

Soil respiration of alpine meadow is controlled by freeze-thaw processes of active layer in the permafrost region of the Qinghai-Tibet Plateau

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Abstract: Freezing and thawing action of the active layer plays a significant role in soil respiration (R_s) in permafrost regions. However, little is known about how the freeze-thaw processes affect the R_s dynamics in different stages of the alpine meadow underlain by permafrost in the Qinghai-Tibet Plateau (QTP). We conducted continuous *in-situ* measurements of R_s and freeze-thaw process of the active layer at an alpine meadow site in the Beiluhe permafrost region of QTP, and divided the freeze-thaw processes into four different stages in a complete freeze-thaw cycle, including summer thawing stage (ST), autumn freezing stage (AF), winter cooling stage (WC), and spring warming stage (SW). We found that the freeze-thaw processes have various effects on the R_s dynamics in different freeze-thaw stages. The mean R_s ranged from 0.12 to 3.18 $\mu\text{mol}/\text{m}^2\text{s}$ across the stages, with the lowest value in WC and highest value in ST. Q_{10} among the different freeze-thaw stages changed greatly, with the maximum (4.91 ± 0.35) in WC and minimum (0.33 ± 0.21) in AF. Patterns of R_s among the ST, AF, WC, and SW stages differed, and the corresponding contribution percentages of cumulative R_s to total R_s of a complete freeze-thaw cycle (1692.98 ± 51.43 gCO_2/m^2) were 61.32 ± 0.32 , 8.89 ± 0.18 , 18.43 ± 0.11 , and $11.29\pm 0.11\%$, respectively. Soil temperature (T_s) was the most important driver of R_s regardless of soil water status in all stages. Our results suggest that as climate change and permafrost degradation continue, great changes in freeze-thaw process patterns may trigger more R_s emissions from this ecosystem because of prolonged ST stage.

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30 **Keywords:** Soil respiration; Different freeze-thaw stage; Q_{10} ; Alpine meadow; Qinghai-Tibet Plateau

1 Introduction

Soil respiration (R_s) is a significant source in estimating terrestrial carbon budget under climate change. It is the second-largest source of carbon emissions to the atmosphere from the terrestrial ecosystem on a global scale (Bond-Lamberty and Thomson, 2010; Schlesinger and Andrews, 2000). In permafrost regions, R_s not only depends on the distribution of vegetation and the content of soil organic matter (SOM) (Ping et al., 2008; Grogan and Chapin III, 2000; Phillips et al., 2011; Jobbágy and Jackson, 2000), but also is regulated by the freeze-thaw process of active layer (Holleisen et al., 2011). Many studies have shown that the winter-time emissions contribute significantly to the

40 annual CO₂ balances. For example, the Arctic tundra ecosystem is becoming a consistent source of
CO₂ because CO₂ emission in winter offsets its uptake in growing season with progressive
permafrost thaw and active layer thickening (Celis et al., 2017). In Alaska, emissions of CO₂ from
tundra during early winter seasons increased by about 73% since 1975, and the Arctic ecosystem
45 has been a net source of CO₂ due to rising temperatures (Commane et al., 2017). For the sub-arctic
tundra ecosystem, the winter-time CO₂ loss has also been increasing due to sustained tundra
warming, and as a result the ecosystem's historical function is shifting away from a carbon sink to
a carbon source (Lüers et al., 2014; Webb et al., 2016). In permafrost regions in the northern
hemisphere, the amount of soil organic carbon (SOC) stored reaches 1832Pg (Ding et al.,
2015; Tarnocai, 2009), among which about 689 Pg distributes in the 0-1 m depth, 1035±150 Pg in
50 the 0-3 m depth and 648 Pg in the 3-25 m depth (Hugelius et al., 2014; Tarnocai et al., 2009). Due
to its high sensitivity to global warming and direct contribution to the atmosphere greenhouse gas
contents, carbon emission from permafrost regions has received worldwide attention (Tarnocai,
2009; Zimov et al., 2009).

Both active layer and whole permafrost distributed in the Arctic and mid-latitude alpine regions
55 are undergoing significant changes due to global warming (Jorgenson and Osterkamp, 2005). The
active layer, which acts as a buffer between permafrost and atmosphere, is highly sensitive and
responsive to climate change (Li et al., 2012). [The exchange of energy and water in permafrost
regions between the land and the atmosphere mainly occurs through the active layer.](#) However, in a
whole freeze-thaw cycle, the active layer will undergo a series of cooling, start freezing to fully
60 freezing, dropping in temperature, rising in temperature but still in frozen state, start thawing to fully
thawing, and rising in temperature but in thawed state (Jiao and Li, 2014). At different developing
stages of freeze-thaw cycling, the heat distribution and transmission in the active layer show
significantly different characteristics (Zhao et al., 2000). Thus the soil physicochemical properties,
microbial activities, and biogeochemical processes at different freeze-thaw stages are also different
65 from each other (Henry, 2007). As such, the dynamics of R_s emission at different freeze-thaw stages
may show apparent differences. Furthermore, the thawing of permafrost and the deepening of the
active layer will expose frozen organic carbon to microbial decomposition, and cause the previously
frozen SOC to become available for mineralization (Walz et al., 2017). This may accelerate a
positive permafrost carbon feedback to climate change (Schuur et al., 2008). A six-year study of
70 CO₂ flux in moist acidic tundra has shown that the active layer thickness is a key driver of NEE,
GPP, and ecosystem respiration (Celis et al., 2017). In high-altitude mountain regions, permafrost
thawing has caused the alpine tundra to release CO₂ from organic carbon stored for a long time to
the atmosphere, exacerbating climate change (Knowles et al., 2019). Therefore, permafrost must be
playing a significant role in carbon-climate feedbacks due to its intensity of climate forcing and its
75 size of the carbon pools (MacDougall et al., 2012; Schneider von Deimling et al., 2012).

The strength and timing of permafrost carbon feedback essentially depend on the freeze-thaw
process of the active layer and the distribution of SOC in permafrost regions. Therefore,
understanding the effects of freeze-thaw actions on R_s at different freeze-thaw stages is critical for
better predicting future climate changes. However, it is still unclear how the freeze-thaw actions at
80 different stages regulate the R_s.

The Qinghai-Tibet Plateau (QTP) of China has the largest extent of permafrost in the low-
middle latitudes of the world and is very sensitive to global climate change (Liu and Chen, 2000; Wu
et al., 2010). Soil organic carbon (SOC) pools in the permafrost regions of QTP were estimated to

85 be 160 ± 87 Pg, which is approximately 8.7% of those in the northern circumpolar permafrost region
(Mu et al., 2015). Recent years have witnessed dramatic changes in the freeze-thaw occurrence,
active-layer thickness, and near-surface permafrost temperature in QTP. In the permafrost regions
distributed with alpine meadow ecosystem in QTP between 2002 and 2012, the average onset of
spring thawing at 50-cm depth advanced by at least 16 days; the duration of thaw increased by at
least 14 days; the active-layer thickness increased by ~ 4.26 cm/a, and the near-surface permafrost
90 temperature at 6 m and 10 m depths increased by ~ 0.13 °C and ~ 0.14 °C, respectively (Wu et al.,
2015). Therefore, the R_s of the alpine meadow is anticipated to be influenced and changed
dramatically due to the variations of freeze-thaw occurrence, active-layer thickness, and near-
surface permafrost temperature.

We took *in-situ* measurements of R_s and freeze-thaw process of the active layer in an alpine
95 meadow from January 2017 to December 2018. The objectives were (1) to determine the dynamics
of the R_s during a complete freeze-thaw process of active layer; (2) to compare the R_s patterns among
the different freeze-thaw stages and their contribution to [total \$R_s\$ emission in a complete freeze-thaw
cycle](#) in this region; and (3) to establish a preferable R_s model to accurately predict the soil CO₂
emission of each freeze-thaw stages.

100 **2 Materials and methods**

2.1 Study site

The experiment was conducted in an alpine meadow ecosystem of the Beiluhe region (34° 49'
25.8" N, 92° 55' 45.1" E), in the hinterland of the QTP, China. The study site represents an area of
151.6 km², with an altitude of 4,600 – 4,800 m, which is underlain by [continuous](#) permafrost with
105 an active layer of 1.1-2.3 m. [The soil types in the study site are primarily classified as MatticGelic
Cambisols \(alpine meadow soil\) in Chinese taxonomy or as Cambisols in FAO/UNESCO taxonomy
\(Wang et al., 2014\).](#) The mean annual temperature is -3.60 °C, which is lower than that of most of
other areas in the QTP (Yin et al., 2017). The mean annual precipitation is 423.79 mm, 80% of which
falls [as rain, sometimes mixed with small hails](#) during the growing season (from May to September).
110 [In winter, little snow falls but is quickly blown away and sublimated off due to high wind and low
air temperature, so the study site is not persistently covered by snow.](#) The air pressure is
approximately 550 hPa. The alpine meadow represents the most common vegetation type in QTP,
which cover more than 70% of whole area (Wang and Wu, 2013; Zhang et al., 2015b). The alpine
meadow ecosystem mainly consists of cold meso-perennial herbs that grow in conditions where a
115 moderate amount of water is available, such as *Kobresia pygmaea* (C. B. Clarke), *Kobresia humilis*
(C. A. Meyer ex Trautvetter) *Sergievskaja*, *Kobresia capillifolia* (Decaisne) (C. B. Clarke), *Kobresia*
myosuroides (Villars) Fiori, *Kobresia graminifolia* (C. B. Clarke), *Carex atrofusca* *Schkuhr* *subsp.*
(minor (Boott) T. Koyama), and *Carex scabriostriis* (Kukenthal) (Chen et al., 2017). On-site
120 surveying and sampling of the experiment set-up showed that soil bulk density, soil organic carbon,
and total N content at the 10-20 cm depth were higher than those at the 0-10 cm depth. The depth
of the active layer was about 1.9 m. The belowground biomass was much greater than that of
aboveground. The average depth of vegetation main rooting zone was around 10 cm (Table 1).

Table 1 Biomass and soil properties at the experiment set-up

Chemical and biological characteristics	Depth (cm)	Values
Bulk density (g cm^{-3})	0–10	0.89 ± 0.2
	10–20	0.98 ± 0.1
Soil organic C (kg m^{-2})	0–10	0.48 ± 0.06
	10–20	1.32 ± 0.04
Soil total N (g m^{-2})	0–10	41.3 ± 7.2
	10–20	117.6 ± 12.8
Above-ground biomass (kg m^{-2})		0.33 ± 0.04
Below-ground biomass (kg m^{-2})		2.41 ± 0.4
Depth of vegetation main rooting zone (cm)		10 ± 3
Active layer depth (m)		1.90 ± 0.2

Values are means ($n = 5$) \pm standard deviation (SD)

2.2 Measurement of the freeze-thaw process of the active layer

In the study site, one flat terrain with vegetation coverage of above 70% was selected to establish the active layer observation site. According to active layer lithology and practical conditions, soil temperature and soil moisture probes were installed at different depths. The installation depths for the soil temperature probes were 5, 20, 50, 80, 120, 150, 180 and 230 cm, and the depths for the soil moisture probes were 5, 20, 50, 80, 120, 150 and 180 cm. Soil temperature was measured using thermistors made by the State Key Laboratory of Frozen Soil Engineering (SKLFSE, China) with the accuracy of $\pm 0.05^\circ\text{C}$. Soil moisture was measured using calibrated soil moisture sensors (EC-5, Decagon USA) with the accuracy of $\pm 0.02 \text{ m}^3\text{m}^{-3}$. Soil moisture measured using the EC-5 probe represents the volumetric water content of liquid water per total soil volume. These measurements were collected automatically every 30 min each day by a data logger (CR3000, Campbell Co., USA).

Utilizing the measurements collected by soil temperature probes and a data logger, soil hourly mean temperature (T_{avg}), maximum temperature (T_{max}), and minimum temperature (T_{min}) of each day at different depths were calculated. Assuming that the soil particle surface energy and the salinity of soil having no influence on the soil freezing temperature (Jiao and Li, 2014), the date on which hourly T_{avg} continued to be lower or higher than 0°C was regarded as the onset freezing or onset thawing date, respectively, according to the T_{avg} values (Yang et al., 2002). If T_{max} was greater than 0°C and T_{min} was less than 0°C in a single day, it was regarded that the soil was undergoing daily freeze-thaw process. That is, the soil absorbs heat and thaws during the daytime, and releases heat and freezes during the nighttime, showing a daily freeze-thaw cycling phenomenon. Based on these criteria, whole freeze-thaw process of active layer can be divided into different stages.

To calculate the freezing or thawing thickness of the active layer in the freeze-thaw process, freeze or thaw depth was estimated by linearly interpolating soil temperature profiles between two neighboring points above and below the 0°C isotherm (Wu et al., 2010). The freezing or thawing thickness of the active layer was estimated from daily soil temperature measurements.

2.3 Soil respiration measurement

For the measurements of R_s , six $5 \times 5\text{m}$ plots were randomly selected around the active layer observation site, and one polyvinyl chloride (PVC) collar (20 cm in internal diameter and 10 cm in

height) was inserted into each plot to a depth of 8 cm into the soil with a chamber offset of 2 cm before the soil froze. All the PVC collars were left in place until the end of the study. R_s flux was measured using an LI-8100A automated soil gas flux system (LI-COR Inc., Lincoln, NE, USA). A standard LI-COR® 20-cm head was applied for measurements with 10-ppm range set and the offset of the program was adjusted to 2 cm. A typical survey measurement protocol was adopted with an observation length of 2 minutes, deadband of 25 seconds, pre-purge of 30 seconds and post-purge of 45 seconds. The chamber volume and the IRGA volume were automatically calculated by the program. Living plants inside the collar were removed carefully on the soil surface at least one day before the measurement. R_s flux was measured for two years covering a complete freeze-thaw cycle of the active layer between 2017 and 2018.

R_s flux was determined once every two or three days during the thawing period and once every seven days during the freezing period due to harsh environmental conditions and lack of manpower. Measurements were taken between 9:00 and 11:30 a.m. local time on every sampling day to represent the daily average flux based on the diurnal measurements (Zhang et al., 2015a). At the same time, soil temperature (T_s) and soil volumetric water content (SWC) at 5cm depth were determined besides the collars using the thermocouple probe and the ECH2O soil moisture sensor (LI-COR, Lincoln, NE, USA) connected to the LI-8100A.

2.4 Temperature sensitivity and scaling for R_s at different freeze-thaw stage

For each freeze-thaw stages, the relationship between R_s flux and soil temperature or soil water content was determined by fitting to exponential and polynomial functions given in equations (1) and (2), respectively (Zhang et al., 2015a).

$$R_s = \beta_0 e^{\beta_1 T} \quad (1)$$

$$R_s = aSWC^2 + bSWC + c \quad (2)$$

where R_s is the measured soil respiration rate ($\mu\text{molm}^{-2}\text{s}^{-1}$); T and SWC are soil temperature and water content at 5cm depth, respectively; β_0 , β_1 , a , b and c are coefficients. The exponential relationship is commonly used to represent soil respiration and soil carbon efflux as functions of temperature (Janssens and Pilegaard, 2003; Davidson et al., 1998). Q_{10} represents the temperature sensitivity of R_s , which is a measure of change in reaction rate at intervals of 10°C and is based on Van't Hoff's empirical rule (Lloyd and Taylor, 1994). Q_{10} based on Eq. (1) was calculated as Eq. (3) (Davidson and Janssens, 2006; Davidson et al., 1998).

$$Q_{10} = e^{10\beta_1} \quad (3)$$

The daily average R_s flux for the different freeze-thaw stages was obtained based on the best fitting equations (1) or (2) and the corresponding daily average soil temperatures or soil water contents at 5cm depth measured by a data logger set up at the study site. The cumulative R_s emission at the different freeze-thaw stage was calculated by computing the sum of products of the average flux rate and the start-stop-time of the different freeze-thaw stage of the active layer as follows (Zhang et al., 2017),

$$SR = \sum_k^m R_{mk} \quad (4)$$

where k and m are the corresponding onset date and end date of each freeze-thaw stage, respectively; R_{mk} is the daily R_s emission over the specific freeze-thaw stage.

2.5 Statistical analysis

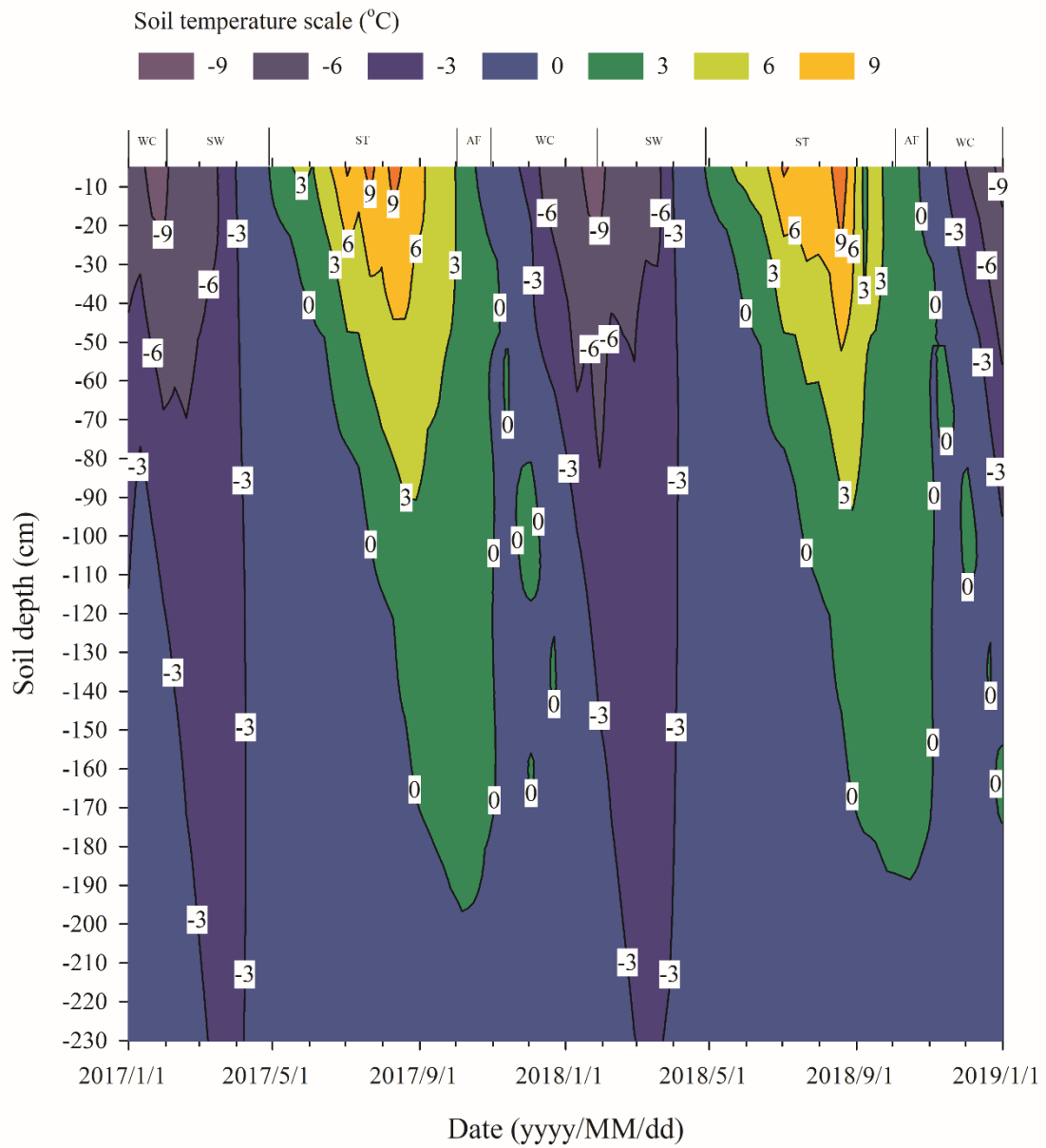
Repeated measures ANOVA was applied for testing the statistical significance of the differences among freeze-thaw stages. Regression analysis was performed between R_s and soil

200 temperature and soil moisture. At different freeze-thaw stages, the fitting equations with higher R^2 values were selected as the preferable models to predict the daily soil CO₂ emission. To evaluate the reliability of the R_s models at the different freeze-thaw stages, root mean squared error (RMSE) analysis was performed. All statistical analyses were carried out at a significance level of 0.05 and were completed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3 Results

205 3.1 Division of different freeze-thaw stages of the active layer

We assumed that the soils began to freeze when the temperature dropped lower than 0°C, and to thaw when the temperature was continuously greater than 0°C, based on the fact that the effects of surface energy of soil particles and salinity in soil on freezing temperature are negligible. Two years' continuous observation on the freezing and thawing of the active layer in the study site showed
210 that the contour outline of 0°C began to slowly develop downwards from the soil surface from late April, and reached the maximum depth in the early October (Fig. 1). The maximum thawing depth of the active layer was 1.98 m in 2017 and 1.89 m in 2018. During this thawing period, the isotherm of 0°C changed gently. However, the isotherm of 0°C changed rapidly from early October to late
215 November, indicating that the whole active layer froze from the surface to the bottom in a short period of time. According to the variations in soil temperature and soil water content in the active layer, the freezing and thawing cycle process of the active layer was divided into four distinctive stages; summer thawing stage (ST), autumn freezing stage (AF), winter cooling stage (WC), and spring warming stage (SW).



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Fig.1. Soil temperature contour outlines of the experimental site in 2017 and 2018

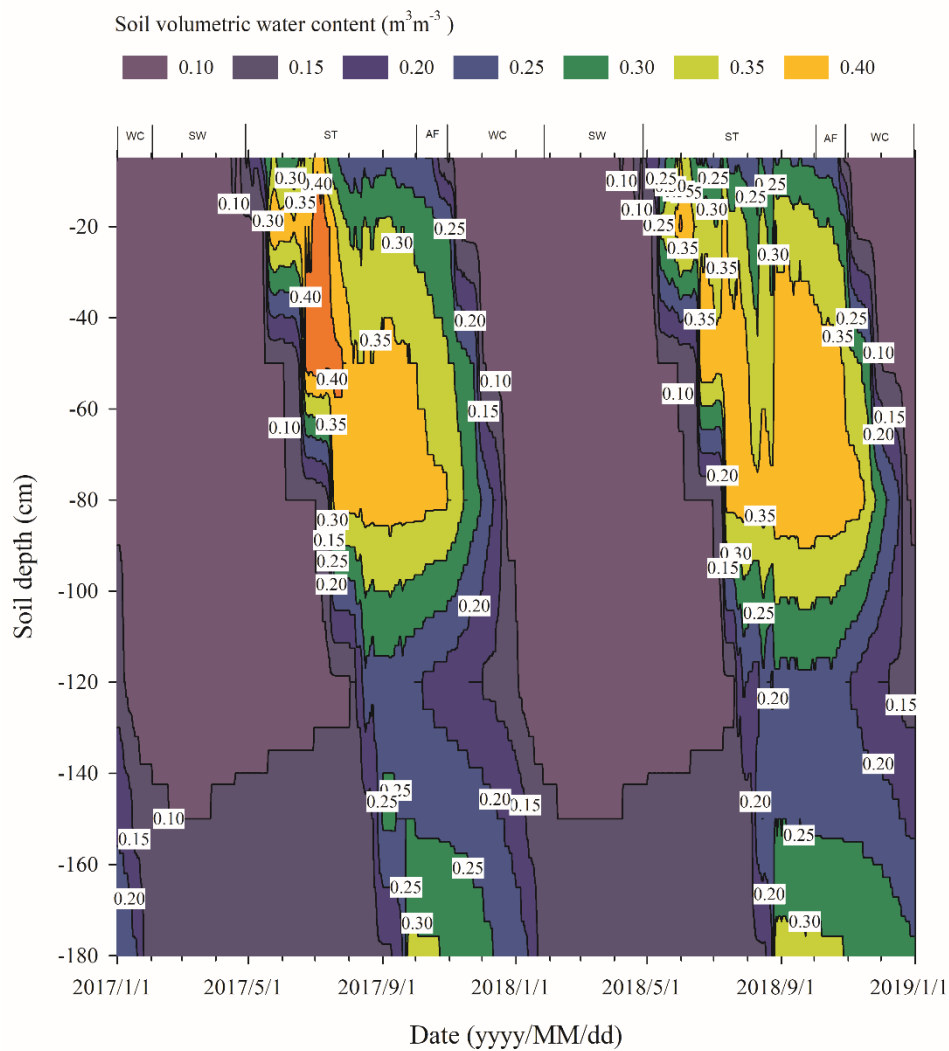


Fig.2. Soil moisture contour outlines of the experimental site in 2017 and 2018

225 The process of ST stage started when the active layer began to thaw downwards from the surface in late April, and preceded to early October when the thawing depth reached the maximum. At this stage, soil temperature decreased along with the soil depth and soil water was mainly transported downward. The whole active layer was in an endothermic process where the heat transferred downwards continuously and the soil thawing front also slowly migrated downwards.

230 At AF stage, once the thawing depth reached the maximum, the soil began to freeze upwards from the bottom of the active layer. Thus, AF lasted until the whole active layer was frozen. At AF stage, soil water, being driven by the temperature gradient, migrated to both sides of the freezing front from the thawing layer and froze there. During AF, the slope of 0°C isotherm was flat. Especially, 0°C isotherm almost paralleled the axis of ordinate between the depth of 50 cm and 160 cm. This

235 phenomenon appeared because the onsets of freezing at different depths had no apparent differences and the whole active layer completed the freezing process in a short time as AF process started. Once the whole freezing process was completed, WC stage quickly started and lasted until mid-late January of the next year. During this process, the soil temperatures were relatively lower in the upper active layer but higher at the bottom; a small amount of soil water near the surface evaporated and

240 the unfrozen water in the active layer tended to migrate upward (Fig.2). However, the amount of
 soil water migration was small because the lower ground temperature limited the content and
 activity of the unfrozen water. The SW stage began in late January as the air-temperature rose, and
 temperature gradients in the active layer gradually decreased. During the SW stage, surface soil
 245 usually underwent daily freezing and thawing cycles in late April. The amount of soil water
 evaporation near the surface increased, and the amount of water migration inside the active layer
 decreased gradually. After the above four freeze-thaw stages were finished, the active layer
 completed a single freeze-thaw cycle. The main characteristics of soil temperature and moisture
 migration at different freeze-thaw stages are summarized in Table 2.

250 Table 2. Characteristics of the different freeze-thaw stages

Stages	Definition	Initiation and termination	Soil temperature/moisture	Total number of measurements
ST	Summer thawing stage	Late April – early October (from when the active layer began to thaw downwards from the surface until the thawing process reached its maximum depth)	Soil temperatures in the active layer decreased from ground surface downwards; Moistures migrated downwards accompanied with the downward movement of the thawing front.	Eight soil depths with 60288 temperature data; seven soil depths with 52752 moisture data
AF	Autumn freezing stage	Initiated when the active layer reached its maximum thawing depth; Terminated when the whole active layer became frozen.	Temperatures of active layer were lower in its bottom or upper part and higher in its middle. Moisture in the thawed part of active layer migrated to both of the upper and lower freezing fronts and froze there.	Eight soil depths with 10752 temperature data; seven soil depths with 9408 moisture data
WC	Winter cooling stage	Initiated when the freezing process finished in late October; Terminated in the mid-late January of the next year.	Temperatures of active layer increased with the increasing depth. Moisture migration was not high due to low ground temperatures.	Eight soil depths with 35328 temperature data; seven soil depths with 30912 moisture data
SW	Spring warming stage	Initiated in early February; Terminated in late April.	Daily freezing and thawing cycles appeared on ground surface in late April. Ground temperature gradient decreased and the rate of unfrozen water	Eight soil depths with 34176 temperature data; seven soil depths with 29904

migration decreased gradually. moisture data
 Moisture content near the
 ground surface showed a
 decreasing trend.

255 Based on the observation data obtained from the experimental site in 2017 and 2018, the initiation and termination points, and the corresponding duration of each stage were calculated (Table 3). ST stage started on April 29, 2017, and ended on October 2, 2017, lasting 157 days. Meanwhile, AF stage was much shorter, lasting about 28 days. WC and SW had a similar duration of 92 and 89 days, respectively.

Table 3. The start-stop-time and duration of different freeze-thaw stages of the active layer

Stage	start-stop time (yyyy/mm/dd)	time of length (days)
ST	2017/4/29-2017/10/2	157
AF	2017/10/3-2017/10/30	28
WC	2017/10/31-2018/1/30	92
SW	2018/1/31-2018/4/29	89

3.2 Dynamics of R_s fluxes in different freeze-thaw stages of the active layer

260 At the Beiluhe experimental site, R_s flux changed as the freeze-thaw processes of active layer developed, showing distinct dynamics in different freeze-thaw stages of the active layer (Fig. 3). R_s flux showed a rapidly increasing trend as the thawing of the active layer intensified in the ST stage. The R_s flux rate rose from 0.26 to 2.77 $\mu\text{mol}/\text{m}^2\text{s}$ in 2017 and 0.53 to 2.82 $\mu\text{mol}/\text{m}^2\text{s}$ in 2018. In AF stage, R_s flux fluctuated between 1.49 and 2.01 $\mu\text{mol}/\text{m}^2\text{s}$, although the number of observations was much smaller due to the short duration of the stage. In the following stage of WC, R_s flux also
 265 decreased rapidly due to the prolonged lowered soil temperature, reaching the minimum values of 0.12 in 2017 and 0.13 $\mu\text{mol}/\text{m}^2\text{s}$ in 2018 by the end of the stage. Then R_s flux began to increase gradually as SW stage proceed. During SW stage, R_s appeared as a small emission peak when the surface of the active layer underwent daily freezing and thawing cycles. Following the small emission peak, R_s flux dropped a little and then started to ascend again quickly once ST arrived.

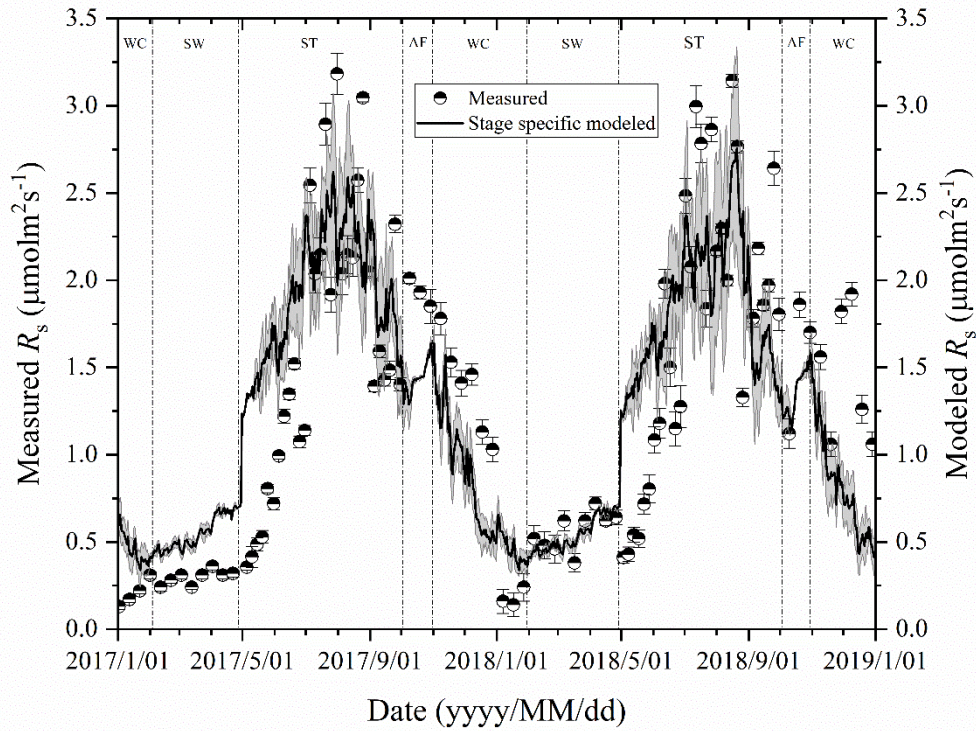


Fig.3. Variations of measured and modeled R_s flux at different freeze-thaw stages in 2017 and 2018. Error bars show standard error of measured R_s ($n=6$)

3.3 Contribution of R_s in different freeze-thaw stages of the active layer

R_s fluxes had significant correlations with soil temperature at 5 cm depth, and the exponential models explained the variations effectively except for AF stage (Fig. 4a). In contrast, soil water content was a poor predictor for R_s with varying relationship for each freeze-thaw stage (Fig.4b). Meanwhile, daily average R_s fluxes modeled by the exponential models for specific freeze-thaw stages were well matched with measured fluxes (Fig. 3). In addition, $RMSE$ analysis showed that the exponential models of soil respiration were preferable for R_s prediction at different freeze-thaw stages ($RMSE < 0.67$, Fig.5). As such, we calculated R_s models, the temperature sensitivity (Q_{10}) and the sum of R_s (SR) based on the Eqs. (1), (3) and (4) in four freeze-thaw stages during a complete freeze-thaw cycle from April 29, 2017 to April 28, 2018 (Table 4). The SR emission during the ST stage ($1041.85 \pm 23.83 \text{ gCO}_2/\text{m}^2$) was much higher than that during the other three stages (150.54 ± 6.80 to $310.69 \pm 12.33 \text{ gCO}_2/\text{m}^2$). The relative contribution of SR during each freeze-thaw stage to the total R_s emission in a complete freeze-thaw cycle (R_{cycle}) ranged from $8.89 \pm 0.18\%$ to $61.32 \pm 0.32\%$. The SR at AF stage was the lowest ($150.54 \pm 6.80 \text{ gCO}_2/\text{m}^2$) and its contribution rate to R_{cycle} was only $8.89 \pm 0.18\%$.

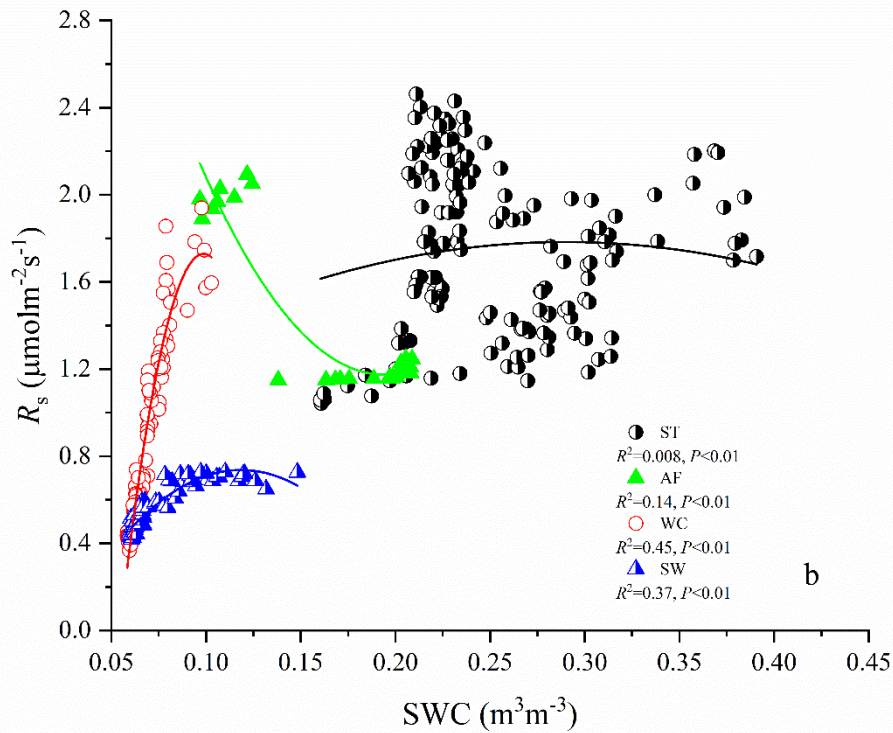
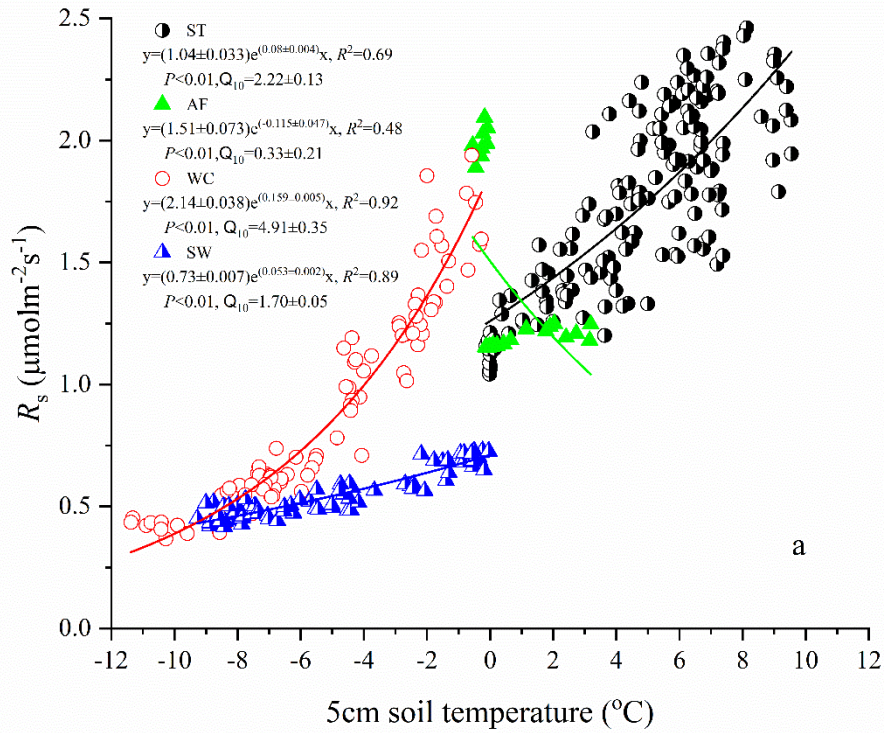


Fig. 4. Relationship between soil temperature (a) and moisture (b) at 5cm depth and measured R_s flux for the summer thawing stage (ST), autumn freezing stage (AF), winter cooling stage (WC),

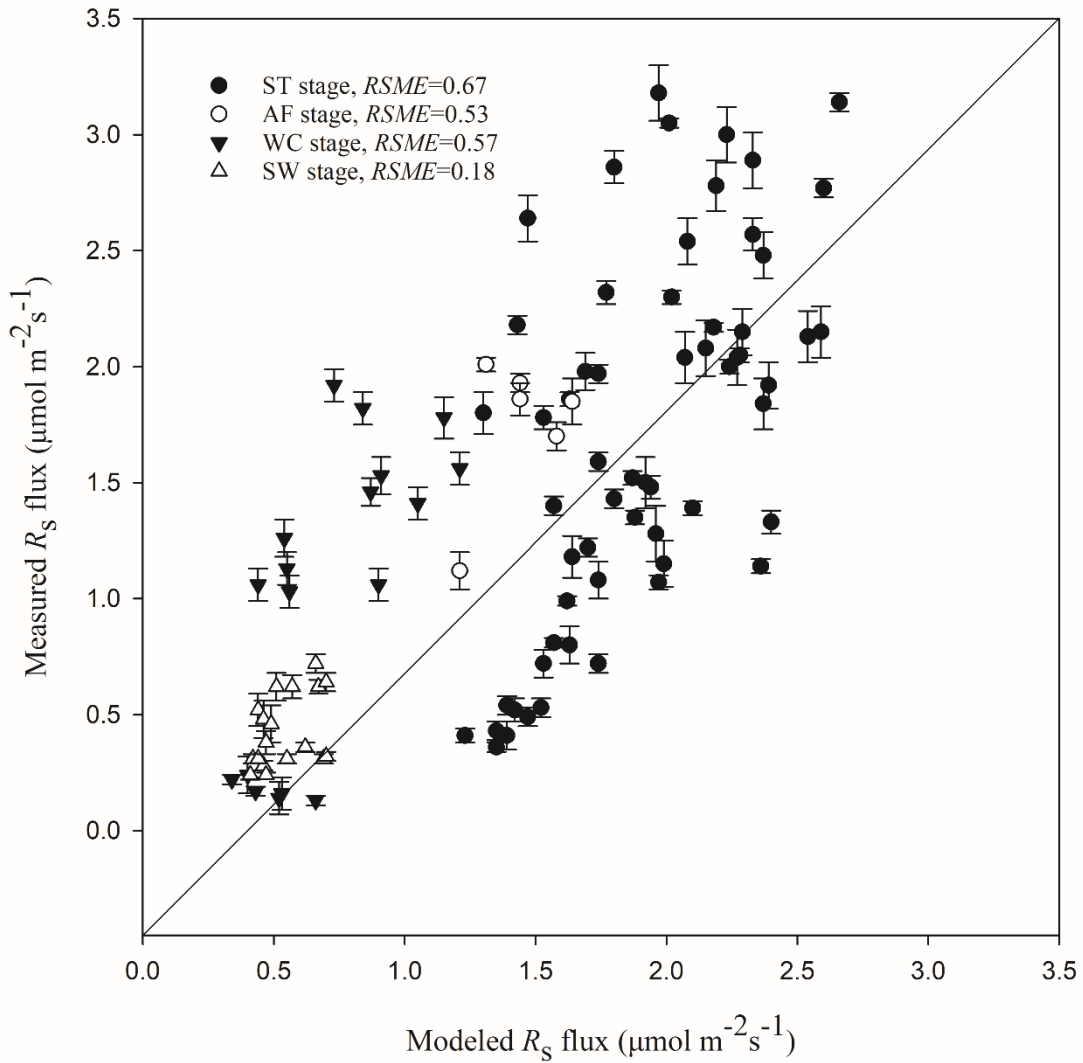


Fig.5. Modeled vs. measured R_s fluxes at different freeze-thaw stages. Error bars represent standard errors of measured R_s flux ($n=6$). The solid line is a 1:1 line.

Table 4. The R_s model, Q_{10} value, SR and its contribution to R_{cycle} in different freeze-thaw stages

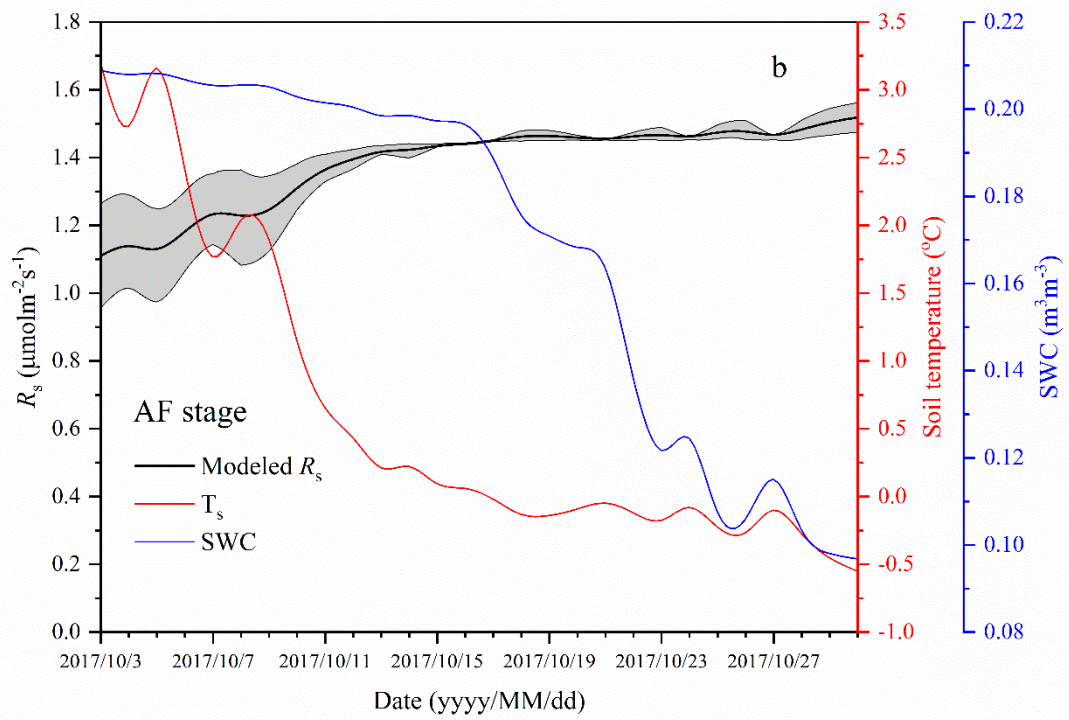
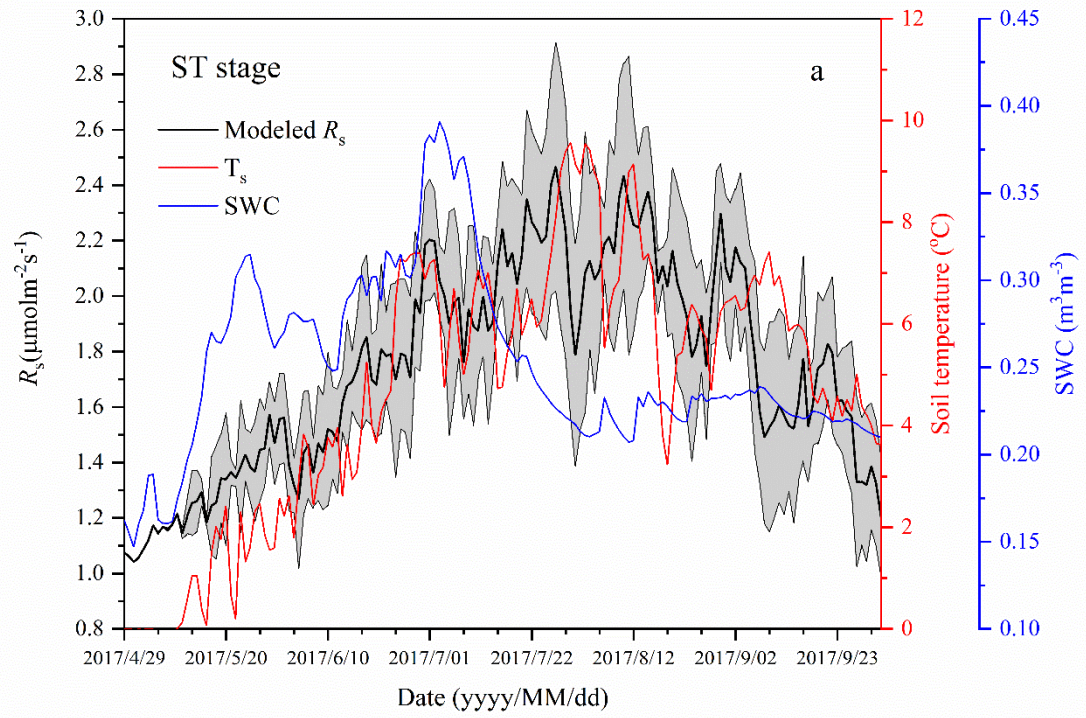
Stages	R_s model		Q_{10}	SR (gCO_2/m^2)	Rate of contribution to R_{cycle} (%)
ST	$R_s = (1.04 \pm 0.033)e^{(0.08 \pm 0.004)T}$	$R^2=0.69$	2.22 ± 0.1 3	1041.85 ± 23.8 3	61.32 ± 0.32
AF	$R_s = (1.51 \pm 0.073)e^{(-0.115 \pm 0.047)T}$	$R^2=0.48$	0.33 ± 0.2 1	150.54 ± 6.80	8.89 ± 0.18

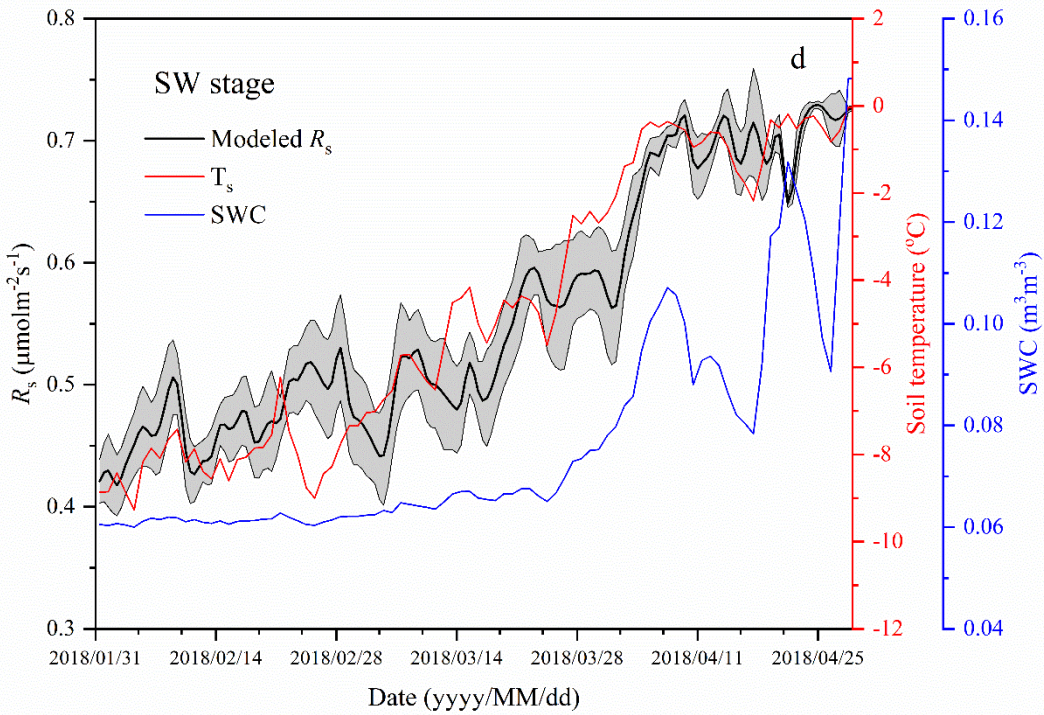
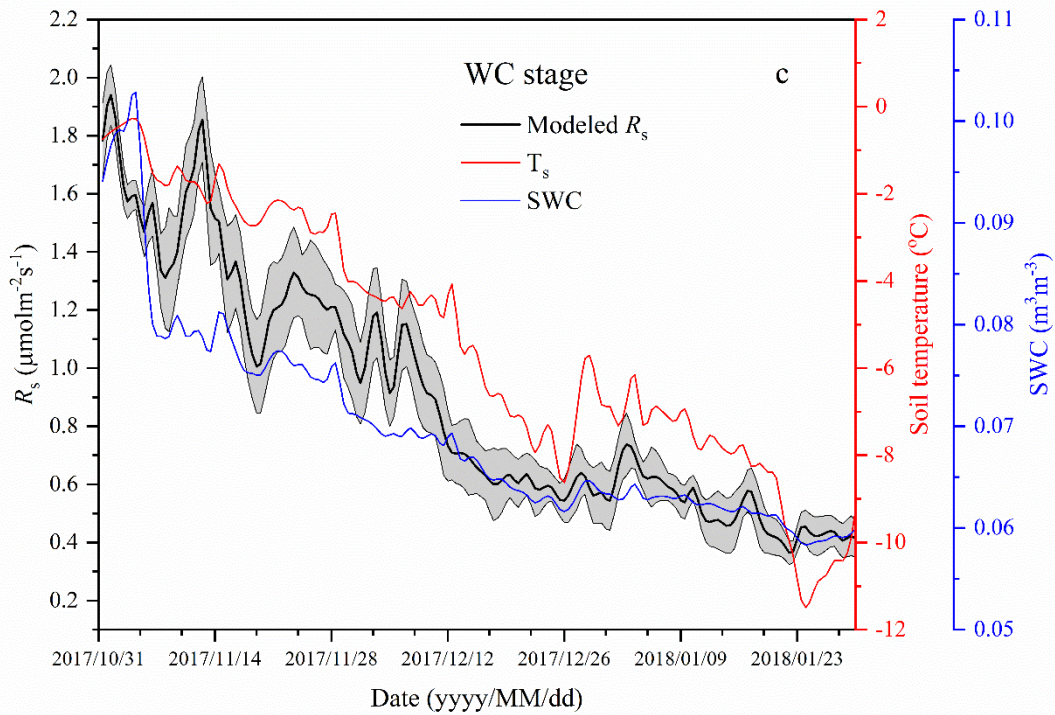
WC	$R_s = (2.14 \pm 0.038)e^{(0.159 \pm 0.005)T}$	$R^2=0.92$	4.91 ± 0.35	310.69 ± 12.33	18.43 ± 0.11
SW	$R_s = (0.73 \pm 0.007)e^{(0.053 \pm 0.002)T}$	$R^2=0.89$	1.70 ± 0.05	189.90 ± 8.47	11.29 ± 0.11
Total R_s emission in a complete freeze-thaw cycle (R_{cycle} , gCO ₂ /m ²)				1692.98 ± 51.4	3

3.4 Factors affecting R_s fluxes in different freeze-thaw stages

305 R_s was positively correlated to soil temperatures, following an exponential relationship with
the 5cm soil temperatures regardless of soil water status during the freeze-thaw stages. When
calculated on the basis of the dataset of each stage, the Q_{10} values were 2.22 ± 0.13 ($R^2=0.69$),
0.33±0.21 ($R^2=0.48$), 4.91 ± 0.35 ($R^2=0.92$), and 1.70 ± 0.05 ($R^2=0.89$) for ST, AF, WC and SW with
soil temperatures ranging from -0.13 to 9.55°C, -0.55 to 3.19°C, -11.38 to -0.28°C, and -9.28 to -
310 0.04°C, respectively (Table 4). The variations of R_s fluxes, determined by the exponential models
we developed, exhibited different characteristics at each freeze-thaw stage during a complete
freezing and thawing circle (Fig.6). In ST stage (Fig.6a), for example, the variations of R_s , T_s , and
SWC were basically consistent. R_s showed an increasing trend as T_s and SWC at 5cm rose due to
the active layer thawing from the surface, and reached the maximum ($2.66 \pm 0.16 \mu\text{mol/m}^2\text{s}$) until
315 August. Then R_s decreased with fluctuations as T_s and SWC dropped. As AF began (Fig.6b),
however, R_s flux did not decrease any longer when it reached its lowest level ($1.11 \pm 0.15 \mu\text{mol/m}^2\text{s}$)
even though T_s and SWC dropped sharply in response to soil freezing. As freeze-thaw process
developed, R_s flux increased slightly and reached a relatively stable state ($1.47 \pm 0.15 \mu\text{mol/m}^2\text{s}$),
although the T_s and SWC continued to lower with fluctuations. When WC stage started (Fig.6c),
320 R_s decreased again with fluctuations as T_s and SWC continuously decreased, although the active
layer was completely frozen. At the end of WC, R_s flux decreased to its lowest level (0.39 ± 0.06
 $\mu\text{mol/m}^2\text{s}$). In SW stage (Fig.6d), R_s flux began to increase with fluctuations as the T_s rose in
response to soil warming, while SWC had no change as the surface soil still remained frozen in
the earlier stage and fluctuated wildly due to daily freeze-thaw process in the later stage.

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Fig.6. Variations in modeled soil respiration (R_s), soil temperature (T_s) and soil water content (SWC) for the four freeze-thaw stages including ST (a), AF (b), WC (c), and SW (d) in a

complete freeze-thaw cycle from later April 2017 to late April 2018. The SWC unit stands for water volume per total soil volume. The error band of modeled R_s stands for 95% confidence interval.

335 4 Discussion

4.1 Impacts of freeze-thaw process on R_s in different stages

Freeze-thaw process plays a significant role in soil biogeochemical processes in most high-latitude and high-altitude ecosystems (Grogan et al., 2004; Liu et al., 2016b). Furthermore, freeze-thaw effects on soil nutrient transformations may substantially influence the C balance of seasonally cold ecosystems (Grogan et al., 2004; Weih, 1998). Although many studies have shown that freeze-thaw events affect R_s in tundra, boreal, and temperate soils (Liu et al., 2016a; Du et al., 2013), variations of R_s in different freeze-thaw stages of active layer has not been studied in permafrost regions on the Qinghai-Tibet Plateau. Our observations clearly demonstrated that freeze-thaw process of active layer strongly affected the R_s dynamics, and that R_s emission models were significantly different for each of the freeze-thaw stages ($P < 0.01$).

In ST stage, the active layer was mainly in a heat-absorbing and thawing condition, where heat was transferred from top to bottom and the thawing front also gradually migrated downwards (Zhao et al., 2000; Jiao and Li, 2014). We observed that R_s was restrained by low temperature onset of the stage and then increased following an exponential correlation with soil thawing (Fig. 6a and Table 4). Such increment of CO_2 fluxes after thawing was also observed in forests, alpine tundra, and arctic heath ecosystems (Wu et al., 2010a; Brooks et al., 1997; Elberling and Brandt, 2003). The rapid increase in R_s flux may be due to the activation of root respiration and rhizospheric microbial respiration as well as heterotrophic respiration by enhanced microbial activities in bulk soil with greater C availability by expansion of thawing depth (Gaumont-Guay et al., 2006). In this stage, the dynamic changes in R_s flux were not always associated with surface soil moisture content, which is reflected in a weak correlation between R_s flux and moisture content ($R^2 = 0.008$, Fig. 4b). Even R_s flux reduced instead when soil moisture content increased quickly. The declining of R_s flux at high soil moisture content conditions may be attributed to the increased free water in the soil clogging the soil pores as the thawed water accumulate at the thawing front (Zhao et al., 2000) and the rainy season of the Tibet Plateau (Ye, 1981). This hampers the two-way exchange of gases involved in respiration, with CO_2 passing upward from the soil to the atmosphere and oxygen moving in the opposite direction (Stepniowski et al., 1994). Although R_s was hindered by a large increase in soil moisture content to some extent, more organic carbon and soluble matrix were exposed and became available to soil microbes as thawing deepened (Ping et al., 2008). As such, higher metabolic rates of soil organisms was stimulated and more soil carbon was released during this wet and humid ST stage (Keith et al., 1997). Furthermore, ten consecutive years of observation has found that the near-surface permafrost is warming in the alpine ecosystem regions underlain by permafrost on Qinghai-Tibet Plateau, increasing the duration of thawing by at least 14 days (Wu et al., 2015), which would emit significantly more R_s in ST stage.

In AF stage, the R_s flux showed a slightly increasing trend at initiation and came to a relatively stable state in the end, although the surface soil temperature declined sharply and the freezing process developed quickly (Fig. 6b). When AF first began, the active layer was still basically an open system. It exchanged air and moisture with the atmosphere at least for some duration during the day, and the upper part of the active layer absorbed heat from the atmosphere during the daytime and

375 released it at night (Zhao et al., 2000). So although the reduction in soil temperature had some
impacts on the soil microbial activities, some of the soil microbes were still active (Monson et al.,
2006) and more nutrient matrix was decomposed and available due to diurnal freezing and thawing
actions (Liu et al., 2016b). Furthermore, the freezing and thawing actions could initiate the activity
of soil microorganisms and promoted the soil respiration (Grogan et al., 2004;Contosta et al., 2013).
380 For these reasons, R_s flux was maintained a relatively stable state accompanied by a slight increase
at the initiation of AF. Although the soil of upper and bottom of active layer became frozen as the
freezing process developed, the soil heterotrophic respiration rate was still higher in the thawing
layer between the two freezing fronts, because the soil remained warm and unfrozen water was
present (Olsson et al., 2003). As the two-way freezing process carried out and moisture migrated
385 towards the freezing fronts (Zhao et al., 2000), the soil pores were constantly filled with ice, and as
a result squeezed out and released the trapped CO_2 in the soil pores. Consequently, the R_s still
showed a relatively stable dynamic at the later period of AF.

In WC stage, once freezing process of active layer was completed, a sudden decline in soil
temperature appeared along with a continuous decrease in soil water content (Fig. 2). The soil
390 respiration at this cooling stage was influenced substantially because the continued decrease in soil
temperature and liquid water content resulted in a partial death of microbes (Walker et al., 2006),
lowered microbial activities and the reduction in substrate affinity (Nedwell, 1999). This appears to
cause a continuous decline in the R_s flux. However, we observed that the R_s rate never reached zero
although the R_s flux continuously declined at this stage (Fig.6c), indicating that the soil
395 microorganisms could maintain their activities at extremely low temperatures (Panikov et al., 2006),
verifying the results of previous studies (Kurganova et al., 2007;Panikov and Dedysh, 2000).
Furthermore, not all cells in the soil were killed or irreversibly damaged by the sustained low
temperature and the dropping in soil water content (Walker et al., 2006), and the cold-adapted
microflora still breathed and consumed the limited liberated nutrients in the frozen soil (Kurganova
400 et al., 2007). Consequently, R_s still maintained a detectable rate.

In SW stage, R_s was still restrained by lower soil temperature and fluctuated at a low rate
although the warming process of the active layer had begun as the air-temperature increased (Jiao
and Li, 2014) before late March (Fig.6d). However, the rapid increase in R_s flux started after late
March, likely a result of the activation of soil microorganisms (Grogan et al., 2004) and the
405 availability of nutrient matrix with increased soil water content (Liu et al., 2016b). This happened
when the diurnal freezing-thawing process within the surface soil initiated and the surface soil water
content increased due to thawing snow in this period (Zhao et al., 2000). This result was consistent
with the reports on the Fenghuoshan region of the Qinghai-Tibet Plateau (Zhang et al., 2015a).

4.2 Dependence of R_s on soil temperature in different freeze-thaw stages

410 Soil respiration is generally known to be controlled by soil temperature, soil moisture, or a
combination of the two. When the soil moisture is not limited, soil respiration is mainly dependent
on soil temperature (Zhang et al., 2015a). In the present study, the variations in soil temperature
explained 48~92% of the freeze-thaw stage specific variabilities in R_s fluxes. Q_{10} values that reflect
the quantitative relationship between R_s and soil temperature differ at each freeze-thaw stage (Table
4). Q_{10} values were higher at WC and SW stages when soil temperatures were lower than other
415 stages (Fig.1) and at ST stage with higher soil moisture content (Fig.2). This result is consistent with
a report from a temperate plantation forest where Q_{10} values tended to be higher under lower
temperature and higher soil moisture conditions (Yan et al., 2019). Negative correlations with soil

420 temperature and positive correlations with soil moisture of Q_{10} values were also reported in a sub-
alpine forest of the Eastern Qinghai-Tibet Plateau (Chen et al., 2010), and in a temperate cropland
(He et al., 2016). However, Q_{10} value was minimal and R_s flux showed weak correlations both with
soil temperature ($R^2=0.48$, $P<0.01$) and the soil moisture ($R^2=0.14$, $P<0.01$) at AF stage. This is
425 mostly likely due to the fact that the active layer became an incomplete open system and hindered
and even blocked the free exchanges of gas and moisture between the active layer and atmosphere
during the later period of AF. Qinghai-Tibet Plateau is anticipated to be warmer and wetter under
global warming (Li et al., 2010), accelerating permafrost thaw. This will enhance the temperature
sensitivities of soil respiration at different freeze-thaw stages, resulting in much stronger response
of the site to global warming in terms of CO_2 emissions.

430 As another influencing factor of soil respiration, soil moisture was reported to exhibit positive,
negative or null effects on R_s fluxes in various ecosystems (Gaumont-Guay et al., 2006; Balogh et
al., 2011; Zhang et al., 2015a). In the present study, R_s flux exhibited a low quadratic (positive)
relationship with soil water content at the different freeze-thaw stages. Soil water content as an
independent variable explained 0.8~45% of the variances of R_s only (Fig.4b), suggesting that soil
435 temperature was the main factor controlling variable for R_s flux regardless of soil water status in all
stages.

4.3 Cumulative R_s in different stages and contributions to total C emission of a complete freeze-thaw cycle

440 The high determination coefficients of exponential equations for R_s fluxes ($R^2\geq 0.48$) and the
low $RMSE$ values ($RMSE\leq 0.67$) between the measured and modeled R_s fluxes suggest that the
freeze-thaw stage specific R_s models proposed in this paper are accurate predictors of soil CO_2
emissions, at least in this region. The large amount of carbon emitted via soil respiration during the
processes of freezing-thawing cycle of active layer suggests that R_s of the Qinghai-Tibet alpine
meadow ecosystem leads a significant carbon loss and may play an important role in global carbon
445 cycle. According to the phenological changes of vegetation and the division of growing season and
non-growing season of the Qinghai-Tibet Plateau (Xu et al., 2008a; Xu et al., 2005), heterotrophic
respiration was most likely the main component of R_s at AF, WC, and SW stages, which lie in non-
growing seasons (from October to the next April); it accounted for almost two third of the ST stage,
which belongs to a growing season (May to September). Because R_s in a growing season differs
450 substantially from those in non-growing seasons not only in quantity but also in key controlling
variables, stage-specific carbon processes should be taken into consideration for accurate estimation
of carbon sink/source of the Qinghai-Tibetan alpine ecosystem.

At ST stage, the modeled R_s fluxes ranged from 1.23 to 2.66 $\mu\text{mol}/\text{m}^2\text{s}$, and the cumulative R_s
(1018.02–1068.68 gCO_2/m^2) was approximately estimated to be 61% of the total R_s emission in a
complete freeze-thaw cycle (1641.55 to 1744.41 gCO_2/m^2). The cumulative R_s of the ST stage and
455 its contribution to the total R_s emission in a complete freeze-thaw cycle in our study were both higher
than those at the Fenghuoshan region on the Qinghai-Tibet Plateau or those from Arctic tundra
(Zhang et al., 2015a; Elberling, 2007). This is likely due to the longer duration (157 days) of ST
stage at our study site and the unique seasonal climate of the plateau. More specifically, the Tibetan
alpine meadow receives more than 60–90% of the total precipitation during ST stage, with less than
460 10% in the other stages (Xu et al., 2008b). In addition, higher soil temperature and water content in
wet and humid summers stimulate microbial activity, inducing higher metabolic rates of soil

organisms and roots (Keith et al., 1997). Furthermore, observation made in ten consecutive years in the alpine ecosystem regions underlain by permafrost on the Qinghai-Tibet Plateau has found that warming of the near-surface permafrost increased the duration of thawing by at least 14 days (Wu et al., 2015), emitting significantly greater amount of R_s in ST stage.

At AF stage, exponential regression analysis was carried out with fewer measured R_s values because the duration was shorter than other stages. The modeled R_s fluxes were generally lower than that measured during this stage (Fig.3). These biases between the measured and modeled R_s fluxes were likely to be caused by sampling scheme. The low sampling frequency (two occasions for daily average data) in the period of AF could increase the variance of aggregated estimates (Ryan and Law, 2005). In addition, measurements during this stage were usually restricted to daytime and dry days, and the sampling would inevitably miss the pulse of microbial or root activity immediately following occasional precipitation (Doff sotta et al., 2004). Thus, the cumulative R_s (143.74 to 157.34 gCO₂/m²) calculated by an exponential model only accounted for about 8.89% of the total R_s emission in a complete freeze-thaw cycle, which probably underestimated the R_s emission during this stage. Although the active layer gradually became a closed system in this stage, it is noteworthy that a proportion of respired soil CO₂ can still be transported via vascular plants, which may function as a conduit for CO₂ from deeper soil layers (Ström et al., 2005). Furthermore, the diurnal freezing and thawing actions occurring in this stage also played an important role on the R_s emissions (Contosta et al., 2013). Therefore, more frequent observations with automated chambers incorporating vegetation function are warranted to refine the estimated R_s at AF stage in this study.

At WC stage, R_s fluxes continuously descended with fluctuation from 1.92 to 0.13 $\mu\text{mol}/\text{m}^2\text{s}$ and the cumulative R_s (298.36–323.02 gCO₂/m²) was estimated to be 18.43±0.11% of the total R_s emission in a complete freeze-thaw cycle. The continued decrease in soil temperature and liquid water content appeared to lead the decline in soil respiration. However, little is known regarding the root function of the alpine meadow and soil microbial activities at this stage. It is generally assumed that lowering soil temperature may hinder microbial activities, but laboratory-based experiments have found that microbial activities can still be substantial at low temperatures of -6°C to -10 °C (Panikov and Dedysh, 2000; Walker et al., 2006). Further research on the physiology and roles of such psychrophilic microorganisms in soil respiration at WC stage is necessary.

At SW stage, the modeled R_s fluxes showed a rising trend in the ranges from 0.42 to 0.72 $\mu\text{mol}/\text{m}^2\text{s}$, and were generally higher than those measured in 2017 (Fig.3). These biases may also be caused by low sampling frequency and simple averaging for daily average data in regression analysis (Ryan and Law, 2005). The diurnal freezing-thawing process during this stage also stimulated the activities of soil microorganisms and promoted the R_s emissions, but the low sampling frequency and the restricted sampling time (between 9:00 and 11:30 a.m. local time) probably missed the peaks and pulses of R_s fluxes of a day. Therefore, the measured R_s flux may underestimate the actual emission rate in this stage. However, it is also reported that biases of chamber-based estimates of R_s can be reduced by using a regression model which is extrapolated with soil temperature and moisture (Ryan and Law, 2005). In addition, the smaller value of *RMSE* at this stage also testified the temperature-driven model was preferable for the R_s prediction (Fig.5). Thus, the cumulative R_s (181.43–198.37 gCO₂/m²) calculated by the exponential model was estimated to be 11.29±0.11% of the total R_s emission in a complete freeze-thaw cycle. Despite of the possible biases between the modeled and measured R_s fluxes, our model is still a reliable estimate for R_s emission during this stage, which is further supported by the same trends in the variations between

the two values. The increasing trend in R_s fluxes can be caused by the following mechanisms: First, the activation of soil respiration was mediated by increased soil microbial activities as soil temperature and water content increased. Furthermore, as spring proceeds with warming of soil, the mobilization of stored carbohydrates enhanced soil respiration (Davidson et al., 2006). Finally, daily freeze-thaw actions in late April may have further enhanced the soil respiration quickly.

5 Conclusions

The freezing and thawing process of active layer significantly controlled the soil respiration of the alpine meadow in permafrost region of the Qinghai-Tibet Plateau. The soil temperature was the key factor affecting soil respiration regardless of soil water status during each freeze-thaw stage. The cumulated soil respiration in different freeze-thaw stages ranged from 150.54 to 1041.85 $\text{gCO}_2\text{m}^{-2}$, and the cumulated soil respiration in ST, AF, WC, and SW stages contributed about 61.32, 8.89, 18.43, and 11.29% to the total R_s emissions in a complete freeze-thaw cycle, respectively. The Q_{10} values were higher at WC and SW stages with lower soil temperatures, and at ST stage with higher soil moisture content. As the Qinghai-Tibet Plateau becomes warmer and wetter (Li et al., 2010), soil respiration at different freeze-thaw stages are predicted to be more sensitive to temperature. Furthermore, in the future climate of warmer temperatures, great changes in freeze-thaw process patterns may have important impacts on R_s . Further research is required to define the regulatory mechanism and its key processes on R_s in different freeze-thaw stages of the active layer. In addition, due to short duration of the AF, more frequent observations should be carried out in order to more accurately evaluate the contribution of R_s at the stage.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (41771080, 41701066, 41003032), the Fund of State Key Laboratory of Frozen Soil Engineering (No. SKLFSE-ZT-36) and the grant of China Scholarship Council. We are grateful to Dr. Yali Liu and Yu Gao for their help in the field measurement of soil CO_2 flux. We gratefully thank the reviewers for their comments. We also thank Jiyoung Kang for English correction.

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