasi C., Meyer H., and Rusalimova, O.. Initial effects of experimental warming on carbon exchange rates, plant growth and microbial dynamics of a lichen-rich dwarf shrub tundra in Siberia. *Plant Soil*, 2008, 307: 191–205.
- [4] Bremner, J.M., Sparks, D.L., and Page, A.L.. *Methods of soil analysis. Nitrogen-total part 3-chemical methods*, 1996, 1085–1121.
- [5] Chang, X.F., Zhu, X.X., and Wang, S.P.. Temperature and moisture effects on soil respiration in alpine grasslands. *Soil Science*, 2012, 177 (9): 554–560.
- [6] Contosta A.R., Frey, S.D., Cooper, A.B., et al.. Seasonal dynamics of soil respiration and N mineralization in chronically warmed and fertilized soils. *Ecosphere*, 2011, 2. DOI: 10.1890/ES10-00133.1
- [7] Cox, P.M., Betts, R.A., Jones, C.D., et al.. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature*, 2000, 408: 184–187.
- [8] Cui, S., Zhu, X., Wang, S., et al.. Effects of seasonal grazing on soil respiration in alpine meadow on the Tibetan plateau. *Soil Use and Management*, 2014, 30(3): 435–443.
- [9] Davidson, E.A., Janssens, I.A.. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 2006, 440: 165–173.
- [10] Fan, J.W., Zhong, H.P., Liang, B., et al.. Carbon stock in grassland ecosystem and its affecting factors. *Grassland of China*, 2003, 25 (6): 51–58.
- [11] Geng, Y., Wang, Y., Yang, K., et al.. Soil respiration in Tibetan alpine grasslands: belowground biomass and soil moisture, but not soil temperature, best explain the large-scale patterns. *PLoS One*, 2012, 7(4): e34968.
- [12] Griffis, T.J., Black, T.A., Gaumont-Guay, D., et al.. Seasonal variation and partitioning of ecosystem respiration in a southern boreal aspen forest. *Agric Forest Meteorol*, 2004, 125: 207–223.
- [13] Grogan, P., and Jonasson, S.. Temperature and substrate controls on intra-annual variation in ecosystem respiration in two subarctic vegetation types. *Global Change Biol*, 2005, 11: 465–475.
- [14] Gu, L.H., Hanson, P.J., Post, W.M., Liu, Q., et al.. A novel approach for identifying the true temperature sensitivity from soil respiration measurements, *Global Biogeochem*, 2008, 22, GB4009. DOI: 10.1029/2007GB003164
- [15] Hagedorn, F., Martin, M., Rixen, C., et al.. Short-term responses of ecosystem carbon fluxes to experimental soil warming at the Swiss alpine treeline. *Biogeochemistry*, 2010, 97: 7–19.
- [16] Heiri, O., Lotter, A.F., Lemcke, G., et al.. Loss on

- ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology*, 2011, 25: 101–110.
- [17] IPCC. Climate Change 2013: The physical science basis: Working Group I Contribution to the fifth assessment report of the intergovernmental panel on climate change. Cambridge Univ. Press, New York, USA, 2014.
- [18] Janssens, I.A., Lankreijer, H., Matteucci, H.G., et al.. Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biol*, 2001, 7: 269–278.
- [19] Kirschbaum, M.U.F.. Will changes in soil organic carbon act as a positive or negative feedback on global warming. *Biochemistry*, 2000, 48: 21–51.
- [20] Klein, J.A., Harte, J., Zhao, X.Q., et al.. Experimental warming causes large and rapid species loss, dampened by simulated grazing, on the Tibetan Plateau. *EL*, 2004, 7(12): 1170–1179.
- [21] Klein, J.A., Harte, J., Zhao, X.Q., et al.. Dynamic and complex microclimate responses to warming and grazing manipulations. *Glob Change Biol*, 2005, 11: 1440–1451.
- [22] Koch, O., Tscherko, D., Kandeler, E., et al.. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils, *Global Biogeochem*, 2007, 21, GB4017.
DOI: 10.1029/2007GB002983
- [23] Körner, C.. Alpine plant life: functional plant ecology of high mountain ecosystems. Springer, Berlin, 1999: 338.
- [24] Li, G.Y., and Sun, S.C.. Plant clipping may cause overestimation of soil respiration in a Tibetan alpine meadow, southwest China. *Ecological Research*, 2011, 26 (3): 497–504.
- [25] Lin, X.W., Zhang, Z.H., Wang, S.P., et al.. Response of ecosystem respiration to warming and grazing during the growing seasons in the alpine meadow on the Tibetan plateau. *Agricultural and Forest Meteorology*, 2011, 151: 792–802.
- [26] Liu, X.D. and Chen, B.D.. Climatic warming in the Tibetan Plateau during recent decades. *International Journal of Climatology*, 2000, 20: 1729–1742.
- [27] Lu, X.Y., Fan, J.H., Yan, Y., et al.. Responses of soil CO₂ fluxes to short-term experimental warming in alpine steppe ecosystem, northern Tibet. *PloS One*, 2013, 8(3): e59054.
DOI: 10.1371/journal.pone.0059054
- [28] Luo, Y.Q., Wan, S.Q., Hui, D.F., et al.. Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, 2001, 413: 622–625.
- [29] Malchair, S., Boeck, H.J., Lemmens, C.M., et al.. Do climate warming and plant species richness affect potential nitrification, basal respiration and ammonia-oxidizing bacteria in experimental grasslands? *Soil Biology and Biochemistry*, 2010, 42 (11): 1944–1951.
- [30] Marion, G.M., Henry, G.H.R., Freckman, D.W., et al.. Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology*, 1997, 3: 20–32.
- [31] Mertens, S., Nijs, I., Heuer, M., et al.. Influence of high temperature on end-of-season tundra CO₂ exchange. *Ecosystems*, 2001, 4: 226–236.
- [32] Molau, U., and Mølgaard, P.. ITEX Manual (Second edition). Printed in Denmark, 1996.
- [33] Moriyama, A., Yonemura, S., Kawashima, S., et al.. Environmental indicators for estimating the potential soil respiration rate in alpine zone. *Ecological Indicators*, 2013, 32: 245–252.
- [34] Moyano, F.E., Vasilyeva, N., Bouckaert, L., et al.. The moisture response of soil heterotrophic respiration: interaction with soil properties. *Biogeosciences*, 2012, 9: 1173–1182.
- [35] Ni, J.. Carbon storage in grasslands of China. *J Arid Environ*, 2002, 50: 205–218.
- [36] Oberbauer, S.F., Tweedie, C.E., Welker, J.M., et al.. Tundra CO₂ fluxes in response to experimental warming across latitudinal and moisture gradients. *Ecol Monogr*, 2007, 77: 221–238.
- [37] Orchard, V.A., and Cook, F.J.. Relationship between soil respiration and soil moisture. *Soil Biol Biochem*, 1983, 15: 447–453.
- [38] Reichstein, M., Tenhunen, J.D., Rouspard, O., et al.. Ecosystem respiration in two Mediterranean evergreen Holm Oak forests: drought effects and decomposition dynamics. *Funct. Ecol*, 2002, 16: 27–39.
- [39] Ren, H., Ma, G.H., Zhang, Q.M., et al.. Moss is a key nurse plant for reintroduction of the endangered herb, *Primulina tabacum* Hance. *Plant Ecol*, 2010, 209: 313–320.
- [40] Ren, J.Z.. Pratacultural science research methods. China Agriculture Press, Beijing, 1998: 56–28.
- [41] Rustad, L.E., Campbell, J.L., Marion, G.M., et al.. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, 2001, 126: 543–562.
- [42] Saier, M.H.. Climate change. *Water Air Soil Poll*, 2007, 181(1–4): 1–2.
- [43] Saleska, S.R., Harte, J., Torn, M.S., et al.. The effect of experimental ecosystem warming on CO₂ fluxes

- in a montane meadow. *Global Change Biol*, 2009, 5: 125–141.
- [44] Schipper, L.A., Baisden, W.T., Parfitt, R.L., et al.. Large losses of soil C and N from soil profiles under pasture in New Zealand during the past 20 years. *Global Change Biology*, 2007, 13: 1138–1144.
- [45] Stenstrom, A., and Jonsdbttir, I.S.. Effects of simulated climate change on phenology and life history traits in *Carex bigelowii*. *Nord. J. Bot*, 2006, 24: 355–371.
- [46] Sun, Z.Z., Liu, M.H., Wu, G.L., et al.. Characteristics of permafrost under a nonpenerative thermokarst lake in Beiluhe Basin on the Tibetan Plateau. *J. Glaciol Geocryol*, 2012, 34 (1): 37–42.
- [47] Wang, J.F., and Wu, Q.B.. Impact of experimental warming on soil temperature and moisture of the shallow active layer of wet meadows on the Qinghai-Tibet Plateau. *Cold Regions Science and Technology*, 2013, 90 (91): 1–8.
- [48] Welker, J.M., Fahnestock, J., Henry, G.H., et al.. CO₂ exchange in three Canadian High Arctic ecosystems: response to long-term experimental warming. *Global Change Biology*, 2004, 10: 1981–1995.
- [49] Wellock, M.L., Rafique, R., LaPerle, C.M., et al.. Changes in ecosystem carbon stocks in a grassland ash (*Fraxinus excelsior*) afforestation chronosequence in Ireland. *J Plant Ecol*, 2014, 7 (5): 429–438.
- [50] Wen, X.F., Yu, G.R., Sun, X.M., et al.. Soil moisture effect on the temperature dependence of ecosystem respiration in a subtropical *Pinus* plantation of south-eastern China. *Agricultural and Forest Meteorology*, 2006, 137: 166–175.
- [51] Xu, Z., Wan, C., Xiong, H., et al.. Initial responses of soil CO₂ efflux and C, N pools to experimental warming in two contrasting forest ecosystems, Eastern Tibetan Plateau, China. *Plant Soil*, 2010, 336: 183–195.
- [52] Zheng, Z., Yu, G., Fu, Y., et al.. Temperature sensitivity of soil respiration is affected by prevailing climatic conditions and soil organic carbon content: A trans-China based case study. *Soil Biol Biochem*, 2009, 41: 1531–1540.
- [53] Zhou, X., Wan, S.Q., Luo, Y.Q., et al.. Source components and inter-annual variability of soil CO₂ efflux under experimental warming and clipping in a grassland ecosystem. *Global Change Biol*, 2007, 13 (4): 761–775.
- [54] Zhou, X.M.. *Kobresia Meadow in China*. Science Press, Beijing, 2001: 188–206.
- [55] Knorr, W., Prentice, I.C., House, J.I., et al.. Long-term sensitivity of soil carbon turnover to warming. *Nature*, 2005, 433: 298–301.