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Freeze-thaw processes of active layer regulate soil respiration of alpine meadow 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 process regulates the R_s dynamics in different stages for the alpine meadow underlain by permafrost on 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 to determine the regulatory mechanisms of the different freeze-thaw stages of the active layer on the R_s. We found that the freezing and thawing process of active layer modified the R_s dynamics differently in different freeze-thaw stages. The mean R_s ranged from 0.56 to 1.75μmol/m^{2}s across the stages, with the lowest value in the SW stage and highest value in the ST stage; and Q_{10} among the different freeze-thaw stages changed greatly, with maximum (4.9) in the WC stage and minimum (1.7) in the SW stage. Patterns of R_s among the ST, AF, WC, and SW stages differed, and the corresponding contribution percentages of cumulative R_s to annual total R_s were 61.54, 8.89, 18.35, and 11.2%, 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 the climate warming 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.

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 between the atmosphere and 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 (Hollesen et al., 2011). What's more, many studies have shown that the winter-time emissions contribute significantly to the annual CO_2 balances. For example, the Arctic tundra ecosystem is becoming a consistent source of CO_2 because of the CO_2 emission in winter offsetting the CO_2 uptake in growing season with progressive permafrost thaw and active layer thickening (Celis et al., 2017). In Alaska, emissions of CO_2 from tundra during early winter seasons increased by about 73% since 1975, and the Arctic ecosystems has been a net source of CO_2 due to rising temperatures (Commane et al., 2017). For the sub-arctic tundra ecosystem, the winter-time CO_2 loss also increases due to sustained tundra warming and the ecosystem historical function is shifting away from a carbon sink to a carbon source (Lüers et al., 2014;Webb et al., 2016). In permafrost regions on northern hemisphere, the amount of soil organic carbon (SOC) stored reaches 1832Pg (Ding et al., 2015;Mu et al., 2015), among which 495.8Pg distributes in the 0-1m depth, 1024Pg in the 0-3m depth and 648 Pg in the 3-25m depth (Mu et al., 2015). Due to its high sensibility to global warming and direct contribution to the atmosphere greenhouse gas contents, the carbon emission from permafrost has received worldwide attention (Tarnocai, 2009;Zimov et al., 2009).

In the scenario of global warming, the active layer and permafrost distributed in the Arctic and mid-latitude alpine regions are undergoing significant changes (Jorgenson and Osterkamp, 2005). The active layer, acting as a buffer between permafrost and atmosphere, is more sensitive and responds more quickly to climate change (Li et al., 2012). The energy and water exchange in permafrost regions between the earth and atmosphere is mainly completed by the active layer. However, in a whole freeze-thaw cycle, the active layer will undergo a series of processes of cooling, start freezing to fully 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 from each other (Henry, 2007). So the dynamics of R_s emission at different freeze-thaw stages will show apparent differences. Furthermore, the thawing of the permafrost and the deepening of the active layer will expose the frozen organic carbon to microbial decomposition and cause previously frozen SOC to become available for mineralization (Walz et al., 2017), and thus may accelerate a positive permafrost carbon feedback on climate (Schuur et al., 2008). A study of six years of CO_2 flux measurements in a 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 the high-altitude mountain regions, thawing permafrost has caused the alpine tundra to release long-frozen CO_2 to the atmosphere, exacerbating climate warming (Knowles et al., 2019). Therefore, permafrost will play a significant role in the carbon-climate feedbacks due to its intensity of climate forcing and the size of the carbon pools in permafrost regions (MacDougall et al., 2012;Schneider von Deimling et al., 2012).

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

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). The soil organic carbon (SOC) pools in the permafrost regions on the QTP were

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estimated to be 160±87 Pg, which was approximately 8.7% of those in the northern circumpolar permafrost region (Mu et al., 2015). However, the freeze-thaw occurrence, active-layer thickness, and near-surface permafrost temperature have been undergoing dramatic changes in recent years. In permafrost regions distributed with alpine meadow ecosystem on the 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 temperature at 6m and 10m depths increased by ~0.13 °C and ~0.14 °C, respectively (Wu et al., 2015). Therefore, the R_s of the alpine meadow must 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 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 its contribution to annual soil CO_2 emission in this region; and (3) to establish a preferable R_s model to accurately predict the soil CO_2 emission of each freeze-thaw stages.

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 km^2, with an altitude of 4600 – 4800 m, which is underlain by permafrost with an active layer at 1.1-2.3m. The mean annual temperature is -3.60 °C, which is colder than that of other areas in the QTP (Yin et al., 2017). The mean annual precipitation is 423.79 mm, 80% of which falls during the growing season (from May to September). The air pressure is approximately 550 hPa. The alpine meadow represents the most common vegetation type in this area (70%) (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 moderate amount of water is available. The ecosystem's vegetation mainly consists of *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 scabriostris* (Kukenthal) (Chen et al., 2017).

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 the 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 230cm, respectively; and the depths for the soil moisture probes were 5, 20, 50, 80, 120, 150 and 180cm, respectively. Soil temperature was measured using thermistors made by the State Key Laboratory of Frozen Soil Engineering (SKLFSE, China), and their temperature accuracy is ±0.05°C. Soil moisture was measured using calibrated soil moisture sensors (EC-5, Decagon USA), and their moisture accuracy is ±2%. The 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 by a data logger (CR3000, Campbell Co., USA).

Utilizing the measurements collected by soil temperature probes and a datalogger, the soil hourly mean temperature (T_{ave}), the maximum temperature (T_{max}), and the minimum temperature (T_{min}) of each day at different depths were calculated. According to the T_{ave} values and 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_{ave} continued to be lower or higher than 0 °C was regarded as the onset freezing or onset thawing date, respectively (Yang et al., 2002). While the date on which if T_{max} was greater than 0 °C but T_{min} less than 0 °C was regarded as the soil was undergoing the daily freeze-thaw process. That is, the soil absorbs heat and thaws during the daytime while it releases heat and freezes during the nighttime, showing a daily freeze-thaw cycling phenomenon. Thus the whole freeze-thaw process of active layer can be divided into different stages.

For the freezing or thawing thickness of the active layer in the freeze-thaw process, the 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 measuring the R_s, six 5×5m measurement 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 at 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). Living plants inside the collar were removed carefully at 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 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 made 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, using the thermocouple probe and the ECH2O soil moisture sensor (LI-COR, Lincoln, NE, USA) connected to the LI-8100A, soil temperature (T_s) and soil volumetric water content (SWC) at 5cm depth were determined as the soil CO_2 flux measurement besides the collars.

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

The dependency of measured R_s flux on temperature was determined by fitting the following equation over soil temperature at 5cm depth for the best regression coefficient (Zhang et al., 2015a),

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

where R_s is the measured soil respiration rate (μmolm^{-2}s^{-1}), T is soil temperature (°C) at 5cm depth, and β_0 and β_1 are constants. This 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} was calculated as the following equation (Davidson and Janssens, 2006;Davidson et al., 1998).

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$$Q_{10} = e^{10\beta_1} \quad (2)$$

The daily average R_s flux at the different freeze-thaw stage was obtained by interpolating the average R_s flux rate between the sampling dates. The total 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),

$$SR = \sum_k^m R_{mk} \times \Delta t \quad (3)$$

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

2.5 Statistical analysis

Repeated measures ANOVA was applied for testing the statistical significance of the difference among the freeze-thaw stages. Regression analysis was performed between R_s and soil temperature and soil moisture. All statistical analyses were performed at a significance level of 0.05 and were completed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 Division of different freeze-thaw stage of the active layer

In the condition that the effects of the surface energy of the soil particles and salinity in the soil on the freezing temperature could be neglected, we assumed that the soils began to freeze when the temperature dropped to be less than 0°C and to thaw when the temperature was continuously greater than 0°C. Basing on the two years' continuous observation on the freezing and thawing of the active layer in the study site, it can be found that from late April the contour outline of 0°C began to slowly develop downward from the soil surface and got to the maximum depth in the early October (Figure 1), indicating that the surface soil of the active layer began to thaw in late April and the thawing depth reach the maximum in early October. The maximum thawing depth of the active layer was 1.98m in 2017 and 1.89m 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 November, showing that the whole active layer froze from the surface to the bottom in a short period of time. According to the variation in characteristics of soil temperature in the active layer, the freezing and thawing cycle process of the active layer can be divided into four different stages: summer thawing stage (ST), autumn freezing stage (AF), winter cooling stage (WC), and spring warming stage (SW).

The process of the ST stage started from the time when the soil of the active layer began to thaw downwards from the surface in late April to that when the thawing depth reached the maximum in early October. In this stage, the temperatures decreased as the soil deepened. The whole active layer was in an endothermic process where the heat transferred downward continuously and the soil thawing front also slowly moved downward. For the AF stage, once the thawing depth reached the maximum, the soil began to freeze upwards from the bottom of the active layer. Thus the AF lasted till the whole active layer was frozen. According to the moving direction of the freezing front, the AF stage again could be divided into two sub-stages, viz. unidirectional freezing process (UF substage) from down to up and "zero-curtain" process (ZC substage). The UF substage started from the moment when the active layer began to freeze upwards from the bottom and ended when the surface soil started to stably freeze. Meanwhile, the ZC substage started from the moment when the surface soil was stably frozen until the end of the whole freezing process. During the AF, the slope

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of 0°C isotherm was small. Especially between the depth of 50cm and 160cm, the 0°C isotherm almost paralleled the axis of ordinate. This phenomenon showed that when the AF process started, the onsets of freezing at different depths had no apparent differences and the whole active layer completed the freezing process in a short time. After the completion of the whole freezing process, the WC process of the active layer 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 in the bottom. The SW stage started in late January as the air-temperature rose, and the temperature gradients in the active layer gradually decreased. During the SW stage, the surface soil usually underwent daily freezing and thawing cycles in late April. After the above four freeze-thaw stages were finished, the active layer completed a complete freeze-thaw cycle.

Based on the observation data obtained from the experimental site in 2017 and 2018, the start-stop-time and the corresponding duration of each stage were calculated (Table 1). It can be seen that the ST stage started from April 29, 2017, and ended on October 2, 2017, lasting about 157 days. Meanwhile, the AF stage was short and only lasted about 28 days, of which the UF substage lasted for 20 days and the ZC substage 8 days. The stages of WC and SW had a similar time of length and they accounted for 92 and 89 days, respectively.

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

At the Beiluhe experimental site, R_s flux changed regularly as the freeze-thaw processes of active layer developed, suggesting that the freezing and thawing processes of active layer regulated the R_s strongly (Figure 2). In the ST stage, R_s flux showed a rapidly increasing trend as the thawing of active layer intensified. The R_s flux rate rose from 0.26 to 2.77μmol/m^{2}s in 2017 and 0.53 to 2.82 μmol/m^{2}s in 2018. After the ST process finished, the active layer went into the AF stage, in which the R_s flux fluctuated acutely. In the UF substage, the R_s flux decreased sharply as the active layer began to freeze from the bottom and the surface soil temperature lowered to freezing point. Thereafter, the active layer went into the ZC substage and the R_s emission rate again began to increase slowly. In the AF stage, the R_s flux fluctuated between 1.49 and 2.01μmol/m^{2}s. After the AF process was completed, the active layer went into the WC stage. As the soil temperature of active layer lowered continuously, the R_s flux also decreased rapidly. When it came to the end of the WC process, the R_s flux reached the minimum value of about 0.12 in 2017 and 0.13μmol/m^{2}s in 2018. Then the R_s flux began to increase gently as the SW process came up. During the SW stage, the R_s appeared as a small emission peak when the surface of the active layer underwent daily freezing and thawing cycles. After the small emission peak passed, the R_s flux dropped a little and then started to ascend again quickly once the ST process arrived.

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

Based on the equations (1), (2) and (3), we calculated the models of the soil respiration, the temperature sensitivity (Q_{10}) and the sum of R_s (*SR*) in the different four freeze-thaw stages during the experimental period in the years of 2017 and 2018 (Table 2). The *SR* emission during the ST stage (1041.85 gCO_2/m^2) was much higher than that during the other three stages (150.54 to 310.69 gCO_2/m^2). The relative contribution of *SR* during each freeze-thaw stage to annual total R_s ranged from 8.89% to 61.54%. The *SR* of the AF stage was the lowest (150.54 gCO_2/m^2), among which the UF-substage and ZC-substage accounted for 89.97 and 60.57 gCO_2/m^2 respectively, and the corresponding annual contribution rates were 5.31% and 3.58% (Figure 3).

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3.4 Factors affecting R_s fluxes in different freeze-thaw stages

The R_s was positively related to soil temperatures, following an exponential (Q_{10}) 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 (R^2=0.69), 1.84 (R^2=0.55), 2.38 (R^2=0.90), 4.90 (R^2=0.80), and 1.70 (R^2=0.61) for ST, UF and ZC substages of AF, WC and SW, respectively (Table 2). The soil temperature modified the R_s dynamics differently in the different freeze-thaw stages (Figure 4). In the ST stage, the variations of R_s, T_s, and SWC were basically consistent. The 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 summit (2.42μmol/m^{2}s) till August. Then the R_s decreased with fluctuations as the T_s and SWC dropped. When it came to the AF stage, the T_s and SWC dropped sharply in response to soil freezing, and the R_s continued to decrease slowly and reached its lowest level (1.14μmol/m^{2}s) in mid-October during the UF substage. However, the R_s increased rapidly to a maximum (2.09μmol/m^{2}s) and then dropped quickly with fluctuations although the T_s and SWC continued to lower during the ZC substage. In the WC stage, the R_s decreased as the T_s and SWC lowered continuously, although the active layer was completely frozen. In the SW stage, the R_s went up again as the T_s rose in response to soil warming although the SWC had no change because the surface soil still remained frozen in the earlier stage and fluctuated wildly due to daily freeze-thaw process in the later stage.

4 Discussion

4.1 Magnitude of R_s in the different freeze-thaw stage of the active layer and its Q_{10}

Although many studies have showed that freeze-thaw events affect soil respiration (R_s) in tundra, boreal, and temperate soils (Liu et al., 2016;Du et al., 2013), the R_s and Q_{10} values in different freeze-thaw stages of the active layer are still rarely reported in permafrost regions on the Qinghai-Tibet Plateau. It can be seen through our observation that the different freeze-thaw stages of active layer strongly regulated the R_s emissions and the R_s emission models and *SR* (sum soil respiration) among the different freeze-thaw stages were significantly different. The mean R_s flux (1.75±0.37μmol/m^{2}s) in the ST stage was 1.20, 1.97, and 3.11 times higher than those in the AF (1.46±0.39), WC (0.89±0.42), and SW (0.56±0.10) stages, respectively. Due to the longer duration (157 days) and higher R_s flux, *SR* in the ST stage was approximately estimated to be 60% of annual soil respiration; and the total *SR* in the AF, WC and SW stages accounted for 40% of annual emissions, being consistent with results for another alpine meadow region on the QTP during non-growing seasons (Zhang et al., 2015a). The higher annual contribution of ST stage to the cumulative R_s is likely due to the unique seasonal climate of the plateau. More specifically, the Tibetan alpine meadow receives more than 60–90% precipitation in the ST stage, but maximum 10% in the other stages (Xu et al., 2008). In addition, higher soil temperature and sufficient soil water content in the wet and humid summers stimulated microbial activity and caused higher metabolic rates of soil organisms and roots (Keith et al., 1997). In the alpine ecosystem regions underlain by permafrost on the Qinghai-Tibet Plateau, ten consecutive years of observation has found that the near-surface permafrost is warming, causing the active layer thickness to increase at 4.26cm/a and the duration of thawing to increase by at least 14 days (Wu et al., 2015), which would emit significantly more R_s in the ST stage. Although

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the AF, WC, and SW stages were in the frozen period, and the durations were short for AF (28 days), WC (92 days), and SW (89 days) stages, the cumulative R_s in these three stages contributed significantly to annual R_s. This may be attributed to the high-temperature sensitivities of soil respiration and the maintenance of soil microbial activities at extremely low temperatures (Panikov et al., 2006), resulting in the high rate of soil organic carbon decomposition (Monson et al., 2006).

Meanwhile, the development of freezing and thawing process also modified the temperature sensitivity of soil respiration (Q_{10}) due to seasonal variations in temperature, water, plant phenology and substrate availability (Jiang et al., 2018;Contosta et al., 2013). In the current study, we found large variations in Q_{10} among the different freeze-thaw stages, with the maximum (4.9) in the WC stage and minimum (1.7) in the SW stage. The Q_{10} value (2.2) in the ST stage was smaller than that in the WC stage but higher than that in the SW stage. It has been previously reported that the Q_{10} values in the frozen period are high. For example, in the freezing dormant season of a semiarid ecosystem in the Loess Plateau of China, the mean Q_{10} value reached 4.0 relative to 1.0 in the warm season (Shi et al., 2012); and the Q_{10} was 10.5 during the freezing process in the boreal forests (Du et al., 2013). In the warm season, the R_s mainly originated from auto- and heterotrophic components, but in cold season R_s was mostly composed by the heterotrophic component. Therefore, this seasonal variation in the composition of the soil microbial community may lead to higher Q_{10} in the frozen period. It was found that microbes living in summer cannot survive in winter below 4℃(Monson et al., 2006), and those who have adapted to freezing conditions grew exponentially with increasing temperature. Furthermore, the soil freezing decreased in liquid water and might invoke a physical limitation to substrate diffusion and render R_s more sensitive to temperature (Schimel and Mikan, 2005). So when the freeze-thaw process shifted from ST stage to AF stage, and to the WC stage, the Q_{10} value increased. When it came to the SW stage, due to the soil still being kept frozen and the labile substrate being consumed in the previous stages, the activation energy supply was low (Wang et al., 2014) and the decomposition of recalcitrant substrates was limited, resulting in a sharp decrease of Q_{10}. Whatever the underlying mechanism, the high performance (R^2=0.55–0.90) of the R_s model in the different freeze-thaw stages, along with the high Q_{10} indicated that the potential magnitude of R_s may be increased with global warming.

4.2 Effects of soil temperature and soil water content in each freeze-thaw stage on R_s

R_s values were sensitive to soil temperature changes during the different freeze-thaw stages. When the soil water content was sufficient, R_s was mainly dependent on the soil temperature. In the present study, we observed that the striking results in Figure 4 showed a significant increase in R_s with a CO_2 emission peak in August as the soil warmed and soil water content arose in the first half of the ST stage. When it came to the latter half of the ST stage, although the soil water content at 5cm decreased dramatically, the R_s still emitted at a high rate and declined with fluctuations with soil temperature. This phenomenon may have been caused by an acceleration of microbial activity because the soil temperature increased, and sufficient soil water content made the organic substrates release largely (Kurganova et al., 2007). In addition, soil water content was not a determinative factor affecting soil respiration under the condition that it was adequate.

When it came to the AF stage, although the surface soil temperature declined sharply and the freezing process began to develop upwards from the bottom of the active layer, the R_s flux was still kept at a relatively stable rate (1.20μmol/m^{2}s) in the UF substage because the soil water content was

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still enough (SWC>14%) and the surface soil was still in a thawed state. While at the transition time between UF substage and ZC substage, the R_s flux decreased suddenly to a minimum (1.14μmol/m^{2}s), and then increased sharply to a maximum (2.09μmol/m^{2}s); it fell again with fluctuation. The mechanism for the dramatical change in R_s efflux in this AF stage is not absolutely clear. One possible explanation is that microbes are warm-adapted and more sensitive to T_s when the freezing process is initiated. Furthermore, the process of the diurnal freezing-thawing cycle of surface soil also happened in the earlier ZC substage, and R_s increased rapidly from a very low level over diurnal cycle, indicating that microbes responded rapidly to minor changes in T_s even within several hours (Liu et al., 2016). As the freezing process developed rapidly downwards from the surface soil and upwards from the bottom of the active layer in the ZC substage, the trapped CO_2 in the soil pores would be squeezed out and released during the transition of soil moisture from the liquid to solid state, leading to a dramatically fluctuating R_s.

In the WC stage, we observed that the R_s decreased synchronously as the soil temperature and water content continued to decline, but the R_s rate never reached zero although the soil of active layer was frozen completely, indicating that the soil microorganisms could maintain their activities at extremely low temperatures (Panikov et al., 2006). This result well agreed with other laboratory researches (Kurganova et al., 2007;Panikov and Dedysh, 2000). The continued decrease in soil temperature and liquid water content resulted in a partial death of microbes (Walker et al., 2006), and the microbial activities and substrate affinity were reduced (Nedwell, 1999), causing a continuous decline in the R_s flux. However, 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 et al., 2007). Consequently, the R_s flux always maintained a level higher than zero.

In the SW stage, we found that although the active layer still remained frozen, the R_s flux and the surface soil temperature (T_s) began to synchronously increase with fluctuation; the soil water content (SWC) changed very little (around 0.06 m^{3}m^{-3}) during the previous stage but varied with great fluctuation during the latter stage because the surface soil underwent spring diurnal freeze-thaw process. This phenomenon indicated that the soil temperature (T_s) was the most important driver of R_s in this stage, and the cold-adapted microbes became much more sensitive to increase in T_s (Tribelli and López, 2018;Razavi et al., 2017), causing the R_s to increase rapidly from a low level. In addition, during the spring diurnal freeze-thaw process in the latter SW stage, the surface soil temperature dropped quickly to <0°C in the night and solar radiation again warmed the surface soil and induced the surface soil to thaw in the day (Wang et al., 2016;Bristow and Campbell, 1984). Therefore the SWC changed with great fluctuation, and the R_s was enhanced and its emission rate was maintained at a high level with fluctuation due to the soil microbes being activated by repeatedly freezing and thawing actions.

5 Conclusions

The freezing and thawing process of active layer significantly regulated the soil respiration of the alpine meadow in permafrost region on 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 60.57 to 1041.85 gCO_2m^{-2}, and the cumulated soil respiration in the ST, AF, WC, and SW stages contributed 61.54, 8.89, 18.35, and 11.22% of annual R_s emissions, respectively. At the transition time of diurnal freeze-thaw cycle, we found that the dramatical variations in R_s in the AF stage may be caused by the inactivated warm-adapted microbes due to rapid dropping in temperature and the extruded CO_2 from soil pores due to freezing, and the vigorous variations in R_s in the SW stage may be attributed to the cold-adapted microbes being activated by repeatedly freezing and thawing actions and becoming much more sensitive to increase in temperature. Furthermore, in the scenario of future climate projections of warmer temperature, great changes in freeze-thaw process patterns may have an 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.

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Figure captions

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

Figure 2. Variations of R_s flux at different freeze-thaw stages in years of 2017 and 2018. Error bars show standard error (n=6)

Figure 3. Sum of R_s (*SR*) and its contribution for the four freeze-thaw stages including summer thawing stage (ST), autumn freezing stage (AF), winter cooling stage (WC) and spring warming stage (SW). The value above the bar is the percentage contribution of each freeze-thaw stage to annual total R_s.

Figure 4. Variations in soil respiration (R_s), soil temperature (T_s) and soil water content (SWC) for the four freeze-thaw stages including summer thawing stage (ST), autumn freezing stage (AF), winter cooling stage (WC), and spring warming stage (SW) (from later April 2017 to late April 2018). The SWC unit stands for water volume per total soil volume.

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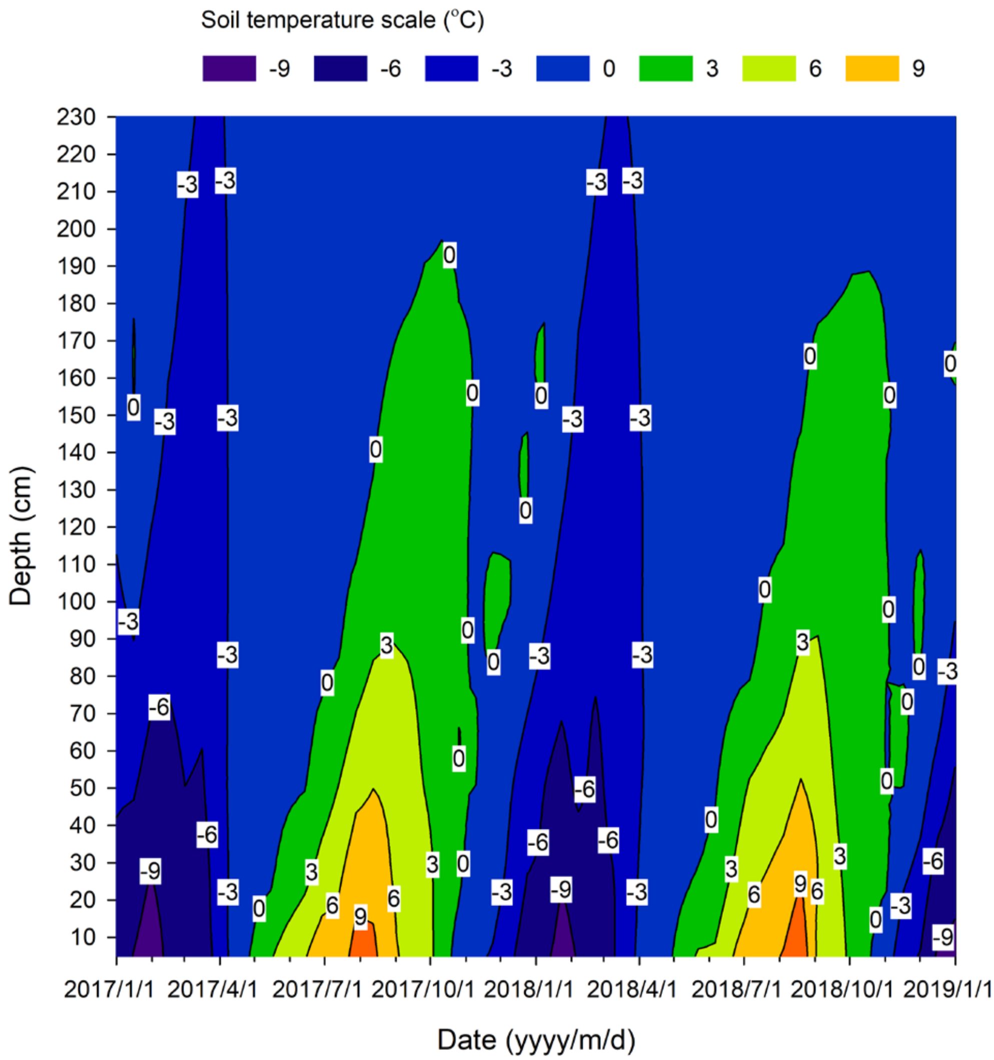

Figure 1

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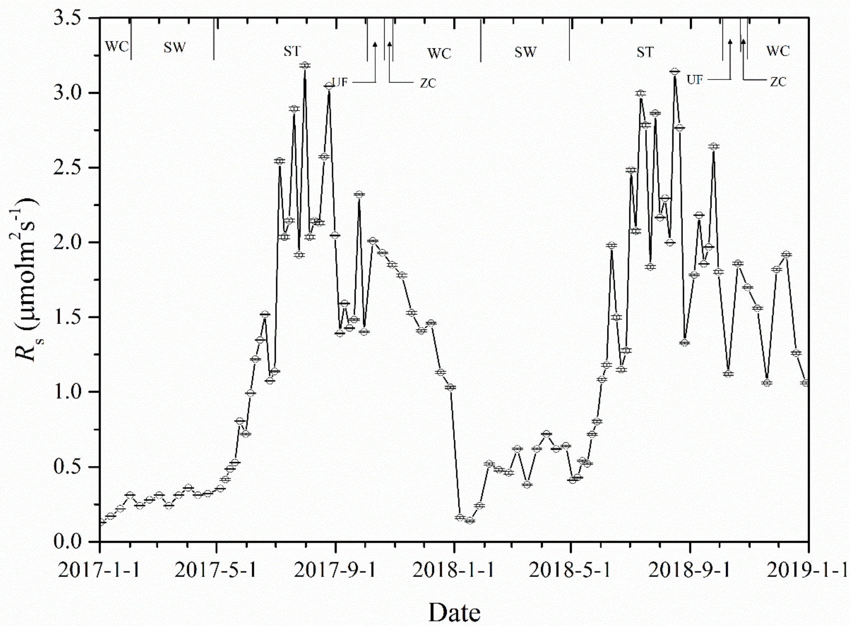

Figure 2

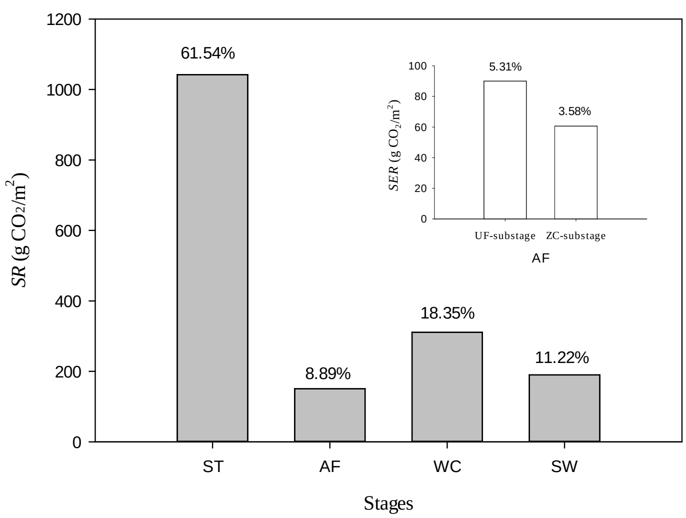

Figure 3

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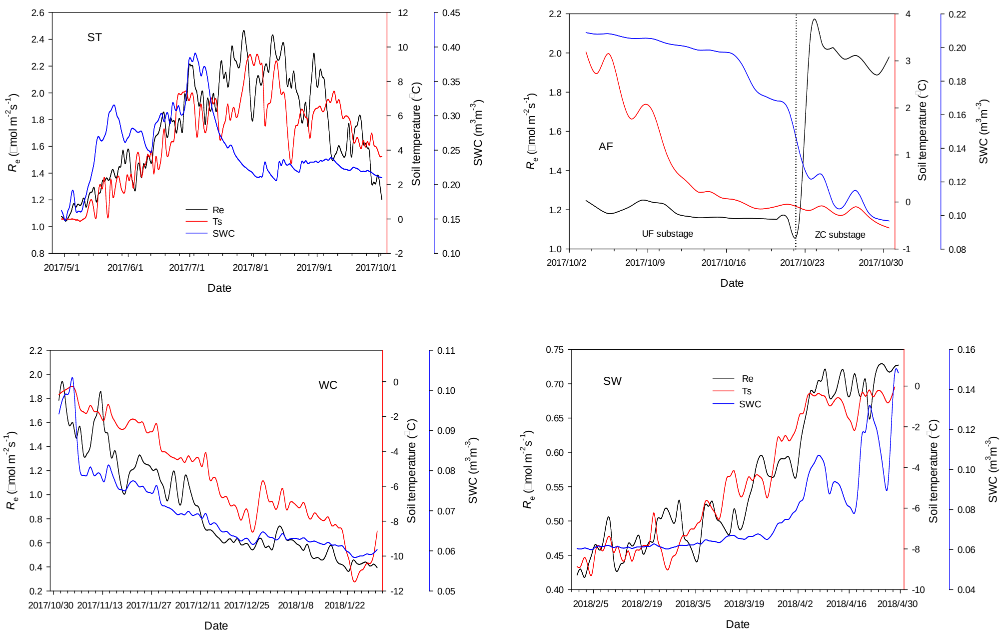

Figure 4