



Plant-mediated effects of long-term warming on soil microorganisms on the Qinghai-Tibet Plateau

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ABSTRACT

Exploring the feedback of the soil microbial community and associated processes to global warming represents a major global challenge. To date, the focus has been placed on the direct effects of warming on soil microbial communities, overlooking how concurrent changes in plant communities may mediate these effects. Additionally, few studies have examined long-term effects of warming in more than one environmental context. In the present study, we conducted a long-term simulated warming experiment to investigate how changes to the plant community within two different environmental contexts affect the responses of soil microorganisms and their respiration to warming. We analyzed the abundance, diversity, and community composition of plants and soil microbes, in addition to soil microbial interaction networks and soil microbial respiration, in two typical ecosystems of the Qinghai-Tibet Plateau. Following long-term warming, the soil microbial composition, structure, and interactions changed, and the shifts depended on the aboveground plant type. Specifically, the co-occurring networks containing different microbial communities tended to be more complex in a shrubland than in a grassland after warming, leading to higher carbon use efficiency. Additionally, long-term warming changed the structure of soil microbial communities, increasing the relative abundances of oligotrophic taxa in the shrubland but not in the grassland. The shifts in community structure and interaction patterns could be explained by vegetation community attributes, highlighting the strong effect of plants on soil microbial responses. These plant-mediated effects on community structure and interactions subsequently could explain changes in soil microbial respiration rates. Microbial respiration showed a positive response to elevated temperature in the grassland but no response to temperature in the shrubland. These results indicate that interactions between soil microbial communities and plant communities determine how soil microbes respond to global warming. Therefore, future research on soil microbial community composition and associated carbon feedbacks to the climate change should include plant-mediated effects, which can provide a scientific basis for effectively mitigating global warming.

Abbreviations: SOC, soil organic carbon; N, nitrogen; DOC, dissolved organic carbon; SL, shrubland; GL, grassland; PLS-PM, partial least squares path model; OTC, open-top chamber; NMDS, nonmetric multidimensional scaling; PERMANOVA, permutational multivariate analysis of variance; GOF, goodness of fit.

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1. Introduction

The balance between terrestrial and atmospheric C regulates the climatic and biological systems of Earth (Friedlingstein et al., 2006); therefore, it is imperative to understand how global warming affects terrestrial ecosystem C cycling. Soil microbes are involved in modulating nutrient cycling processes, including greenhouse gas emissions (Canarini et al., 2017). Therefore, it is important to explore how soil microbes respond to elevated temperatures and to understand whether the terrestrial ecosystem will be a C source or sink in the future.

Global warming affects soil temperature and moisture, directly affecting soil microorganisms and their long-term activities. Soil temperature and moisture potentially affect greenhouse gas emissions by altering (1) soil microbial biomass and physiology (e.g., the activities of extracellular enzymes) (Baldrian et al., 2010) and (2) soil microbial community structure (DeAngelis et al., 2015). Specifically, a decrease in soil moisture caused by warming was found to suppress soil microbial biomass and activities of extracellular enzymes (Allison and Treseder, 2008). However, long-term warming potentially increases the abundance of bacteria and changes the beta diversity by providing more or alternative niche spaces (DeAngelis et al., 2015). Moreover, plant communities are also affected by global warming and may indirectly mediate the effects of global warming on soil microbial communities and biogeochemical cycles (Bragazza et al., 2015). Changes in plant community compositions in response to warming could affect soil microbial communities and respiration through changes in litter and root inputs (i.e., C allocation patterns and plant-derived organic matter quality) (Ward et al., 2015). Additionally, changes in plant communities can affect soil pH or the high levels of polyphenols produced by its litters, affecting the catabolic processes of soil microorganisms (Rydin and Jeglum, 2006; Ward et al., 2015).

Plant communities are changing concurrently with global warming; however, how the interaction between plant community changes and global warming affects soil microbes and their activities under natural conditions is yet to be fully understood. Additionally, the direct effects of global warming and indirect effects mediated by plants on microbial community composition and function still need to be disentangled (Kardol et al., 2014). Given that the composition and respiration rates of most soil microbial communities are affected by both global warming and plant community composition, it is important to establish their combined effects on soil microbial carbon feedbacks under long-term global warming.

Similarly to that in aboveground organisms, a series of complex negative (competition, predation, and parasitism) and positive (mutualism and commensalism) interaction modes are common to soil environments (de Menezes et al., 2017). Previous studies have provided crucial insights into how global warming affects the composition of soil microbial communities (Ward et al., 2013, 2015). However, these studies were based on pure cultures and genetic studies, with a primary focus on single properties or related ecological functions (Deng et al., 2012). Additionally, each organism was typically studied in isolation or only in its taxonomic group (Tao et al., 2018). Consequently, community interactions are overlooked by these approaches, and therefore, understanding the mechanisms underlying the response of networks of co-existing and interacting soil microbes to climate disturbances is a key knowledge gap (Zhou et al., 2011). In recent years, there has been increasing research on the influence and mechanism of soil microbial interactions on community structure, species distributions, and functions (Xiao et al., 2018; Li et al., 2021). However, further work is required to determine whether complex microbial interaction networks change in response to global warming, which in turn, would affect C feedbacks.

Global climate change represents a major threat to the Qinghai–Tibet Plateau because the temperatures in this region are rising more rapidly than the global average temperature (Kang et al., 2010). Consequently, this region exhibits higher ecological sensitivity to global climate

change (Jing et al., 2016). Large quantities of soil organic C (SOC) are stored in this region (Jing et al., 2016). Over the past 20 years, global climate change has already induced striking impacts on ecosystem C and N processes on the Qinghai–Tibet Plateau (Chen et al., 2013). While the effects of plant species and community attributes on soil microbial communities have been widely explored, knowledge remains limited regarding how plants with different physiological and ecological characteristics mediate the effects of long-term warming on soil microorganisms. Thus, in the present study, we evaluated how long-term warming affects two typical plant communities [shrubland (SL) and grassland (GL) with different litter quality] of the Qinghai–Tibet Plateau and related soil C losses by soil microbial respiration. Specifically, we aimed to (1) quantify, over 21 years, how soil microbes (biomass, diversity, community composition, and community interactions) and plant communities (species composition and diversity) respond to warming and (2) provide new information regarding how ecological variables (soil properties and altered plant community composition) shape the community composition of soil microbes and their respiration by partial least squares path modeling (PLS-PM). We hypothesized that (1) the effects of long-term warming on above- and belowground ecological variables (plant and soil microorganism communities, and the interactions among microorganisms) would vary depending on plant type, and (2) microbial responses to long-term warming in different ecosystem types would be driven by plant community attributes.

2. Materials and methods

2.1. Study area and sample collection

This study was conducted at the Haibei Alpine Meadow Ecosystem Research Station (37° 36' N, 101° 19' E, 3220 m above sea level) (Fig. 1), where the climate is plateau continental. The annual mean temperature is -2°C , and the annual mean precipitation is 500 mm, of which the majority occurs during summer. The growing season extends from May to September. Mollic-Cryic Cambisol is the main soil type (Zhao and Zhou, 1999). There are two dominant ecosystems widely distributed in this region: meadow habitats dominated by *Kobresia humilis* and shrub habitats dominated by *Potentilla fruticosa*. One study site of $30\text{ m} \times 30\text{ m}$ was established in each of two ecosystems, which are hereafter referred to as the GL and SL sites, respectively. The elevation difference between the two study sites was not more than 30 m. There were several other plant species at the sites, including *Gentiana straminea*, *Kobresia pygmaea*, *Stipa aliena*, and *Elymus nutans*.

Within each site, we established six replicate control plots and six replicate warming plots. The plots were established in 1997 and protected from grazing animals by fencing. Warming was simulated using 1.0-mm thick Sun-Lite HP fiberglass (Solar Components Corporation, Manchester, NH, USA) open-top chambers (OTCs; 0.4 m height and 1.5 m diameter; Fig. S1). Each plot covered a total area of 1.77 m^2 , with a 2-m buffer zone between each plot. The mean daily temperature of air (at a height of 10 cm) and the mean temperature of soil (at a depth of 12 cm) increased by $1.0\text{--}2.0^{\circ}\text{C}$ and $0.3\text{--}1.9^{\circ}\text{C}$, respectively, and $< 3\%$ of the soil moisture content was reduced from May to September in the OTCs (see details at Klein, 2003; Klein et al., 2004; Zhang et al., 2017a). Moreover, the OTCs' effects on temperature differed and depended on habitat. For example, the warming effect at the SL site was generally weaker than that at the GL site (Klein, 2003).

Soil samples were collected from the two sites in August 2018, 21 years after the warming treatment began. After removing surface litter and O horizon material, four soil cores (5 cm diameter, 0–5 cm depth) from each plot were randomly collected and transferred to the laboratory in zip-lock plastic bags placed in ice boxes. In total, there were 24 samples (2 ecosystems \times 2 treatments \times 6 replicates). All soil samples from the same plot were mixed and then sieved with a 2.0-mm sieve to remove coarse ($>2\text{ mm}$) and fine ($<2\text{ mm}$) roots, debris, and stones and were separated into two sub-samples. One sub-sample was stored at –

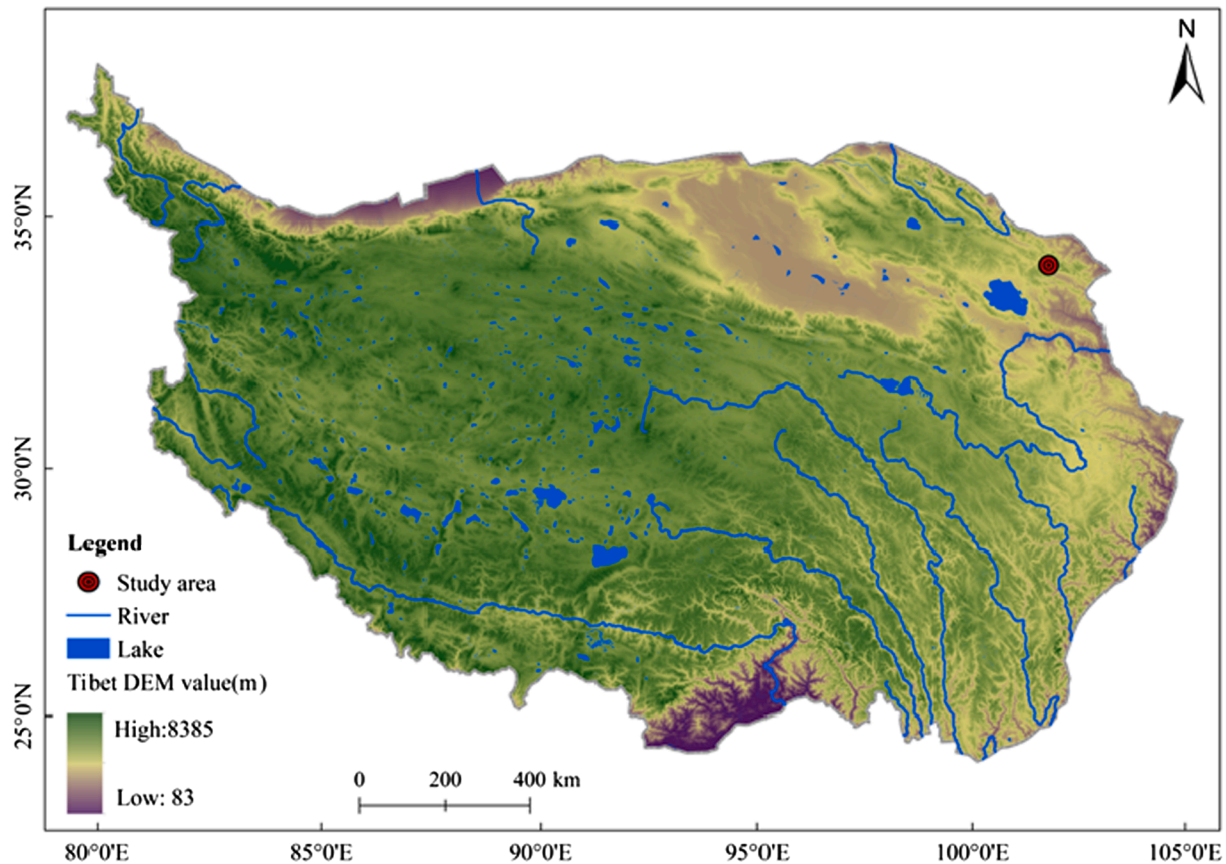


Fig. 1. Location of the study site (Haibei Alpine Meadow Ecosystem Research Station) on the Qinghai-Tibet Plateau, China. DEM, digital elevation model.

20 °C for analyzing microorganisms. The other subsample was stored at 4 °C for analyzing soil properties within 1 week.

2.2. Soil and vegetation properties

We measured soil weight before and after drying at 105 °C for approximately 2 days to calculate soil water content. Before measuring soil properties, soil samples were air-dried and finely ground. A SOC content assay was performed by $\text{H}_2\text{SO}_4\text{-K}_2\text{Cr}_2\text{O}_7$ oxidation (Nelson et al., 1982). The soil total nitrogen concentration was assayed using the Kjeldahl method (Bremner and Mulvaney, 1983). The soil total phosphorous concentration was assayed colorimetrically after H_2SO_4 and HClO_4 digestion. We measured soil pH with a pH meter (1:5 soil to water ratio) (Metrohm 702, Herisau, Switzerland). A continuous flow auto-analyzer (AutAnalyel, Bran + Luebbe GmbH, Norderstedt, Germany) was used to determine soil ammonium ($\text{NH}_4^+\text{-N}$) and nitrate-nitrogen ($\text{NO}_3^-\text{-N}$), which were extracted in 1 M KCl (Wu et al., 2021). Dissolved organic carbon (DOC) concentration in fresh soil was measured with a Liquid TOC II analyzer (Elementar Analyses System, Hanau, Germany) after extraction with distilled water (Jones and Willett, 2006).

When plant biomass peaked in August 2018, a 0.25 m² quadrat was randomly placed in each of the six plots (1.77 m²) to measure the number of plant species, plant height, and plant cover. The species richness and cover of plant communities were measured by visual estimation. Briefly, we first set a permanent quadrat (0.25 m²) above the canopy, with 100 uniformly distributed grids on it. The percentage cover of species in all grid cells was then visually estimated. The number of species in each quadrat represented species richness. In each plot, biological plants on the ground were sampled in a 0.25 m² quadrat to estimate plant biomass. All samples were transferred to the laboratory and oven dried for 48 h at 65 °C, then weighed as aboveground biomass.

2.3. Microbial respiration rate

Microbial respiration rate was estimated by a 2-day laboratory incubation. One incubation subsample (40 g) from each plot was adjusted to 60% field capacity and then placed at 25 °C for 7 days in 500-ml mason jars in the dark for pre-incubation. After the pre-incubation, we measured the changes in the slope of microbial respiration (ppm) (at 08:00, 12:00, and 16:00; three times a day to obtain an accurate calculation of the average daily respiration rate) in real-time using an automatic system (G2301, Picarro, USA) in the dark, which was the real microbial respiration rate of the sample. The soil microbial respiration rate was calculated as follows (He et al., 2013; Chen et al., 2020):

$$R = C \times V \times \alpha \times \beta/m$$

where R represents soil microbial respiration rate ($\mu\text{g C g}^{-1} \text{d}^{-1}$), C represents the CO_2 concentration production slope ($C = \Delta\text{CO}_2 / \Delta t$ (ppm s^{-1}), ΔCO_2 represents the total amount of carbon dioxide concentration produced in Δt ($t_1 - t_0$), V represents the gas tube and incubation jar volume, m represents the weight of soil samples (g), α represents the conversion coefficient of CO_2 mass (to transform from CO_2 to C, 44/12), and β represents a time conversion coefficient (to transform from seconds to hours, 1/3600).

2.4. DNA extraction, amplification, and sequencing

Microbial DNA was extracted from frozen soil samples (0.25 g) using a Power Soil DNA Isolation kit (MO BIO Laboratories). DNA concentration and purity were estimated by a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Then, 1% agarose gel electrophoresis was used to check DNA quality. The DNA samples were stored at -80 °C until further assaying and processing. PCR was used to

amplify the V3–V4 region of the bacterial 16S rRNA gene with the following universal primers: forward primer 338F (5'-ACTCCTACGG-GAGGCAGCA-3') and reverse primer 806R (5'-GGAC-TACHVGGGTWTCTAAT-3') (Wang et al., 2019). For fungal community analysis, the internal transcribed spacer 1 (ITS1) region was amplified with the forward primer ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and reverse primer ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3'). A QIAquick gel extraction kit (Qiagen, Germany) was used to purify the PCR products. Quant-iT™ dsDNA HS Reagent was used to quantify the PCR products, which were then pooled and sequenced using the Illumina HiSeq 2500 platform (2 × 250 paired ends).

2.5. Processing of sequencing data

After removing the adaptor and primer sequences, the raw sequences were assembled for each sample using a unique barcode. FLASH V1.2.7 was used to merge the paired-end sequences (Magoč and Salzberg, 2011). Sequences were quality-filtered using QIIME (Bokulich et al., 2013). The fungal and bacterial sequences were compared with reference databases (Unite database, <https://unite.ut.ee/>; Gold database, http://drive5.com/uchime/uchime_download.html, respectively) using UCHIME to detect chimera sequences, which were then removed (Edgar et al., 2011). The remaining sequences were clustered by UPARSE software and were assigned to OTUs at 97% similarities. Bacterial taxonomic identity was determined using the Silva reference database (<http://www.arb-silva.de>) with the RDP classifier, and fungi were identified using the Unite database (<https://unite.ut.ee/>) with the BLAST tool and QIIME software (<http://qiime.org/index.html>).

2.6. Network analysis

Network analysis was conducted with the Molecular Ecological Network Analyses Pipeline (<http://ieg2.ou.edu/MENA/main.cgi>). Details are provided in Deng et al. (2012) and He et al. (2017). We constructed fungal and bacterial networks under warming and no warming (control) at the two sites with different plant communities. In brief, the following steps were taken. (1) The OTU table was square-root transformed, and the OTU taxonomy file and the environmental metadata were prepared to satisfy analytical requirements. (2) Based on Spearman's Rho between pairwise OTUs, we constructed a similarity matrix. The cutoff value for the similarity matrix was automatically set using default settings. We calculated "individual nodes' centrality," "global network properties," and "modularity and module separation." (3) We visualized the co-occurrence networks using Gephi 0.9.2. (4) We compared the characteristics of the random network (constructed by running "Randomize the network structure") and empirical networks. (5) To find the key driver of the network and their eigengenes, we conducted a "module-eigengene" analysis to elucidate the relationships among soil, plant properties, and network characteristics.

2.7. Statistic analysis

The Shannon–Wiener diversity index of plants was calculated as: $H = -\sum p_i \ln p_i$, where p_i is the relative importance of the i th species out of S species. Pielou's evenness J' of plants were calculated, indicating the species evenness of the plant community: $J' = H'/\ln S$, where S is the total number of species observed. The differences between the treatments' (warming vs. control) species richness and Shannon–Wiener diversity index values and soil microbial respiration rate were analyzed by independent sample t-tests. These analyses were conducted via the "t.test" function in the statistical package in R.

Differences in plant and soil characteristics (e.g., aboveground plant biomass, plant cover, soil physicochemical properties, soil respiration rate, microbial diversity, and relative abundance at the phylum level) between treatments were detected using t-tests. Significance was established at the $P < 0.05$ level. Variables were transformed using

natural logarithms to improve normality and homogeneity of variance. Microbial alpha diversity (OTU richness, Shannon diversity, ACE, and Chao1) was calculated via the *alpha_diversity.py* script by QIIME software. Nonmetric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA) were used to determine the influences of warming on the composition of bacterial and fungal communities between treatments at the OTU level based on Bray–Curtis distances. We also used NMDS and PERMANOVA to assess how warming affected the plant community at the species level.

PLS-PM was used to discern the potential path by which plants mediate the effects of global warming on the composition and respiration rate of the microbial community. Specifically, we defined plant characteristics by the indexes of the above- biomass of plants, community features (species diversity, abundance, evenness index), and the community structure. We defined soil characteristics by the indexes of the soil chemical properties and defined microbial community structure by indexing the bacterial and fungal community structure. We defined the network properties by the indexes of the degree, clustering coefficient, path length, betweenness, connectivity, and the number of nodes and edges. The community compositions of plant, bacteria, and fungi were represented by the first and second principal components of NMDS at the OTU level. We evaluated the goodness-of-fit (GOF) of PLS-PM models. The Mantel test was performed to discern the relationships between monolithic soil properties and microbial community interaction patterns. All statistical analyses were performed in the R 3.2.1 software system (<https://www.r-project.org/>), using the *vegan* and *pls-pm* packages (Sanchez and Trinchera, 2012).

3. Results

3.1. Plant and soil properties

After 21 years, warming affected the plants at the GL site differently from those at the SL site. In the GL site, biomass was significantly greater in warming plots than in control plots, but the abundance of plants was significantly lower ($P < 0.05$). However, long-term warming had no significant effect on the other indices (Table 1). At the SL site, warming significantly decreased evenness, diversity, and abundance; however, the relative cover and aboveground biomass did not differ between the warming and control sites ($P < 0.05$; Table 1). Warming significantly influenced community composition at both sites after 21 years of experimental warming but in opposite directions. This phenomenon was visualized using species-level NMDS ordination (Fig. 2).

At the GL site, SOC and DOC content significantly decreased ($P < 0.05$) after long-term warming. However, at the SL site, no differences were detected between the warming and control plots. No other soil properties changed significantly in response to warming at the GL and SL sites (Table 2).

Table 1

Analysis of plant between warming and control at two sites.

	GW	GCK	SW	SCK
Relative Coverage	1.002 ± (0.167)	1.159 ± (0.161)	1.055 ± (0.214)	1.138 ± (0.127)
Evenness index	0.926 ± (0.048)	0.940 ± (0.082)	0.787 ± (0.059)b	0.953 ± (0.027)a
Diversity Index	2.601 ± (0.23)	2.911 ± (0.339)	1.550 ± (0.403)b	2.914 ± (0.238)a
Abundance	16.833 ± (3.251)b	22.167 ± (2.927)a	7.667 ± (3.386)b	21.500 ± (3.987)a
Above-ground biomass (g/m ²)	92.561 ± (35.351)a	53.655 ± (21.408)b	138.494 ± (55.285)	99.174 ± (27.535)

Abbreviations: GW, Grass Land -warming; GCK, Grass Land -control; SW, Shrub Land -Warming; SCK, Shrub Land -control; lowercase letters indicate differences between four treatments, values represent means based on six replicates ± SD.

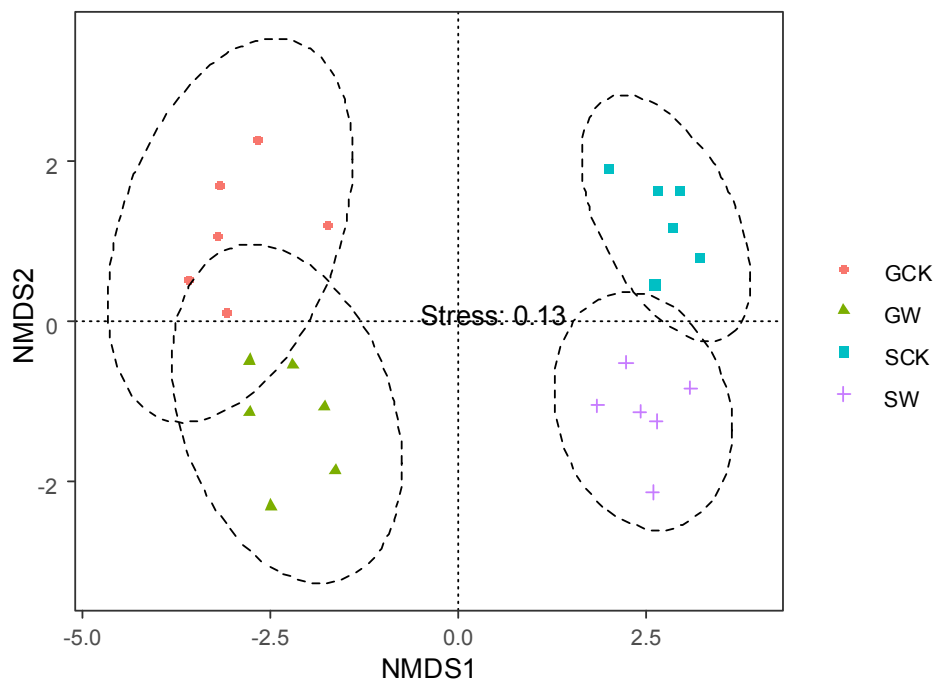


Fig. 2. Non-metric multidimensional scaling patterns of community dissimilarities. Dissimilarity was estimated using data on plant species composition weighted by species relative abundance. All community data (25 cm × 25 cm quadrats) are included. GW, Grass Land-warming; GCK, Grass Land-control; SW, Shrub Land-warming; SCK, Shrub Land-control.

Table 2
Comparison of soil variables between warming and control at two sites.

	SOC (mg g ⁻¹ soil)	TN (mg g ⁻¹ soil)	TP (mg g ⁻¹ soil)	pH	NO ⁻ ₃ -N (μg g ⁻¹ soil)	NH ⁺ ₄ -N (μg g ⁻¹ soil)	DOC (μg g ⁻¹ soil)
G-W	56.79 ± 7.22b	4.73 ± 0.6 ns	0.77 ± 0.1 ns	7.88 ± 0.1 ns	34.91 ± 5.3 ns	18.671 ± 2.9 ns	69.28 ± 23.59b
G-CK	65.60 ± 4.90a	4.89 ± 0.5 ns	0.90 ± 0.1 ns	7.66 ± 0.3 ns	39.74 ± 16.2 ns	22.1 ± 3.145 ns	129.15 ± 24.35a
S-W	74.28 ± 11.44 ns	4.69 ± 1.1 ns	0.87 ± 0.1 ns	6.65 ± 0.2 ns	32.88 ± 10.1 ns	20.00 ± 4.4 ns	128.84 ± 26.51 ns
S-CK	71.23 ± 19.59 ns	4.43 ± 1.2 ns	0.97 ± 0.12 ns	6.52 ± 0.1 ns	26.11 ± 10.8 ns	19.33 ± 5.1 ns	101.84 ± 34.54 ns

Abbreviations: G-W, Grass Land -warming; G-CK, Grass Land -control; S-W, Shrub Land -Warming; S-CK, Shrub Land -control; SOC, soil total organic carbon, TN, soil total nitrogen. TP, soil total phosphorous. DOC, Dissolved organic carbon. Soil ammonium (NH⁺₄-N) and nitrate N(NO⁻₃-N), lowercase letters indicate differences between four treatments, values represent means based on six replicates ± SD.

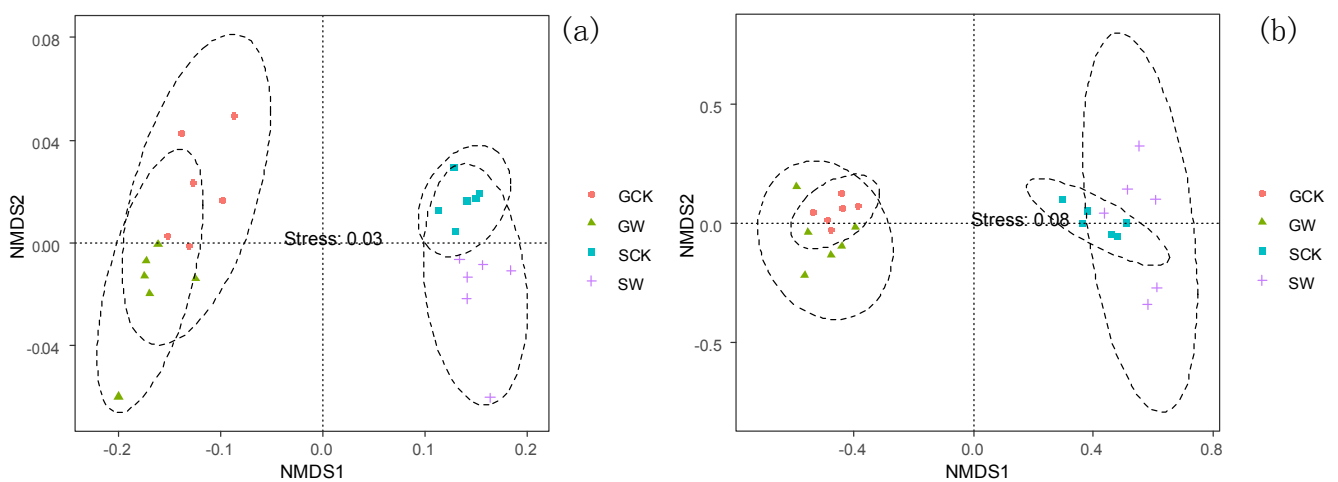


Fig. 3. Non-metric multidimensional scaling patterns of community dissimilarities. Dissimilarity (Bray-Curtis) was estimated using data on soil microorganism composition (a: bacteria, b: fungi) weighted by relative abundance. GW, Grass Land-warming; GCK, Grass Land-control; SW, Shrub Land-Warming; SCK, Shrub Land-control.

3.2. Soil microbial community

At the GL site, bacterial alpha diversity (ACE, Chao1, and OTU richness) was significantly lower in warming plots compared to in control plots ($P < 0.05$) (Table S1). Similarly, fungal alpha diversity (ACE, Chao1, OTU richness, and Shannon diversity index) was significantly lower in warming plots compared to in control plots ($P < 0.05$). However, at the SL site, there were no significant changes in species richness and diversity under long-term warming ($P < 0.05$). The composition of fungal and bacterial microbial communities shifted during long-term warming both in two ecosystem (Fig. S2a, b), which was revealed with NMDS ordination based on OTUs (Fig. 3a, b). In particular, warming significantly changed the relative abundances of Leotiomycetes, Acidobacteria, and Chloroflexi in both the GL and SL sites (Table S2). PERMANOVA demonstrated that the overall community structure of fungi and bacteria significantly differed ($P < 0.05$) between warming plots and control plots in both sites (GL: bacteria, $R^2 = 0.145$, $P < 0.01$; fungi, $R^2 = 0.12$, $P < 0.01$. SL: bacteria, $R^2 = 0.123$, $P < 0.05$; fungi: $R^2 = 0.137$, $P < 0.01$). The structure and composition of soil fungal and bacterial communities were significantly altered by long-term simulated warming in both the GL and SL sites ($P < 0.05$; Fig. 3a, b).

3.3. Effects of biotic and abiotic factors on microbial communities

We subsequently confirmed the potential direct and indirect effects of plants on microbial community structure using PLS-PM models (Fig. 4a, b). At the GL site, a plant-mediated effect on microbial community structure was only realized through soil properties (path coefficient = 0.826). At the SL site, plants directly affected the microbial community (path coefficient = -0.875).

3.4. Interaction patterns of soil microorganisms

We constructed four microbe co-occurrence networks to identify potential bacterial-fungal interactions under the warming treatments

based on significant Spearman's correlations ($P < 0.05$) (Fig. 5). Similarity threshold values were set to 0.99. Network degree for microbial nodes obeyed a power-law distribution (GW: $P < 0.001$; GCK: $P < 0.001$; SW: $P < 0.001$; SCK: $P < 0.001$), indicating a non-random distribution pattern (Fig. S3). The typical modular and small-world characteristics of co-occurrence networks, compared to respective random networks, reflected the higher values of the average clustering coefficient, characteristic values of average path length, average clustering coefficient, and modularity of molecular ecological co-occurrence networks. Thus, the molecular ecological network was stronger than random distributions (Table S3).

At the GL site, the number of associations in the network tended to decline with warming (2254 edges in the control plots and 1721 edges in the warming plots). This phenomenon was caused by decreasing fungi-fungi and fungi-bacteria correlations in the soil (Table 3), which were driven by soil properties (Fig. 4, Table S4). However, warming at the SL site increased potential interactions (2478 edges in the warming plots and 2150 in the control plots) (Table 3). This phenomenon was largely attributed to increasing bacteria-bacteria and fungi-fungi correlations (Table 3). Additionally, at the SL site, positive correlations tended to increase with long-term warming. However, these correlations tended to decline in the GL site.

3.5. Effects of biotic and abiotic factors on soil microbial respiration rate

At the GL site, soil microbial respiration rate was significantly higher in warming plots ($226.09 \mu\text{g C g}^{-1} \text{ soil d}^{-1}$) than in control plots ($194.03 \mu\text{g C g}^{-1} \text{ soil d}^{-1}$) ($P < 0.05$). In contrast, there was no significant difference between the warming plots and control plots at the SL site ($P > 0.05$) (Fig. 6). PLS-PM analysis was used to determine how the plant community influenced soil microbial respiration rate via the following pathways: plant community properties (diversity, richness, above-ground biomass, and community composition), soil chemical properties, soil microbial community composition, and the co-occurrence of network properties (interaction patterns among microorganisms) (Fig. 4). After retaining the most significant variables, PLS-PM path

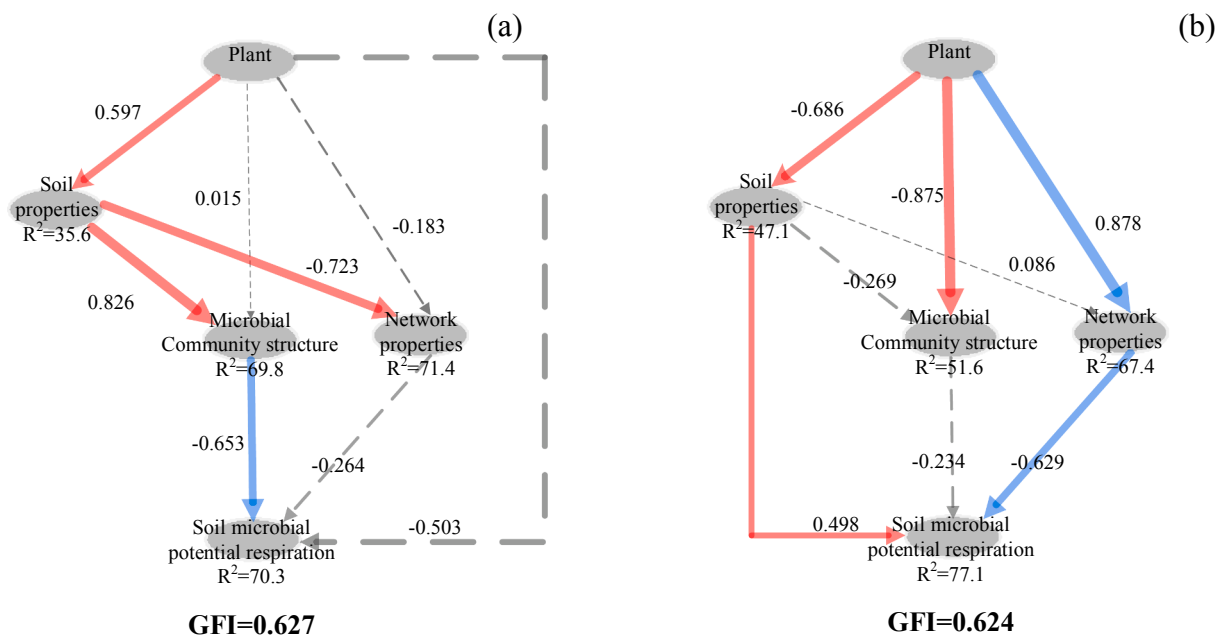


Fig. 4. Partial least squares path models (PLS-PM) of long-term warming effects on the vegetation-soil-microbe system in grass-land(a) and shrub-land(b) sites. The model was evaluated using goodness of fit (GFI). The width of the arrows is proportional to the strength of the causal relationship, and numbers are the correlation coefficients. Bold and dashed lines indicate significant ($P < 0.05$) and non-significant ($P > 0.05$) pathways, respectively. Blue solid lines indicate positive relationships and red solid lines indicate negative relationships. R^2 values represent the explained variance of each factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

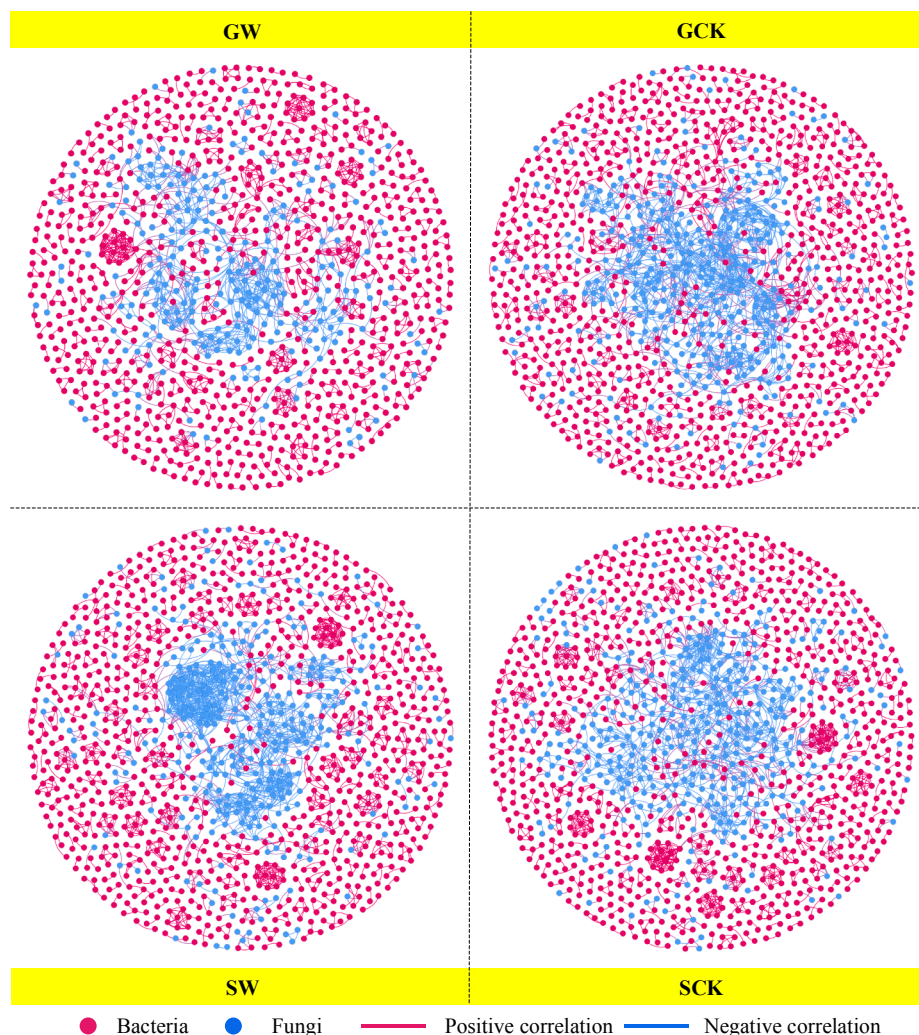


Fig. 5. Co-occurrence network interactions of the soil microorganism community (bacteria and fungi) at the OTU level. GW, Grass Land-warming; GCK, Grass Land-control; SW, Shrub Land-Warming; SCK, Shrub Land-control.

Table 3

Properties of bacterial and fungal co-occurrence networks in two sites between control and warming.

Sites	Total Nodes	Edges		B-B Edges		F-B Edges		F-F Edges	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
GW	1087	965	756	580	296	217	136	168	324
GCK	1249	1125	1129	517	256	295	211	313	662
SW	1230	1535	943	845	208	182	111	508	624
SCK	1267	1200	950	635	325	272	153	293	472

Abbreviations: GW, Grass Land -warming; GCK, Grass Land -control; SW, Shrub Land -Warming; SCK, Shrub Land -control.

B-B, links between bacterial taxa (bacteria-bacteria); F-F, links between fungal taxa (fungi-fungi); F-B, links between fungal and bacterial taxa (fungi-bacteria).

modeling explained 70.3% and 77.1% of the variation in microbial respiration rate at the GL and SL sites, respectively. At the GL site, plants indirectly affected soil microbial respiration rate by altering the microbial community composition (path coefficient = -0.653). At the SL site, plants indirectly affected soil microbial respiration rate by altering microorganism interaction patterns (path coefficient = -0.629) and soil nutrient contents (path coefficient = 0.498). By comparing the effects of plants on soil respiration rate in response to long-term warming, we demonstrated that shrubs have a more direct effect on respiration rate than grasses.

4. Discussion

4.1. Shifts in soil microbial community diversity and composition

After 21 years, warming did not significantly alter soil microbial diversity at the SL site; conversely, the soil microbial diversity at the GL site was significantly lower in warming plots than in control plots. The effects of climate change on soil organisms will largely depend on plant types (Johnson et al., 2011; Chakraborty et al., 2012; Ward et al., 2015). That is, the influence of global warming on soil microbes is largely indirect via changes to plants. Previous studies carried out at the same sites demonstrated a rapid decrease in vegetation diversity during the

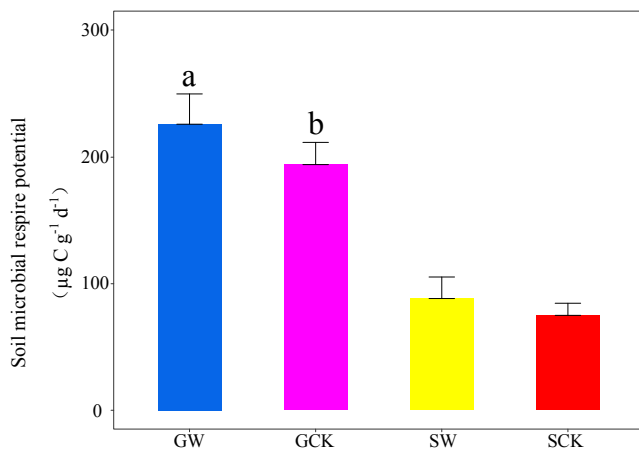


Fig. 6. Soil microbial respiration potential in grass-land and shrub-land sites. Different letters indicate significant differences between control and warming sites in each ecosystem (Independent sample test, $P < 0.05$). GW, Grass Land-warming; GCK, Grass Land-control; SW, Shrub Land-Warming; SCK, Shrub Land-control, values are means \pm standard deviation ($n = 6$).

initial stages of warming, followed by a long period of significant recovery (Klein, 2003; Klein et al., 2004; Zhang et al., 2017a). Given their reliance on plants, it is likely that belowground communities respond strongly to plant diversity, which can partly explain the changes in microbial community structure after 21-years of warming. Plant-mediated effects on soil microbial communities may be driven by the quantity and quality of available resources or the synchrony between vegetation and microorganisms (Bardgett and Wardle, 2010; Zhang et al., 2017b). Trophic interactions between aboveground and belowground organisms are a crucial factor for soil microbial community assembly. Changes in the quantity of plant-derived organic substrates can affect the activity of specific species and the competitive dynamics between species, due to the varied nutrient needs of soil microbes, which further shape soil microbial community structure. The increase in aboveground plant biomass in the GL site may have also led to an increase in belowground plant biomass. Therefore, the different effects of warming on different vegetation types could have led to differences in nutrient availability for soil microbes. SOC is an important mediator of soil microbes and plants and drives the composition of soil microbial communities (Eilers et al., 2010). Consistent with the results of previous studies, we found that soil C availability (e.g., DOC) and N availability regulate microbial community structure (Cederlund et al., 2014; Liu et al., 2014; Xiao et al., 2018). Moreover, changes to plant community composition in response to global warming may result in changes to the quality of litter input (e.g., C:N ratio, the content of polyphenol and nitrogen), which can mediate the effect of temperature on soil microbes (Bardgett and Wardle, 2010; Yang et al., 2015). Rinnan et al. (2007) observed a long time-lag between the onset of warming and changes to soil microbial communities, indicating that temperature was not the only driving factor and that plant also contributed. Moreover, Xue et al. (2016) revealed that soil substrate and plant community characteristics explained 52% of the total variance in the soil microbial community's response to warming, which was far higher than that of soil temperature and moisture (8%). In our study, the effects of warming on soil microbial community structure were mediated by plant types and the interaction between warming and plant characteristics, as demonstrated by our PLS-PM model.

4.2. Effects of warming on soil microorganism interactions

Complex interactions among soil microorganisms can determine how they respond to disturbances. For example, it was demonstrated that mycelial fungi can promote bacterial activity by supplying scarce

resources under high stress, and this interaction could therefore help maintain ecosystem function (Worrlich et al., 2017). Moreover, soil microbe–microbe interactions play an important role in ecological functions (Barberán et al., 2012). Thus, researching species separately, rather than evaluating them as part of a functional meta-community, restricts our understanding of how they impact ecological functions. Network analysis is an efficient tool for exploring potential biotic interactions and related ecosystem functions (Xiao et al., 2018). Thus, we used network analysis to explore how the effects of warming on soil ecosystem functions varied under different plant communities. In the current study, networks tended to be more complex at the SL site, indicating that soil microorganisms in the SL established more interactions, which could indicate greater C-use efficiency (Morrién et al., 2017). Additionally, the topological features of microbial networks were correlated with soil nutrient contents, which differed depending on the plant type owing to the influence of plant communities on the soil micro-environment. The change of environmental available resources lead to the topological change could be explained by that the effect of environmental filtering lead to shifts in competition and cooperation patterns among different microbial taxa with the different environmental adaptability (Barberán et al., 2012; Deng et al., 2016; Tao et al., 2018; Ma et al., 2020; Li et al., 2021).

4.3. Effects of warming on soil microbial respiration rate

Fungi and bacteria are the main drivers of biogeochemical cycling in soil, affecting the balance between the C stored in soil and C respired into the atmosphere as CO₂. In the present study, warming affected soil microbial respiration differently under each of the two plant communities. Warming affected soil microbial respiration via changes to microbial community structure and weakened more rapidly at the SL site compared to the GL site. The PLS-PM demonstrated that plants mediated the effects of global warming on soil microbial respiration through influencing microbial community composition or via interactions among soil microbes.

Microbial community structure can affect soil functional processes (Trivedi et al., 2013). Microbes have various abilities and strategies for utilizing soil organic matter; consequently, shifts in the composition of the soil microbial community likely influence decomposition processes and CO₂ emissions (Monson et al., 2006; Balser and Wixon, 2009; Lipson et al., 2009; Liptzin et al., 2009; Keiblinger et al., 2010). A recent study revealed that it is important to classify ecological functional groups (e.g., copiotrophs and oligotrophs) as a basis for generalizing taxa with different C-use efficiencies (Liu et al., 2018). In the current study, under warming at the SL site, there was an increase in the relative abundances of Actinobacteria and Acidobacteria, which are considered classic oligotrophs (i.e., they have lower respiration rate and higher C-use efficiency than copiotrophs). This shift in the SL towards an oligotrophic-dominated community resulted in lower soil respiration rates, a pattern which has been observed in other ecosystems (Luo et al., 2001; Romero-Olivares et al., 2017). Thus, the effects of warming on soil C respiration rates may be mediated by plants via shifts in abundances of microbial taxa with different life strategies. Additionally, plants can influence soil microbial respiration via changes to soil microbial community structure and microbial interactions. At the GL site, warming resulted in a shift towards a copiotrophic community and a less connected network, which could indicate that the community had a lower C-use efficiency and could result in a positive feedback to warming, as in the previous study (Morrién et al., 2017).

5. Conclusions

Long-term warming (21 years), affected soil microbial communities and the interactions among soil microbes at both the SL and GL sites. These effects depended on the plant type and were likely mediated by litter inputs and live plant effects (e.g., root exudates) and subsequently

resulted in different soil microbial respiration rates at each site. Importantly, the different soil microbial responses in the two ecosystems could be explained by the measured plant community attributes, highlighting the effect of plants on soil communities in situ. These results highlight the important role of plant-mediated effects of warming on decomposers and soil microbial potential. Therefore, it is important to consider plant communities in future studies on the effects of warming on soil microbial communities. This is important for predicting future soil C loss potential on the Qinghai–Tibet Plateau.

CRedit authorship contribution statement

WJC, HKZ, SX and XQZ design the experiments. WJC, YW, YZL, LLQ, JYZ, YHS, and ZWZ contributed to sample collection. WJC, YW, and ZHJ performed the experiments. WJC and JW analyzed the data. WJC and HKZ wrote the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that may have influenced the work reported in this paper.

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