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Sustained increase in soil respiration after nine years of warming in an alpine meadow on the Tibetan Plateau

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ABSTRACT

Soil microbes are key determinants of soil carbon (C) dynamics. The response of the soil microbial community to climate warming modulates the feedback between ecosystem C cycling and future climate change. We conducted a long-term manipulative warming (1.6 °C increase of the soil temperature at 5 cm) experiment to examine the soil respiration, microbial biomass, and community composition at an alpine meadow site on the Qinghai-Tibetan Plateau. After nine years of warming, soil respiration (3.5 μmol m^2 s^{-1} in control in the growing season) increased in the warmed plots. In the early growing season, the increase in heterotrophic respiration (R_h) accounted for more than 90% of the increase in soil respiration. The warming effect gradually decreased during the mid and late growing season (46%). Microbial biomass C and nitrogen declined significantly in the 0–10 cm to the 30–50 cm layer. Warming did not significantly affect microbial biomass C and N in any soil depth layer. Metabolic activity of microbes in terms of R_h per unit microbial biomass C significantly increased by 66% in warmed plots. The bacterial and fungal community composition did not significantly change in the warmed plots. The relative abundance of *Actinobacteria* decreased at 20–30 cm and 30–50 cm soil depths, but that of *Cercozoa* increased in all four soil layers. The relative *Actinobacteria* abundance was negatively correlated with R_h and metabolic activity in the 10–20 and 20–30 cm layers. Our results indicate a decrease in *Actinobacteria* abundance, increases in metabolic activity, and no substrate limitation sustained the positive warming effect on soil respiration throughout the last 9 years. This implies that climate warming could trigger a substantial loss of soil C to the atmosphere in the alpine meadow on the Qinghai-Tibet Plateau.

1. Introduction

Soil respiration (R_s), the efflux of carbon dioxide from the microbial decomposition of soil organic matter (heterotrophic respiration, R_h) and root respiration, is an important component of terrestrial carbon (C) fluxes (Luo and Zhou, 2006). The magnitude and direction of the response of R_s to climate change determines the terrestrial C balance and is key in developing future climate projections (Cox et al. 2000; Melillo et al., 2002; Friedlingstein et al., 2006; Koven et al., 2011). Regions with colder climates are considerably more responsive to climate warming than warmer regions (Carey et al., 2016) because the magnitude of warming is stronger and cold regions store a huge amount of C in the soil, especially in permafrost regions (Liu and Chen, 2000; Liu et al., 2012; Biskaborn et al., 2019).

Although most warming experiments stimulated R_s in various ecosystems (Rustad et al., 2001; Carey et al., 2016), differences in R_s responses to short-term and long-term warming remain largely controversial and inconsistent (Luo et al., 2001; Reth et al., 2009; Melillo et al., 2011, 2017; Metcalfe, 2017). The gradual decline in increased R_s from warming has been observed in field experiments (Wan and Luo, 2003; Knorr et al., 2005; Pold et al., 2017) and in a lab incubation experiment (Li et al., 2019). The decreased positive warming effect on R_s could be attributed to 1) changes in decomposable substrate availability (Wan and Luo, 2003), 2) changes in substrate quality due to

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shifts in plant community dominance (Classen et al., 2015), and 3) changes in the microbial community, corresponding functional genes, and the resilience/acclimation of the microbial community (Griffiths and Philippot, 2013; Crowther and Bradford, 2013; Cheng et al., 2017). However, a sustained stimulation of R_s after ten years of warming has also as well been observed, likely due to a lack of soil water limitation and enhanced microbial growth and activity (Reth et al., 2009).

The soil microbial community is an important link in ecosystem C and nitrogen cycling, thus changes in microbial community composition and physiological processes can modify ecosystem C and nitrogen processes (Allison et al., 2010). The soil microbial community may exhibit strong resilience, in that in the long-term it may return to pre-warming state after a short-term change in community composition or function in response to climate warming (Classen et al., 2015). The resilience of the soil microbial community occurs because 1) many fast growing microorganisms quickly recover after a disturbance, 2) physiologically flexible microbes can acclimate to the new abiotic conditions over time and return to their original abundance even if the relative abundance of some taxa decreased initially, and 3) if physiological adaptation is not possible, the rapid evolution (through mutations or horizontal gene exchange) may allow microbial taxa to adapt to new environmental conditions and recover from disturbance (Allison and Martiny, 2008; Classen et al., 2015). However, other studies also reported no difference between short and long-term warming on the microbial community (Wang et al., 2014; Romero-Olivares et al., 2017), which implies that the microbial communities can be resistant to warming.

The soil of the Qinghai-Tibetan Plateau (QTP) contains 2.5% of the world's soil C pool (Liu et al., 2012), and is predicted to emit substantial amounts of C to the atmosphere under future warming scenarios (Crowther et al., 2016). The soil microbial community structure here was found to change in the short-term warming (Xiong et al., 2014a,b), but how soil microbes adapt to long-term warming and how long-term warming influences soil C decomposition needs to be further investigated. A previous warming experiment in the QTP found no thermal acclimation of R_s and reduced soil moisture (Peng et al., 2014a,b, 2015). Although a warming-induced decline in soil moisture was observed to limit soil microbial activity in a montane meadow (Saleska et al., 1999), the relatively higher soil organic matter and soil water availability during the thawing of permafrost led us to hypothesize that the increase of R_s and R_h in an alpine meadow on the QTP is sustained. The adaptation of microbes to extreme environments makes the soil microbial community more resistant to climate disturbance (Contosta et al., 2015). Thus, we also hypothesized that the soil microbial community of the alpine meadow soil is resistant to long-term warming. The objectives of our study were to determine 1) whether the positive effect of climate warming on R_s and R_h declines after 9 years of artificial warming, 2) whether the microbial community was altered due to nine years of warming, and 3) how the microbial community composition response relates to changes in R_s and R_h in an alpine meadow of the permafrost area in the QTP.

2. Materials and methods

2.1. Study site and the experimental design

The study site was in the Yangtze River headwaters region (92°56E′, 34°49′N), with a mean elevation of 4635 m. Our experiment was carried out at the Beiluhe Permafrost Observation Station. We used a randomized block experiment design to conduct manipulative warming starting in July 2010, and there were five blocks. Paired control and warming plots (2 × 2 m) were set up in each block. Infrared heaters (MR-2420, Kalglo Electronics Inc., USA) were used to heat the plots year-round with a radiation of 150 $W{\cdot}m^{-2}$ at a height of 2 m. Mean annual temperature was −3.8 °C (2010–2019) with monthly mean air temperature ranging from −27.9 °C in January to 19.2 °C in July. Soil development is weak and belongs to the alpine meadow soil taxonomy in China and is classified as Cryosols in the World Reference Base for soil. The parent soil material is of fluvio-glacial origin and sand ($\Phi > 0.05$ mm) content reaches 95%. Permafrost thickness near the experimental site was 60–200 m and the active layer thickness (ALC) was 2.0–3.2 m. The active layer begins to thaw in mid-May and starts to freeze at the end of September (Peng et al., 2014a,b; Xue et al., 2014). The study area is dominated by alpine meadow sedges (*Kobresia capillifolia*, *Kobresia pygmaea*, *Carex moorcroftii*) with a mean height of 5–10 cm in summertime. Plant roots are mainly in the first 20 cm of soil with an average soil organic C of 1.5%.

2.2. Soil microclimate, soil respiration measurement, and soil sampling

Daily soil moisture and soil temperature measurements were taken automatically using thermal probes (Model 109, Campbell Scientific, Inc. USA.) and frequency domain reflection sensors (Sentek Pty Ltd. Stepney, Australia) at different soil depths. Monthly, we measured the R_s and R_h in the growing season (May to September) from 2010 to 2019 using a Licor-6400 attached to a R_s chamber (Li-Cor 6400-09, NE, USA). A PVC tube (50 cm in depth and 12 cm in diameter) was inserted into the soil and living roots were curt in the collar at each plot in 2010. Our previous studies found that after one growing season (Peng et al., 2015), measured CO_2 efflux in the deep collar was lower than the shallow collar (5 cm in depth). Thus, the difference between the CO_2 efflux measured in the deep and shallow collar can be redeemed as R_h. Soil samples for microbial biomass and composition measurement were collected at 0–10, 10–20, 20–30, and 30–50 cm depth with a soil auger on June 27th, 2018. We put a part of the soil samples into tubes that were sent from the DNA sequencing service company, and then placed the tubes in a cooler and sent them to Biomarker (Biomarker Technologies Corporation, Beijing) for DNA sequencing. Another part was stored in sampling bags and sent to a lab for microbial C (MBC) and nitrogen (MBN) measurement.

2.3. Aboveground biomass measurement

In the growing season (May–September), plant coverage and height in each experimental plot were measured once per month. Each plot (2 m × 2 m) was diagonally divided into four subplots. In each subplot, the coverage was measured using a 27 cm × 27 cm frame (two replicates). In each subplot, the height of ten randomly selected plants was measured. Thus, eight plant coverage measurements and 40 plant height measurements were collected for each plot. Coverage and height measurements were averaged for each plot across each month, and the averages were used to estimate the aboveground biomass (AGB) using an empirical model for our study site (Peng et al., 2015). The AGB in August was used for analyzing the AGB difference among different treatments because August was the peak of the plant growth.

2.4. Soil microbial biomass and available nitrogen measurement

The MBC and MBN contents were measured using the chloroform fumigation-extraction method (Brookes et al., 1982). The concentrations of ammonia nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) were determined using a flow autoanalyzer (SEAL analytical AutoAnalyzer 3, Germany) after extraction by 1 M KCl solutions.

2.5. DNA extraction, PCR and DNA sequencing

DNA was extracted from 0.5 g soil using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. The quality and quantity of the extracted DNA were assessed by a Nanodrop spectrophotometer (ND-1000, NanoDrop Technologies, USA). The V3-4 hypervariable region of the bacterial 16S rRNA gene was amplified with the universal primers 338F (5′-ACTCC

TACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Fungal ITS1 region was amplified with the primers ITS1F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS2 (5′-A-TCCTCCGCTTATTGATATGC-3′). Polymerase chain reaction (PCR) amplification products were purified and recovered using a 1.8% agarose gel electrophoresis method. The 16S rRNA and ITS1 gene amplicons were sequenced using Hiseq 2500 PE 100 (Illumina, Inc.; San Diego, CA, USA). The 16s and ITS1 rRNA gene sequences associated in this study were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (accession no. PRJNA563013).

2.6. Data analysis

We used repeated measures ANOVA analysis to test warming and its interaction with measuring time on R_s and R_h. Both R_s and R_h meet the sphericity. Greenhouse-Geisser correction was used to indicate the within-group statistical significance. The two-way ANOVA analysis was used to test warming and its interaction with soil depth on the MBC, MBN, NH_4-N, NO_3-N, microbial C to microbial nitrogen ratio (MBC/MBN), and the alpha diversities of the bacterial and fungal community. Alpha diversity indices included in the analysis were the number of operation taxonomic units (OTUs), Chao1, Simpson, and coverage. The non-metric multidimensional scaling (NMDS) ordination analysis was first used to see whether there was a clear difference in the soil microbial community among different treatments and soil depths. Then, the permutation variance analysis (PERMANOVA) was used to test and compare the centroid and dispersal of the microbial community of different groups. In the study, we did the test with warming as the fixed factor at four depths. The NMDS and PERMANOVA analyses were based on Bray-Curtis distance for both the microbial and fungal community. If significant differences were tested by the PERMANOVA analysis, the Latent Dirichlet Allocation Effect Size (LefSe) analysis was used to determine the biomarker at different taxon. All above analyses were based on the relative abundance of bacteria and fungi at the phylum level. The correlation analysis was conducted to analyze the relationship between the R_h and metabolic activity with the relative abundance of bacteria and fungi at the phylum level.

The amount of CO_2-C produced per unit microbial biomass is defined as metabolic quotient (Anderson and Domsch, 1985). In our study, measured R_h (μg CO_2-C m^{-2} s^{-1}) and corresponding MBC (μg C g^{-1} dw) when R_h measured were used to calculate the metabolic quotient. MBC was measured at 0–10, 10–20, 20–30, and 30–50 cm depths. Thus, the MBC at 0–50 cm was used to calculate the metabolic quotient.

3. Results

3.1. Microclimate, aboveground biomass, and available nitrogen

Compared to the previous years (2010–2017), the year 2018 had the highest total annual precipitation (525 mm) in 2010–2019. The soil moisture of the samples that we collected for microbial biomass measurement at the 0–10 cm depth decreased from 19.1 ± 0.03 v/v % in the control to 13.4 ± 0.01 v/v % in the warmed plots ($P < 0.05$). The soil moisture in the 0–10 and 10–20 cm layers was higher than that at 20–30 and 30–50 cm ($P = 0.082$). The annual average daily soil moisture at 0–10 cm depth was significantly higher ($P < 0.01$) in the control plots (15.7 v/v %) than in the warmed plots (13.8 v/v %) in 2018 (Fig. 1a), but there was no difference in soil moisture in the control and warmed plots for the other three layers. The mean annual soil temperature was significantly higher ~0.6 °C at 5 and 30 cm depths (Fig. 1b) in warmed plots in 2018. The warming-induced change in soil moisture and soil temperature varied seasonally. The temperature increases at all the three depths were lower in May-October than in the other months (Fig. 1b).

In June, the aboveground biomass in the control was 222.8 ± 14.3 g dry matter m^{-2}, and was 68.3 g m^{-2} higher in the warmed plots. In August 2018, the aboveground biomass in the warming plot was 51 g m^{-2} more than in the control. Although non-significant, the NH_4-N at 0–10 cm and 10–20 cm layers was lower in warmed plots. They were 26.3 ± 3.6 versus 19.3 ± 3.6 mg g^{-1} at 0–10 cm and 32.4 ± 3.6 versus 24.7 ± 3.6 mg g^{-1} in the 10–20 cm layer in the control and warmed plots, respectively. There was no difference in NO_3-N between control and warmed plots at the four soil layers.

3.2. Soil respiration

On average, mean R_s and R_h from June to August significantly increased (Table 1) in 2018 by 40% (3.5 μmol m^2 s^{-1} in control) and 20% (1.87 μmol m^2 s^{-1} in control), respectively (Fig. 2a, b). The warming-induced increase in R_s significantly decreased (Fig. 2c) from June to August. In June, the R_h increase accounted for 92% of the change in R_s, but it only accounted for 46% of the R_s increase in August.

3.3. Soil microbial biomass and community

MBC, MBN, and MBC/MBN varied significantly ($P < 0.05$) whereas NH_4-N and NO_3-N showed no difference among different soil depths. The average MBC (509 ± 63.1 μg g^{-1}) and MBN (163 ± 35.1 μg g^{-1}) of the control and warmed plots were the highest in the 0–10 cm layer (Fig. 3a, b). The highest MBC/MBN occurred at 10–20 cm both in the control (3.0 ± 0.6) and warmed plots (3.2 ± 1.4) (Fig. 3c). Warming and its interaction with soil depth did not affect MBC, MBN, NH_4-N, or NO_3-N (Table 1). Warming marginally decreased the MBC in the 0–10 cm layer, but non-significantly enhanced MBC in the 10–20 and 20–30 cm layers (Fig. 3a).

Warming treatment, soil depth, and their interaction did not significantly affect the number of OTUs, Chao, Simpson, or Shannon diversity indices for both bacteria and fungi at the phylum level (Table 2). Only the Simpson diversity index of bacteria was significantly higher at 0–10 cm than the other three depths in both the control and warmed plots ($P < 0.001$, Table 2). NMDS analysis did not show a clear differentiation of the bacterial and fungal community among the warmed and control plots at all four depths (Fig. 4). PERMANOVA results revealed no significant difference in the bacterial and fungal community at the phylum level between control and warmed plots at all four depths (Fig. S1).

When grouped by warming treatment regardless of the soil depth, the beta diversity of the fungal community was significantly different between the control and warmed plots (Fig. S2), but the between-group variation in the control and warmed plots only contributed to 7% of the community variation in 24 samples (Fig. S2). No biomarker taxon was identified by the LefSe analysis.

On the phylum level, the relative abundance of *Actinobacteria* was lower in warmed plots, especially at 20–30 and 30–50 cm depths (Fig. 5a), and the relative abundance of *Cercozoa* was significantly higher in the warmed plots at all four depths (Fig. 5b). R_h was negatively correlated with the abundance of *Actinobacteria* (Fig. 6a) but positively correlated with *Chlorobi* at 10–20 and 20–30 cm depths in June (Table 3). R_h was also positively correlated with *Bacteroidetes*, *Verrucomicrobia*, and *Chlorobi* but negatively correlated with *Gemmatimonadetes* and *Chloroflexi* at 20–30 cm depth (Table 3). No correlations between the relative abundance of *Cercozoa* and other fungal phyla and R_h were observed (Table 3).

The metabolic quotient was negatively correlated with the relative abundance of *Actinobacteria* in the 10–20 and 20–30 cm layers (Fig. 6b). The metabolic quotient of the microbial community was 0.0008 g CO_2-C h^{-1} per g^{-1} MBC (bulk density of 1 g cm^{-3} was used to convert the R_h from area based to mass based) in the control plots, which was significantly higher by 66% in June in the warmed plots (Fig. 7).

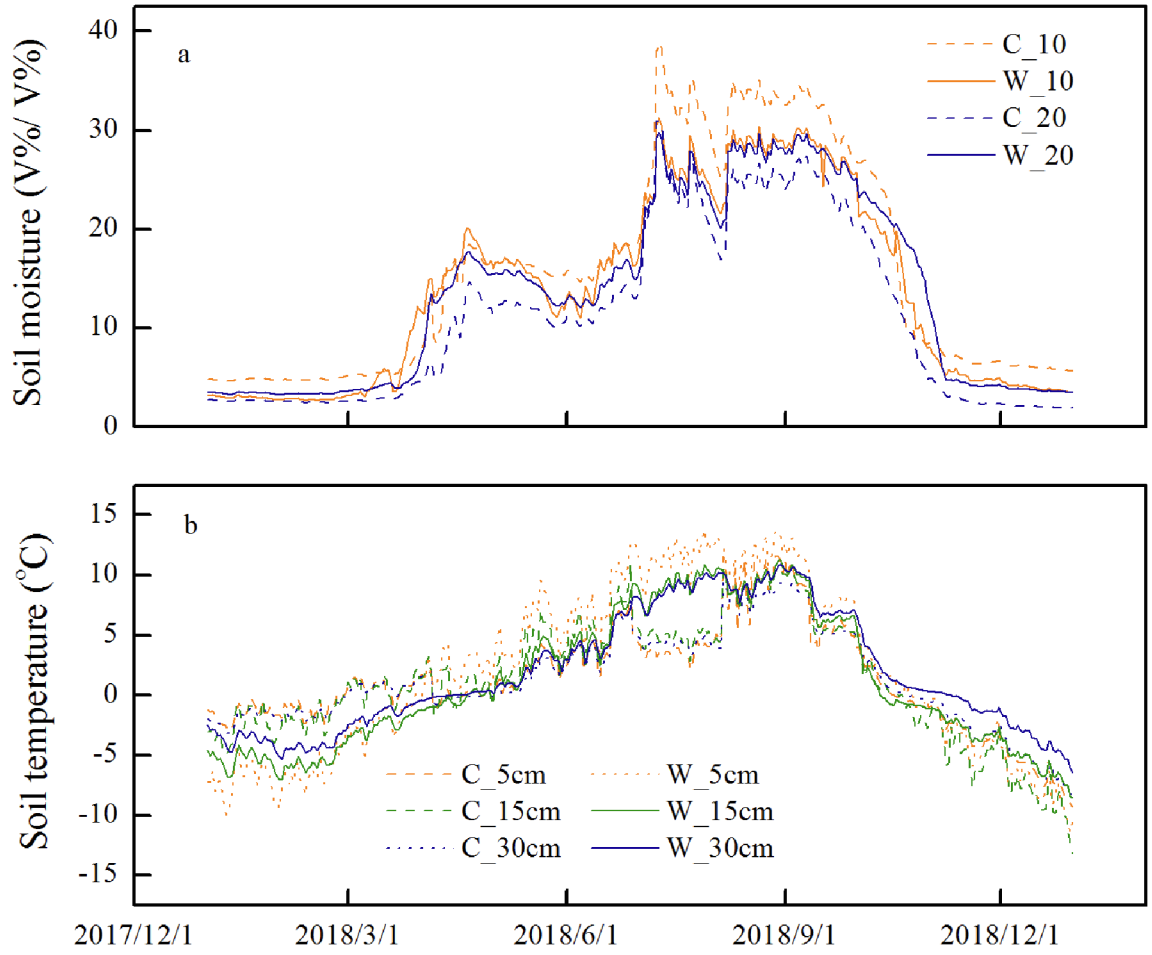

Fig. 1. The annual precipitation in the study site from the beginning of the experiment (2010) to the end of 2019 (a), mean daily soil moisture at 0–10 and10-20 cm depths (b), and mean daily soil temperature at 5, 15, and 30 cm depths (c) in control and warmed plots.

Table 1
P value of the variance analysis of warming, soil depth, months and their interaction on the soil respiration (Rs), heterotrophic respiration (Rh), soil microbial carbon (MBC), soil microbial nitrogen (MBN), soil ammonia nitrogen, nitrate nitrogen and microbial carbon to microbial nitrogen ratio (MBC/MBN). The MBC to MBN ratio was square-root transformed because the raw data don't meet the variance homogeneity.

Variance Resource	R_s	R_h	MBC	MBN	Ammonia	Nitrate	MBC/MBN
Treatment	0.03	0.059	0.28	0.57	0.45	0.87	0.83
Depth			< 0.01	0.03	0.39	0.46	< 0.01
Month	< 0.01	0.06					
Treatment × Month	0.68	0.38					
Treatment × Depth			0.77	0.80	0.23	0.81	0.78

4. Discussion

4.1. Soil respiration responses to climate warming

The temporal dynamics of R_s response to warming is directly affected by the temperature increase. In addition to the direct effect of temperature, the warming-induced change in soil moisture (Reth et al., 2009), decomposable substrate availability, plant community composition (Rinnan et al., 2009, 2007), permafrost thaw (Schuur et al., 2013), and the microbial community adaption (Classen et al., 2015) could also determine the temporal dynamics of the response of R_s to climate warming (Melillo et al., 2017). In our study, we continued to see higher rates of R_s and R_h in the warmed plots after nine years of warming (Fig. 2a b). The sustained increase in R_s and R_h differed from the results reported by other long-term warming studies in an alpine grassland (Saleska et al., 1999), a forest (Melillo et al., 2011), and a tall grass prairie (Luo et al., 2001). At our study site, R_s had a polynomial relationship with soil moisture, which reaches the maximum at a soil moisture of ~13 v/v % (Peng et al., 2014a,b; 2015). Even though the mean soil moisture of the growing season decreased, it remained higher than 13% (Fig. 1) in the warmed plots partly due to the high precipitation in 2018. The relatively higher soil moisture even in the warmed plots indicates a lack of water limitation, thereby contributing to the sustained increase of R_s in 2018. At the beginning of the growing season, the permafrost thaw will release a large amount of decomposable C (Shang et al., 2016), which can lower the substrate limitation restricting R_s and R_h. The similar relative increasing magnitudes between R_s and R_h in June (Fig. 2c) indicate low root activity. Although low root activity will not provide much root exudates, root debris and other sources of C from thawed soil could ensure no C limitation for soil microbial decomposition in the early growing season. We did not measure the labile soil organic C, but in our previous study we found a

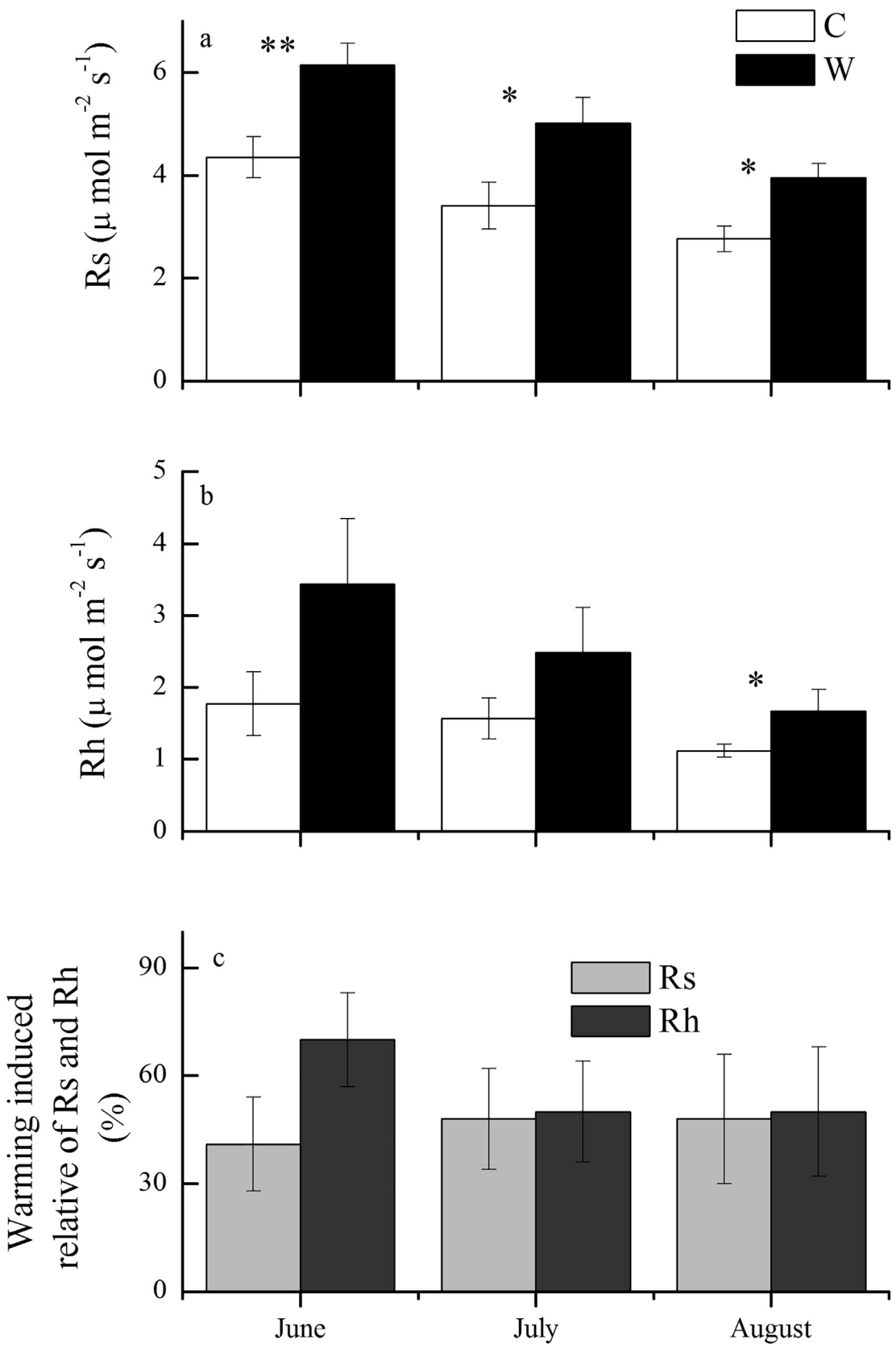

Fig. 2. Soil respiration (R_s, a), heterotrophic respiration (R_h, b) and warming-induced change of R_s and R_h (c) in June, July, and August. ** above columns represent significant difference at $P < 0.05$, and **, $P < 0.10$.

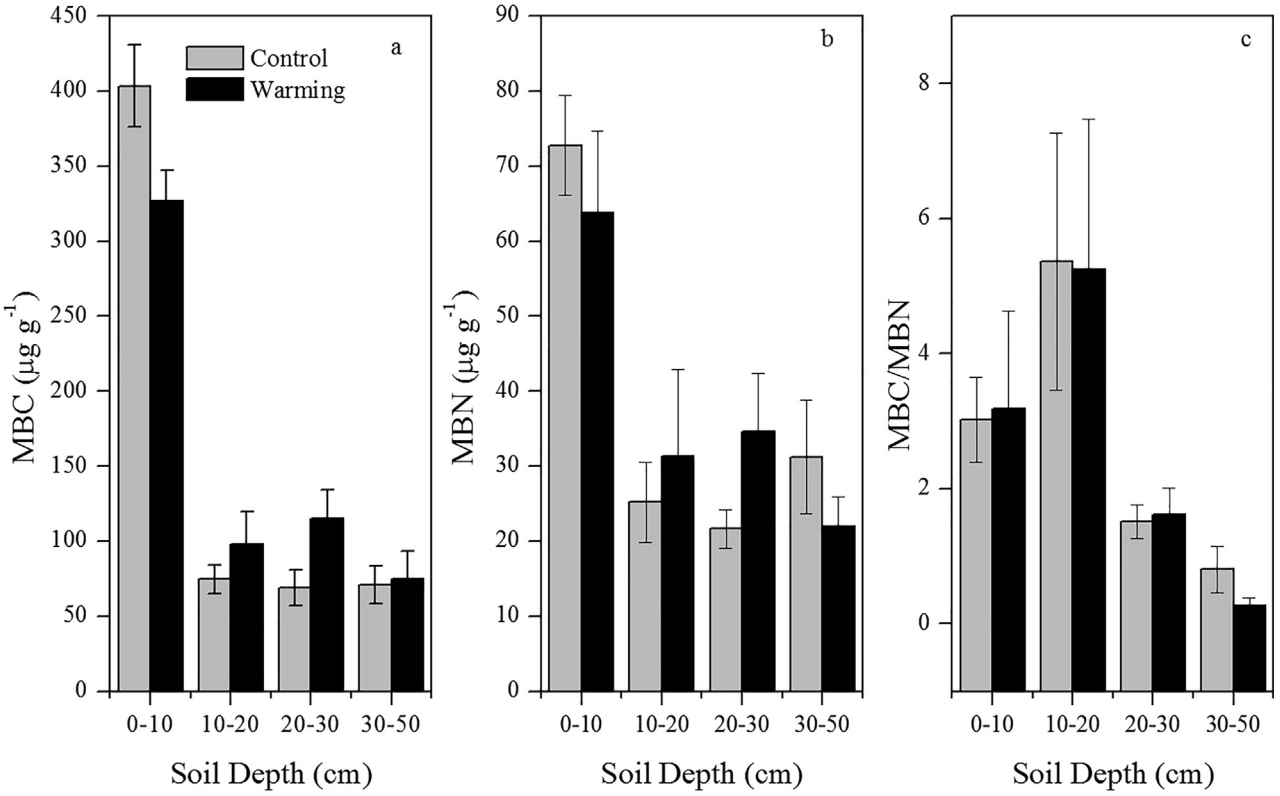

Fig. 3. The average microbial biomass C (MBC, a), microbial biomass nitrogen (MBN, b), and MBC to MBN ratio (MBC/MBN, c) at 0–10, 10–20, 20–30, and 30–50 cm layers in the control (light gray column) and warmed treatments (black column).

higher labile C in the 30–40 and 40–50 cm layers, mainly due to increased root biomass in these two layers in warmed plots (Peng et al., 2015). The increase in root biomass in the middle and end of the growing season (Xu et al., 2015) may provide abundant C for microbes and thereby sustain the increase in R_s in warmed plots in August (Fig. 2). The increase in aboveground biomass could enhance the litterfall, which can also help to alleviate the substrate depletion in warmed plots. But in a dry year, warming-induced reduction of soil moisture can limit plant growth (Peng et al., 2014a,b), and consequently suppress the microbial decomposition of soil C. Therefore, our results should be interpreted with some caution since 2018 was a wet year.

The lack of a change in the copiotrophic bacterial phyla, such as *Proteobacteria, Bacteroidetes,* and the fungal phylum *Ascomycota* (Fierer et al., 2007, Fig. 5), and a lack of a change in the oligotrophic bacterial phylum like the *Acidobacteria* (Fig. 5), suggested that there was no substrate limitation on the microbial decomposition of the soil organic matter. Similar to our results, Reth et al. (2009) and Schindlbacher et al. (2015) reported a sustained increase of R_s after 9 years of soil warming. In their study, the aboveground biomass was similar in control and warmed plots, therefore the substrate depletion resulting from the labile and recalcitrant kinetic model cannot explain the sustained stimulation in R_s. They attributed the persistent increases in R_s to no soil moisture limitation and changes in microbial growth and activity (Reth et al., 2009; Schindlbacher et al., 2015). In our study, the sustained, higher rates of R_s in the warmed plots likely was due to the lack of soil water limitation, increased aboveground biomass in the growing season, and thawed decomposable C in the early growing season.

Microorganisms can dispose of C via overflow respiration as CO_2 (respiration without the production of energy (Russell and Cook, 1995)) to make the substrate meet their nutritional demands when growing in N-poor conditions (Spohn, 2015). When growing on N-poor substrate, microorganims do not have enough N to build up as much as biomass as the C concentration would allow (Spohn, 2015). The overflow respiration is one alternative explanation for the positive relationship between metabolic quotient and litter C to N ratio (Spohn, 2015). Overflow respiration has been observed in microbial species in lab incubation (Russell and Cook, 1995) but is still questioned by several ecosystem studies. In our study, the reduction of NH_4-N at the 0–10 and 10–20 cm layers might make the N limitation more progressive. Given the limited N availability, overflow respiration might be an alternative or additional explanation for the increase in R_h.

4.2. Microbial biomass and microbial community composition

In line with previous studies, our results confirmed that

Table 2
P values of the two-way ANOVA analyses for the diversity indices of the bacterial and fungal community under the warming treatments (warmed and control) and at different soil depths (0–10, 10–20, 20–30, and 30–50 cm).

	Bacteria						Fungi					
Variance source	OTU	ACE	Chao1	Simpson	Shannon	Coverage	OTU	ACE	Chao1	Simpson	Shannon	Coverage
Treatment	0.429	0.482	0.530	0.936	0.601	0.959	0.966	0.940	0.832	0.555	0.901	0.855
Depth	0.663	0.644	0.753	< 0.001	0.128	0.426	0.811	0.640	0.542	0.116	0.055	0.115
Treatment × Depth	0.766	0.692	0.720	0.319	0.785	0.991	0.432	0.222	0.605	0.674	0.718	0.982

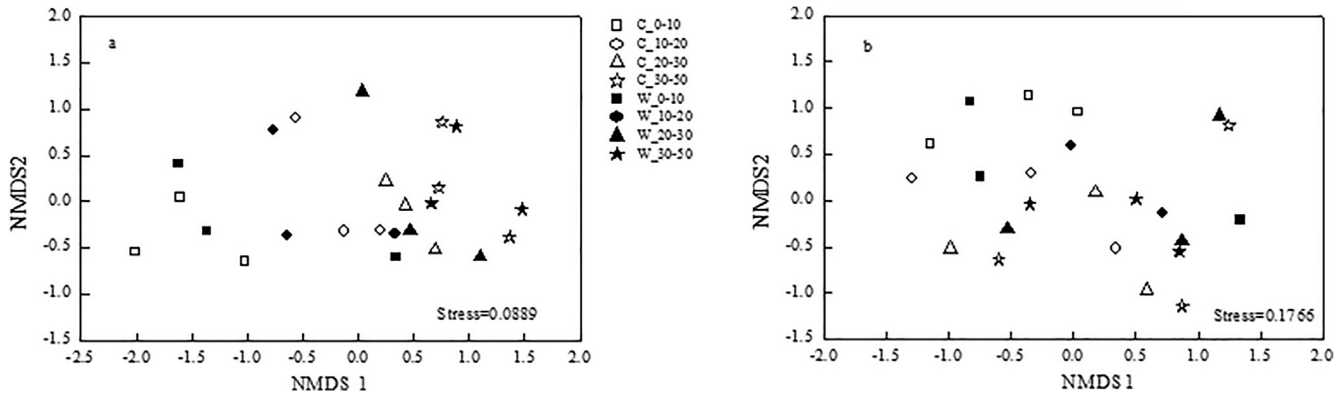

Fig. 4. NMDS of the bacterial (a) and fungal (b) communities based on Bray-Curtis distance at 0–10, 10–20, 20–30, and 30–50 cm in control and warmed plots.

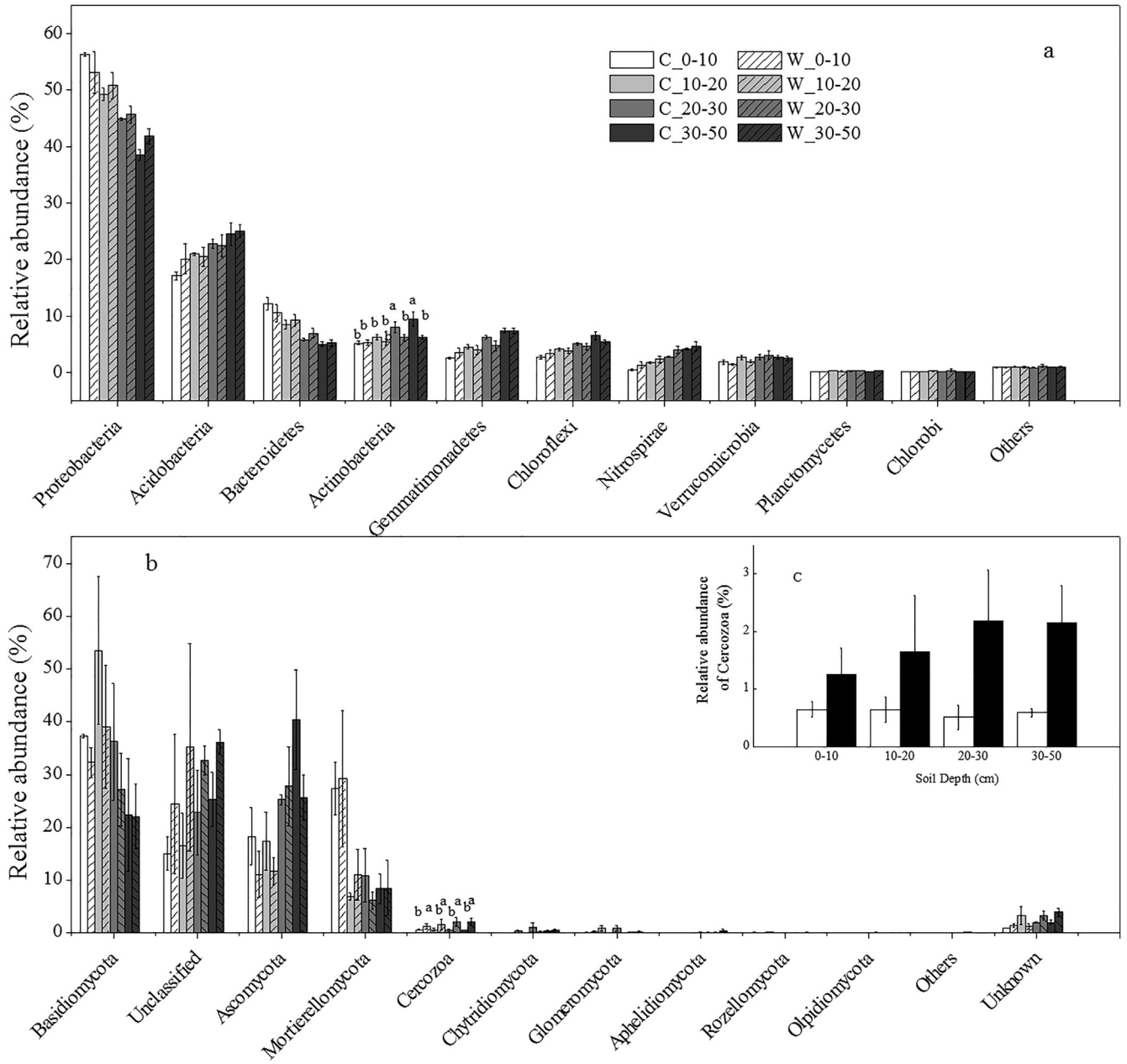

Fig. 5. Relative abundance of bacterial and fungal phyla control and warmed plots. The presented relative abundances are based on the 97% similarity clusters of the OTUs. Bars with different letters mean significant difference between control and warmed plots at $P < 0.05$, n = 3.

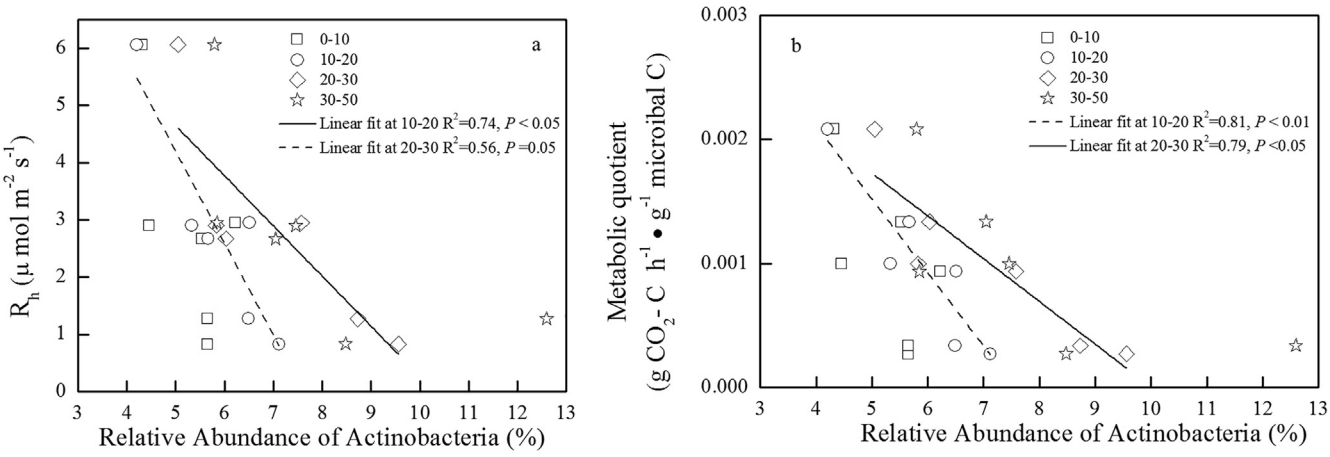

Fig. 6. The relationship between the relative abundance of *Actionobacteria* with R_h (a) and metabolic quotient (b) at different depths.

Proteobacteria, *Acidobacteria*, *Actinobacteria*, and *Bacterioidets* (Fig. 5) were the most abundant taxa in the soil of alpine grassland (Zhang et al., 2016). Only the relative abundance of *Actinobacteria* (Fig. 5a) decreased, and *Cercozoa* (Fig. 5b) increased particularly in deep layers. Our results are in contrast to other studies, which that found *Actinobacteria* increased after 20 years warming in a metagenomics study (Pold et al., 2017), in Arctic soils warmed for 18 years (Deslippe et al., 2012), and in a temperate mountain forest soil (Liu et al., 2017). *Actinobacteria* is found to be positively correlated with temperature and soil organic matter (Siles and Margesin 2016; Yergeau et al., 2012). Increased C availability in warmed soils favors the fast-growing microorganism like *Actinobacteria* (Fierer et al., 2007). Thus, the increase in aboveground biomass but the decrease in relative abundance of *Actinobacteria* seems to contradict previous studies. Both the association of *Actinobacteria* with labile (Ali et al., 2018) and recalcitrant C (Barret et al., 2011) were reported. When *Actinobacteria* is associated with recalcitrant C, the increase in labile C due to the increase in root biomass might lead to the reduction in *Actinobacteria* (Fig. 5c). The negative correlation between the relative abundance of *Actinobacteria* and R_h (Fig. 6a), increase in R_h, and no C limitation indicates the association of *Actinobacteria* with recalcitrant C.

For the soils on the QTP, a clear decrease in bacterial diversity with soil depth was observed in the first 20 cm, which was probably due to plant growth and the input of nutrients only in the topsoil (Ollivier et al., 2014), soil moisture, and pH (Wu et al., 2017). The significant impact of soil depth on alpha diversities of bacterial and fungal phyla (Table 2) suggested a shift in microbial community structure with soil depth. No interactive effects between soil depth and warming on the microbial diversities at the phyla and class levels (Table 2) imply similar responses of microbial community to warming among different soil depths, which agrees with results of an open top chambers warming experiment near our study site (Zhang et al., 2014) but differs with observations in tundra where the carbohydrate utilization genes increased in the 15–25 cm layer and methanogenesis increased in 45–55 cm (Johnston et al., 2019). The higher soil moisture in their study (~75% in 15–25 cm and 30–40% in 45–55 cm layers) may lead to the increase of methanogens in the deep layer (Johnston et al., 2019), while in our study we don't expect anaerobic conditions due to the relatively low soil moisture (Fig. 1).

Individual experiments discovered a lagged microbial community response to climate warming, especially in tundra (Rinnan et al., 2007, 2009; Johnston et al., 2019) and alpine ecosystems (Zhang et al., 2016). In these cold regions, it takes more than a decade to detect first changes in the soil microbial community composition (Rinnan et al., 2007, 2009). Warming-induced changes in soil edaphic and plant properties rather than the temperature increase itself may regulate the lagged response of the soil microbial community to warming (Allison et al., 2010; Li et al., 2016; Zhang et al., 2016). As we discussed in Section 4.1, there was likely no substrate limitation on R_s and R_h. Thus, the effect of substrate availability on the soil microbial community was negligible. The relatively stable soil substrate would, therefore, result in unchanged microbial functional diversity and structure (Tang et al., 2019). The high aridity of our study site (with annual precipitation around 300 mm) could also contribute to the lack of a change in the microbial community (Fig. 4) since bacterial composition is found to be resistant to warming when annual precipitation is less than 515 mm (Sheik et al., 2011). The microbial adaption to temperature extremes and C and nutrient availability will also render them resistant to warmer conditions (Contosta et al., 2015). In our study site, the microbes experience dramatic daily temperature variation (with a daily temperature range of 20 °C in summer), thus the adaptation to this harsh environment could make them resistant to the nine years of consecutive warming. The fungal community grows slowly and is found to be resistant to short-term warming in Tibetan grassland soils (Li et al., 2019; Xiong et al., 2014a). The fungal community may respond to warming only when the labile substrate is depleted (Frey et al., 2008), as fungi predominantly control the decomposition of recalcitrant organic matter (de Boer et al., 2005). In our study, the absence of labile C limitation, as indicated by a sustained increase in R_s after nine years, implies the same use of recalcitrant C in both warmed and control plots led to a lack of change in fungal community (Fig. 4).

Most warming experiments showed no changes in alpha diversity but did find changes in beta diversity of the bacterial and fungal community (Xiong et al., 2014a,b; Zhang et al., 2016) in the short-term through taxonomic shifts (Zhang et al., 2016). Fifteen months of warming in our study site caused a significant increase in some phyla like *Actinobacteria*, *Alphaproteobacteria*, and Basidiomycota-affiliated groups (Xiong et al., 2014a,b). But after nine years of warming, there was no difference in the beta diversity and microbial composition in the control and warmed plots (Fig. 4. Table 2), which was similar to observations in 3 years of warming in an alpine meadow in Haibei (Li et al., 2016).

4.3. The change in metabolic activity of microbes and heterotrophic respiration

A global meta-analysis detected a positive relationship with microbial metabolic activity and temperature (Ye et al., 2019). In our study, the absence of C limitation and a unchanged soil microbial community structure (Fig. 4) indicated that the increase in soil microbial metabolic activity may be the primary factor driving the sustained high rates of in R_s and R_h in the warmed plots. The metabolic quotient can be expressed as a mass-based respiration rate (Schindlbacher et al., 2011), which was found to increase with incubation temperature but

Table 3
Correlation coefficients between Rh in June and relative abundance of bacteria and fungi in the phylum level (n = 6).

Bacteria													
	Proteobacteria	Acidobacteria	Bacteroidetes	Actinobacteria	Gemmatimonadetes	Chloroflexi	Nitrospirae	Verrucomicrobia	Planctomycetes	Chlorobi	Others		
0–10	0.22	0.03	−0.04	−0.64	−0.21	−0.20	0.15	−0.36	−0.06	−0.23	−0.53		
10–20	0.62	−0.20	0.01	**−0.91***	−0.75	−0.54	0.62	0.02	0.10	**0.91***	−0.42		
20–30	0.64	−0.41	**0.84***	**−0.85***	**−0.98***	**−0.91***	0.66	**0.85***	0.61	**0.84***	**0.90***		
30–50	0.32	−0.08	0.37	−0.66	−0.03	−0.47	0.74	0.48	0.55	−0.17	0.76		
Fungi													
	Basidiomycota	Unclassified	Ascomycota	Mortierellomycota	Cercozoa	Chytridiomycota	Glomeromycota	Aphelidiomycota	Rozellomycota	Olpidiomycota	Unknown		
0–10	−0.29	−0.17	0.56	0.58	−0.10	−0.42	−0.27	−0.60	−0.42	−0.58	−0.15		
10–20	−0.03	−0.06	−0.02	0.63	0.01	−0.41	−0.44	−0.47	−0.62	−0.40	−0.38		
20–30	−0.49	0.28	0.70	−0.08	−0.02	−0.46	−0.45	−0.31	−0.72	−0.02	−0.09		
30–50	0.06	0.39	−0.25	−0.09	−0.09	−0.39	−0.36	−0.17	0.48	0.03	0.09		

Numbers in bold are significant correlation at $P < 0.05$.

Fig. 7. Microbial metabolic quotient expressed as heterotrophic respiration rates per microbial biomass carbon (g CO_2-C h^{-1} g^{-1} microbial C, n = 3) at control and warmed plots in June 2018.

was not different between the control and warmed soils (Schindlbacher et al., 2015). By contrast, the microbial C use efficiency will decrease, which indicates that some energy-demanding processes outbalanced the microbial C gain at increasing temperatures. Hagerty et al. (2014) proposed that the apparent decline in microbial C use efficiency at higher soil temperatures is caused by increased microbial turnover rates. With a higher microbial turnover, a strong decrease in the microbial biomass pool was observed in the longer-term warming experiment (Hagerty et al., 2014). In our study, although the MBC decreased non-significantly in the 0–10 cm soil layer, it may indicate a higher turnover of soil microbes and a decline in microbial C use efficiency in the surface layer. Microbial growth efficiency is also generally expected to decline due to increased microbial maintenance costs at higher temperatures. The higher maintenance cost in warmed plots could thus lead to increased metabolic activity (Fig. 7). The relative abundance of *Actinobacteria* was negatively correlated with the respiration quotient at 10–20 and 20–30 cm depth (Fig. 6b). The respiration quotient was calculated using the MBC in the entire 50 cm soil column. Therefore, the positive correlation between respiration quotient and the relative abundance of *Actinobacteria* (Fig. 6b), and the decrease in the 20–30 cm layer, suggested that R_h is mainly from the mid- to deep soils. The change in a small subset of the soil microorganisms might therefore be one reason for the sustained higher rates of R_s and R_h.

5. Conclusion

R_s and R_h maintained a positive response throughout nine years of manipulative warming of the meadow ecosystem. The microbial community composition was largely unaffected by warming at the soil surface down to the 50 cm depth. The increase in the aboveground biomass could ensure the availability of substrate for microbial decomposition and the associated slight decline in the soil moisture in the soil surface layer apparently did not negatively affect R_s rates. The intensification of metabolic activity in terms of R_h per unit MBC indicated an increase in maintenance respiration. The relative abundance of

Actinobacteria at the deep layers was negatively correlated with the metabolic activity of soil microbes. The abundant C supply, increase in metabolic activity, and a change in a small subset of the soil bacteria suggest a sustained increase in soil C emission to the atmosphere in an alpine meadow under climate warming.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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