



# Short-term warming increases root-associated fungal community dissimilarities among host plant species on the Qinghai-Tibetan Plateau

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## Abstract

**Background and aims** Root-associated fungi link resource fluxes between the soil and roots, thus influencing plant growth and ecosystem function. However, at present, the impact of global warming on these fungi and their plant host specialization, especially in ecosystems on the Qinghai-Tibetan Plateau is obscure.

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**Methods** Here, pot experiments were conducted to examine the root-associated fungal community structure of 14 host plant species undergoing two years of experimental warming on the Qinghai-Tibetan Plateau. Infrared heaters were used to raise the soil temperature to 2.0 °C higher than the relative ambient temperature. Subsequently, the endophytic and rhizoplane fungal communities were explored by sequencing the fungal internal transcribed spacer (ITS) region.

**Results** A total of 1564 OTUs were identified, which were dominated by ascomycetes (82.71%). Similar root-associated fungal diversity and community composition were identified under ambient and warming environments. The root-associated fungal community composition significantly varied among different host plant species, and this dissimilarity was enhanced under experimental warming. Root N concentration was essential in shaping the structure of the root-associated fungal community.

**Conclusions** Although the root-associated fungal community was resilient to short-term warming, our study highlights that climate warming can induce higher host specificity of root-associated fungal communities in the alpine meadow ecosystem.

**Keywords** Root-associated fungi · Global climate change · Plant–microbe interaction · Alpine meadow ecosystem

## Introduction

Climate change has the potential to influence plant–microbe interactions. This phenomenon is particularly of significance in the extreme environments of the Qinghai-Tibetan Plateau, where plants rely more on associated microbes than elsewhere. In the past few decades, the Qinghai-Tibetan Plateau has experienced rapid climate change (IPCC 2019), which has attracted research interest on the response of plants (Ma et al. 2017; Meng et al. 2019) and soil microbes (Che et al. 2019; Shi et al. 2020). However, the impact of climate change on root-associated fungi, which link aboveground and belowground ecosystems (De Deyn and van der Putten 2005), has received far less attention (Rudgers et al. 2020). Understanding the interactions between plants and root-associated fungi and their responses to climate warming could improve our predictions for ecosystem structures and functions under climatic disturbances.

Root-associated fungi, including fungi residing in and on plant roots (e.g., mycorrhizal fungi, endophytes, and pathogens), have important roles in influencing plant performance and ecosystem processes (Almario et al. 2017; Clemmensen et al. 2013; Robinson et al. 2020; Trivedi et al. 2020). Some root-associated fungi have negative effects on plants, such as pathogenic fungi, which can cause severe plant diseases resulting in decreased plant biomass, whereas plant mutualists, such as mycorrhizal fungi, can enhance plant nutrient uptake and improve disease resistance (van der Heijden et al. 2006). Many studies have focused on the impact of climate warming on specific fungal groups, such as arbuscular mycorrhizal fungi (Birgander et al. 2017) or ectomycorrhizal fungi (Fernandez et al. 2017), yet disparate fungal groups could colonize the same segment of plant roots. There is evidence that different groups of root-associated fungi respond differently to climate change (Olsrud et al. 2009), and distinct environmental conditions could switch the interaction between different fungal groups from cooperative to antagonistic (Abrego et al. 2020a, b; Faust and Raes 2012). As such, it is necessary to obtain systematic insights into the responses of the entire plant root-associated fungal community to climate warming.

The responses of root-associated fungi to climate warming are less clear and likely depending on the

plant species. Generally, root-associated fungi benefit from the photosynthates produced by the plant. Thus, shifts in the taxonomy of root-associated fungi would be expected since climate warming alters the root dynamics and increases the amount of plant carbon transferred to roots (Hawkes et al. 2008; Wang et al. 2017; Kilpeläinen et al. 2020). However, some field studies found no (Fujimura et al. 2008) or weak (Lorberau et al. 2017) effects of climate warming on root-associated fungi. Despite this inconsistent response of root-associated fungi, the mechanism by which elevated temperatures impact root-associated fungi largely depend on the plant response to climate warming. For instance, Bunn et al. (2009) found that the colonization of arbuscular mycorrhizal fungi in psychrophilic plants was generally higher at an elevated soil temperature, but thermophilic species did not show any difference under different temperatures. It is well documented that plant response to climate warming varies among different species (Dolezal et al. 2020; Krab et al. 2018). Thus, studies seeking to predict how root-associated fungi respond to climate warming should examine multiple plant species within the specific ecosystem.

While plant species identity have been shown to be major drivers of root-associated fungal communities, the relative contributions of this effect may be mediated by the environment. Although numerous studies have reported the host specificity of root-associated fungi (Koyama et al. 2019; Maciá-Vicente et al. 2020; Toju et al. 2013; Wang et al. 2019), low or no host specificity has also been observed in some ecosystems, especially at low-temperature regions, such as Arctic ecosystems (Botnen et al. 2014; Walker et al. 2011). Recently, Gao and Yang (2016) estimated the diversity of root-associated fungi on herbaceous species in three alpine meadows on the Qinghai-Tibetan Plateau; and they did not observe host specificity at the site with the lowest annual mean temperature. These inconsistent findings suggest that the host specificity of root-associated fungi may be influenced by temperature, thus climate warming could switch the interactions between plants and root-associated fungi from facultative to obligate (Rudgers et al. 2020). Overall, plant growth in cold environments with short growing seasons is limited by low temperature, and association with generalist fungi may reduce the plant costs for conservative growth and increase the plant

ability to tolerate such environments (Botnen et al. 2014). Therefore, if plants produce more biomass with a stronger competitive growth strategy under climate warming, they will recruit specific soil fungi that increase their capacity to exploit available resources, leading to higher host specificity. To date, how host specificity of root-associated fungi responses to climate warming has been largely underexplored.

With an average altitude above 4000 m, the Qinghai-Tibetan Plateau is characterized by low temperatures, low atmospheric pressures, and short growing seasons. Under this cold and hypoxic environment, an intimate relationship between plants and root-associated fungi is expected because root-associated fungi could promote plant survival. Besides, given the relatively high diversity of plant species and the positive correlation between plant and soil fungal diversity (Yang et al. 2017), diverse root-associated fungi may inhabit these ecosystems on the Qinghai-Tibetan Plateau. However, we still lack adequate evidence about the composition and relationships of root-associated fungal communities with their host plants in this ecosystem (Harrison and Griffin 2020).

This study explored the root-associated fungal communities on the Qinghai-Tibetan Plateau under ambient and an increased temperature. Fourteen host plant species from different phylogenetic lineages were chosen to determine the effect of climate warming on the diversity of root-associated fungi and understand the interactions between root-associated fungi and host plants. We proposed two hypotheses: (i) Climate warming would increase the root-associated fungal diversity as a result of increased C allocation to the belowground, causing a significant variation in community composition, and this effect would vary among plant species; (ii) Climate warming would increase the host specificity of root-associated fungi due to the increase in plant biomass.

## Materials and methods

### Study site

The study was conducted at the Haibei Alpine Grassland Ecosystem Research Station (37°36'N, 101°19'E, c. 3,215 m a.s.l.), located on the Qinghai-Tibetan Plateau of China. The site has a typical continental

climate, with long, cold winters and short, cool summers. The annual average precipitation is 488 mm, with 80% occurring in the growing season from May to September. The annual average temperature is -1.1 °C and ranges from a minimum of -15.2 °C in January to a maximum of 9.9 °C in July. Alpine meadow is the primary vegetation in the present study site, dominated by *Kobresia humilis*, *Elymus nutans*, and *Stipa aliena*.

### Experimental design

A mesocosm experiment was established in 2016 to explore the influence of climate warming on the composition of root-associated fungal communities associated with 15 plant species (Table S1). The targeted plant species were all dominant at the sample site and phylogenetically diverse. There were six replicates for each plant species under warming or control treatments, resulting in a total of 180 mesocosms. Six blocks were designed to arrange the mesocosms. In each block, mesocosms under the same treatment were placed together (Fig. S1). We used infrared heaters (1200 W, 220 V, 1 m long, 0.22 m wide) hung 1.2 m above the ground to increase the soil temperature in the top 5 cm layer by c. 2.0 °C above the ambient temperature. Meanwhile, the control plots contained dummy heaters.

At the beginning of the growing season in 2016, whole plant samples, including the rhizosphere soil, were collected. All the collected plant species were at the seedling stage, and each plant species was as homogenous in size as possible. Seedlings were planted in 18.85-L pots (20 cm diameter and 60 cm depth) with a mixture (2:1, v/v) of soil collected from the location and sand. Each pot consisted of one plant species with four replicate seedlings, and the pots were buried in the field after planting. No supplementary irrigation or other practices was done during the 2-year growth period, except that non-target plants were removed manually every month to ensure that only the targeted plant species grew in each pot.

### Sampling procedure

After two years of warming, all individual plants were harvested to determine the oven-dried biomass and collect fine root samples. We measured the key

plant traits like specific leaf area (SLA), specific root length (SRL), root length, root density, and root diameter in each mesocosm. Dry roots were ground to a fine powder to determine the C and N concentrations by combustion on a CHN elemental analyzer (2400 CHN elemental analyzer, PerkinElmer, Boston, MA, USA). Then, fine root samples were gently washed to remove soil debris and stored at  $-20\text{ }^{\circ}\text{C}$  until used for DNA extraction.

#### DNA extraction, PCR, and Illumina sequencing

Genomic DNA was extracted from 0.1 g of homogenized dry roots using a Plant DNA Extraction kit following the manufacturer's instructions (Tiangen Biotech Beijing, China). After quality evaluation, DNA samples were sent to Genesky Biotechnologies, Inc., (Shanghai, China) for Illumina MiSeq sequencing. The fungal ITS2 region was amplified using ITS3/ITS4 primers (White et al. 1990). The volumes of PCR mixtures were 30  $\mu\text{l}$  with 12.5  $\mu\text{l}$  of 2 $\times$ Taq PCR MasterMix, 1  $\mu\text{l}$  of forward (5  $\mu\text{M}$ ) and reverse primers (5  $\mu\text{M}$ ), and 30 ng of template DNA. The amplification conditions were as follows: 5 min at 95  $^{\circ}\text{C}$ , 35 cycles of 45 s at 95  $^{\circ}\text{C}$ , 50 s at 58  $^{\circ}\text{C}$  and 45 s at 72  $^{\circ}\text{C}$ , and a final 10 min elongation at 72  $^{\circ}\text{C}$ . Amplicons were purified using the QIAquick PCR purification kit (QIAGEN) and quantified by a NanoDrop ND-1000 (Thermo Scientific, USA). Subsequently, equimolar concentrations of samples were sequenced on the Illumina MiSeq PE250 platform.

#### Bioinformatics analysis

All raw sequences were processed using the MOTHUR 1.44.2 software (Schloss et al. 2009) following a modified MiSeq SOP pipeline. Briefly, sequences with a low average quality score ( $<20$ ) and short length ( $<200$  bp) were quality filtered before further analysis, and chimeric sequences were identified by the VSEARCH (Rognes et al. 2016) in "uchime\_ref" mode against the UNITE-based fungal chimera dataset. The remaining sequences were clustered into OTUs (operational taxonomic units) at a  $\geq 97\%$  similarity level; the most abundant sequence in each OTU was taken as a representative sequence. All singletons (clusters of size 1) were removed. Taxonomic classification of the OTUs was assigned based on the UNITE 8.0 database (Kõljalg et al. 2013).

Representative sequences that had no match against the UNITE database were subjected to a BLAST search against the International Nucleotide Sequence Database (INSD). All non-fungal sequences (e.g., Streptophyta, Cercozoa) were removed before downstream analyses, leaving 2006 OTUs represented by 5,974,829 high-quality reads. Since the reads varied among plant species, the raw data matrix was rarefied to the same reads (3000), and samples with fewer reads were excluded. Eventually, 147 samples corresponding to 14 host plant species were retained; samples of *Saussurea pulchra* were discarded due to insufficient sequencing length. Fungal OTUs were then assigned to different functional groups based on data file S2 in Tedersoo et al. (2014), which annotates fungal OTUs into different trophic guilds, such as saprotrophs, plant pathogens, arbuscular mycorrhizal (AM) fungi, ectomycorrhizal (EcM) fungi, and others based on the genus level of classification. Representative sequences of each root-associated fungal OTU were submitted to the GenBank under accession numbers MW049363-MW050926.

#### Statistical analysis

All statistical analyses were conducted in R 3.5.3. Before analysis, the raw data of all variables were tested for normality and log-transformed using 'box-cox' when needed. We estimated the effect of warming and host plant species using mixed linear models with block as a random effect. The analysis was performed in R using the 'lmer' command in the 'lme4' package; the corresponding *F* and *P* values were derived from the 'anova' function in the 'lmerTest' package. The correlations between root-associated fungal richness and different plant variables were analyzed by Pearson correlation.

To summarize patterns of fungal community structure, we carried out a Principal coordinate analysis (PCoA) ordination of Bray–Curtis dissimilarities among all samples using the function 'pcoa' from the R package 'ape'. The PCoA ordination was plotted from the mean scores of PCoA axes 1 and 2 of each plant species to clearly show the dissimilarities of fungal communities among the 14 host plant species. Significance of the effect of warming and host plant species on fungal community composition were confirmed by permutational multivariate analysis of variance (PERMANOVA; 'adonis' function from

R package ‘vegan’). Meanwhile, the relationships between each plant variable (Euclidean distance) and root-associated fungal dissimilarity (Bray–Curtis distance) were assessed using the Mantel test (‘mantel’ function from R package ‘vegan’), and the *P*-value was adjusted according to the Bonferroni correction. Variation partition analysis was applied to analyze the relative contribution of host plant species and plant traits in determining the community composition of root-associated fungi.

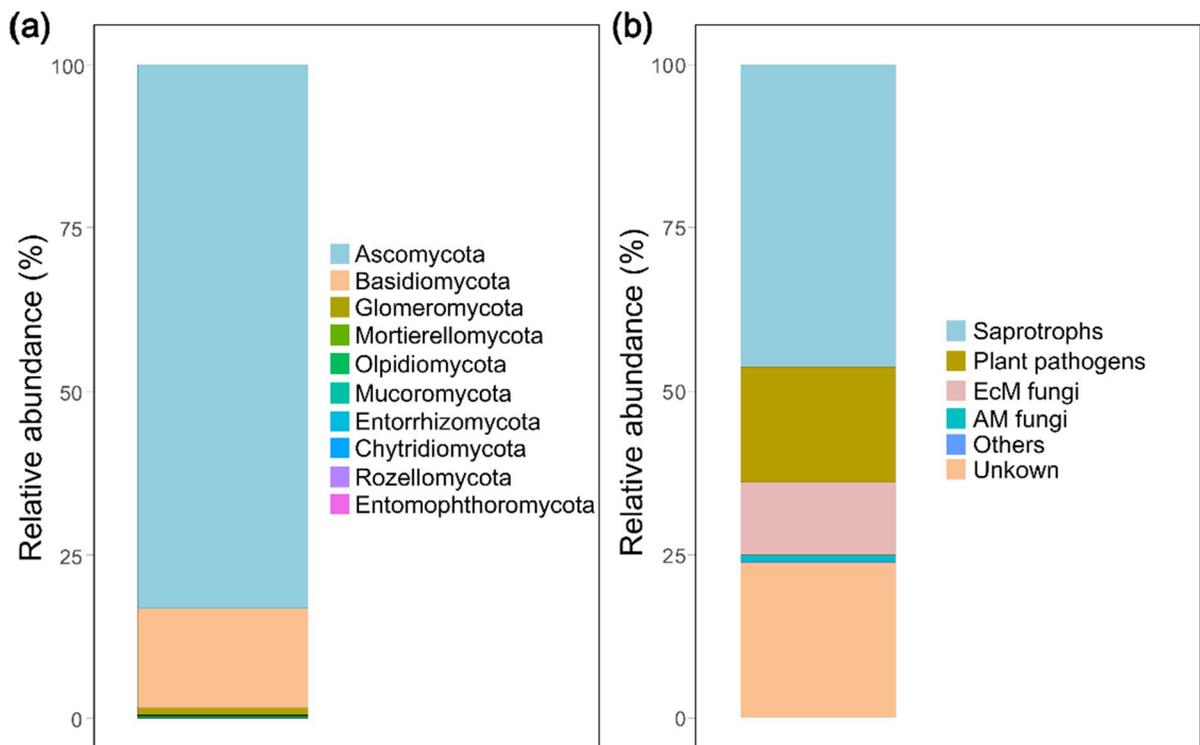
We used the Mantel tests to analyze whether host plant genetic distance is associated with fungal Bray–Curtis dissimilarities. The phylogenetic relationship of the 14 species was obtained from the online program Phylomatic (version 3, Webb and Donoghue, 2005; <http://www.phylodiversity.net/phyloomatic>). The relationships between the plant phylogenetic distance and fungal dissimilarity under different treatments were analyzed by linear regression. Student’s *t*-tests (two-tailed) were used to determine whether the two regression slopes were significantly different. For each host plant species, the mean

Bray–Curtis dissimilarity values between control and warming treatments within the same plant species or among different plant species were also compared.

## Results

### Taxonomic composition and guild classification

Our analysis of 147 root samples yielded 1564 fungal OTUs, which were classified into ten phyla. The root-associated fungal communities in all the host plant species were mainly dominated by Ascomycota with an average of 82.71% of reads, followed by Basidiomycota with 15.20% of reads (Fig. 1a). Although contributing to only 1.09% of the total reads, we also detected 202 Glomeromycota OTUs. The remaining OTUs contributed <1% of the total reads (Fig. 1a). At the genus level, 309 genera were identified in this study; the three most abundant were *Tetracladium* (10.43%), *Ilyonectria* (5.25%), and *Cistella* (5.03%). In total, 843/1564 OTUs were assigned to different



**Fig. 1** Relative abundances (% , proportion of sequencing reads) of each phyla (a) and fungal functional guilds (b) of root-associated fungi across 14 plant species

fungus functional categories. Saprotrophs were the most abundant fungal functional guild of these OTUs, representing 45.93% of the total reads. Taxa identified as plant pathogens, EcM fungi, and AM fungi represented about 20.73%, 9.00%, and 1.09% of the total reads, respectively (Fig. 1b).

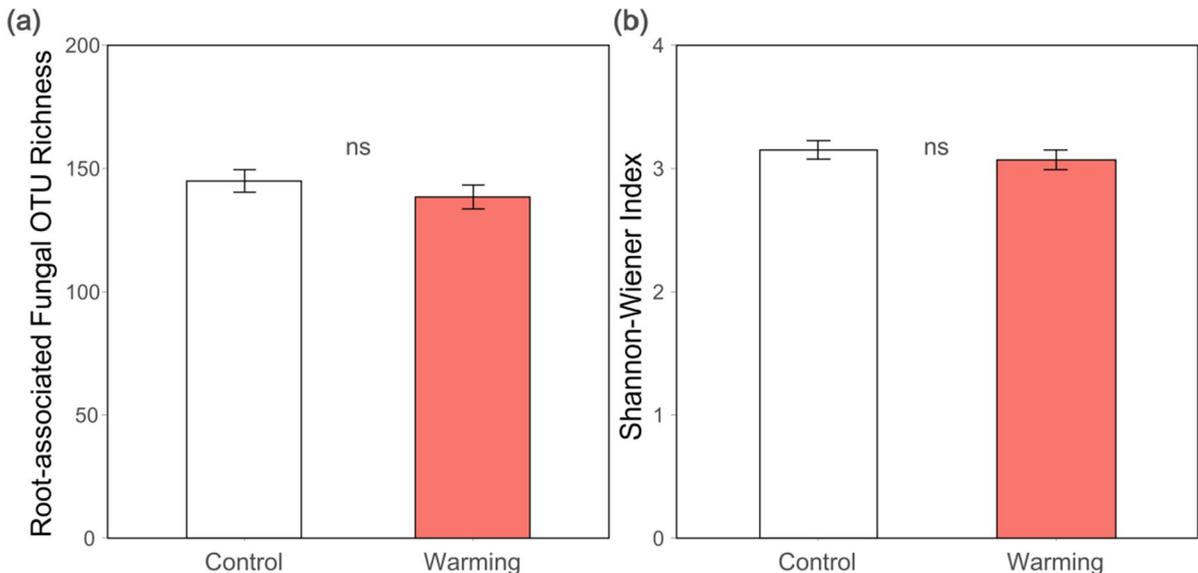
#### Root-associated fungal community diversity pattern

We did not observe a significant effect of warming on root-associated fungal richness across the different plant species ( $F=1.24$ ,  $P=0.29$ ; Fig. 2a). Only one (*Potentilla fruticosa*) of the 14 species exhibited a significant decrease ( $P=0.02$ ; Fig. S2) in root-associated fungal richness after warming. Also, the Shannon–Wiener index was not influenced by warming ( $F=0.96$ ,  $P=0.34$ ; Fig. 2b). For fungal functional categories, warming had no significant effect on the richness of saprotrophic fungi ( $F=0.92$ ,  $P=0.36$ ), plant pathogenic fungi ( $F=0.007$ ,  $P=0.93$ ), AM fungi ( $F=1.02$ ,  $P=0.33$ ), and EcM fungi ( $F=0.027$ ,  $P=0.87$ ).

Both PERMANOVA and PCoA analysis showed that warming did not influence the root-associated fungal community composition (PERMANOVA:  $F=0.84$ ,  $R^2=0.006$ ,  $P=0.18$ ; Fig. 3a). Within the

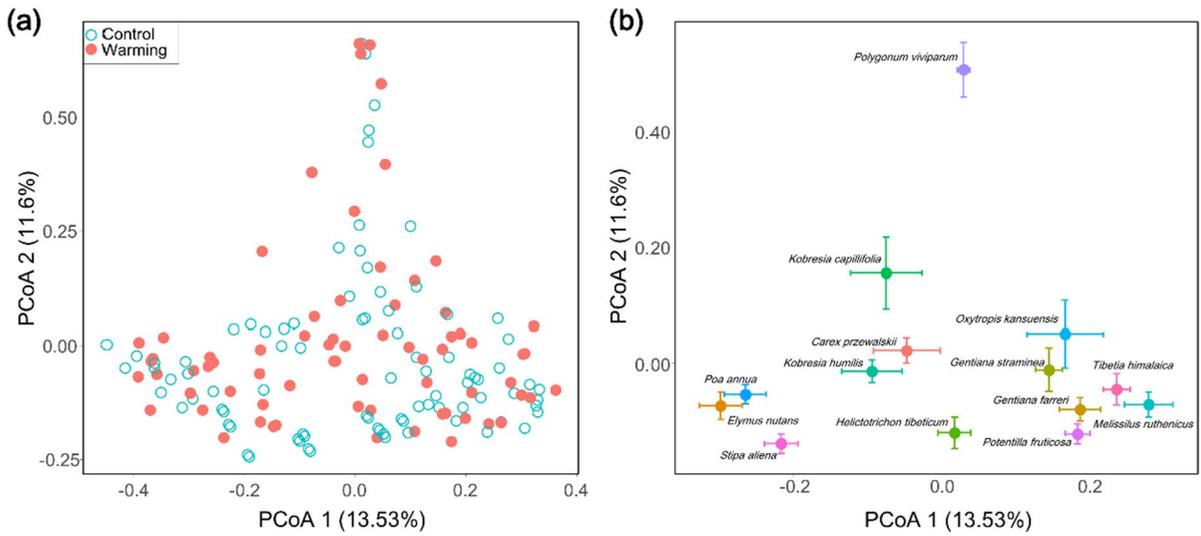
14 plant species, only *Melissilus ruthenicus* showed a significant change due to warming (PERMANOVA:  $F=2.07$ ,  $R^2=0.21$ ,  $P=0.03$ ; Table S2). In addition, we did not observe a significant change in the community composition of saprotrophic fungi, plant pathogenic fungi, AM fungi, and EcM fungi between the different treatments (all  $P>0.05$ ).

Compared with warming, we observed a stronger effect of host plant identity on root-associated fungal richness ( $F=9.41$ ,  $P<0.001$ ) and community composition (PERMANOVA:  $F=6.54$ ,  $R^2=0.39$ ,  $P<0.001$ ). Based on the variation partitioning, plant identity also accounted for the most part of the variation (29.8%, Fig. S3). As shown on the PCoA plot (Fig. 3b), plant species with closer phylogenetic distances harbored relatively similar root-associated fungal communities. The Mantel test results confirmed that the plant phylogeny was significantly correlated with the root-associated fungal composition (Mantel  $r=0.444$ ,  $P=0.001$ ; Fig. 4). Besides, saprotrophic fungi (Mantel  $r=0.367$ ,  $P=0.001$ ) showed the most substantial host specialization, followed by plant pathogenic fungi (Mantel  $r=0.341$ ,  $P=0.001$ ), while AM fungi (Mantel  $r=0.102$ ,  $P=0.001$ ) and EcM fungi (Mantel  $r=0.090$ ,  $P=0.001$ ) were similar and had the least host specialization.



**Fig. 2** Root-associated fungal richness (a) and Shannon–Wiener index (b) across 14 plant species under control and warming treatments. Bars represent means  $\pm$  SEs. Significant dif-

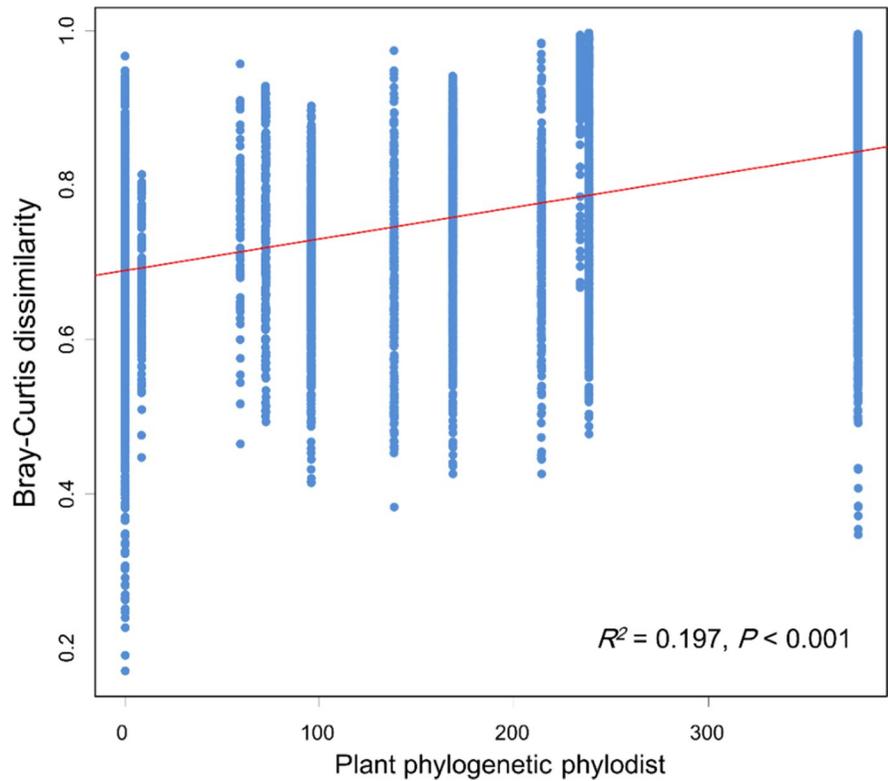
ferences between different treatments were determined using linear mixed-effects models, and analysis showed no significant (both  $P>0.05$ ) difference between different treatments

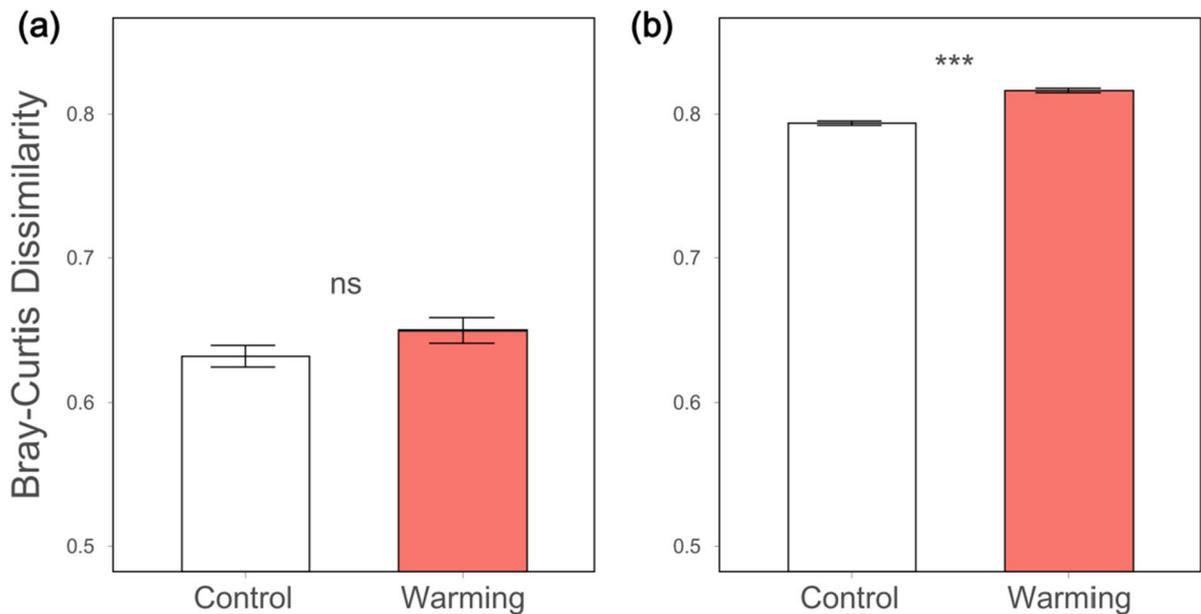


**Fig. 3** Principal coordinate analysis ordination (PCoA) plot based on a Bray–Curtis dissimilarity matrix of root-associated fungal communities under different treatments (a) or among different host plant species (b). For (a), the open circles represent control treatment, the solid circles represent warming treatment. For (b), the points represent the mean scores of PCoA axis 1 and axis 2 in each plant species; bars represent the standard errors of PCoA scores

resent control treatment, the solid circles represent warming treatment. For (b), the points represent the mean scores of PCoA axis 1 and axis 2 in each plant species; bars represent the standard errors of PCoA scores

**Fig. 4** The relationship between plant phylogenetic distance and root-associated fungal Bray–Curtis dissimilarity. Linear models (shown as a red solid line) and associated correlation coefficients are provided





**Fig. 5** Root-associated fungal Bray–Curtis dissimilarities between different treatments at the intra-plant level (a) and the inter-plant level (b). Bars represent means  $\pm$  SEs. Signifi-

cant differences between the treatments were determined after undertaking the *t*-test. Significance levels: ns, non-significant; \*\*\*,  $P < 0.001$

Warming significantly increased the Bray–Curtis dissimilarity of root-associated fungi between different host plants at the inter-specific level (Fig. 5b), rather than the intra-specific level (Fig. 5a). Comparing the regressions of fungal community dissimilarity against host plant phylogenetic distance at the inter-specific level showed that the slope under the warming treatment was significantly higher than the control treatment ( $P < 0.001$ ; Fig. S4). In this case, warming increased the beta-diversity of root-associated fungal communities between host plant species.

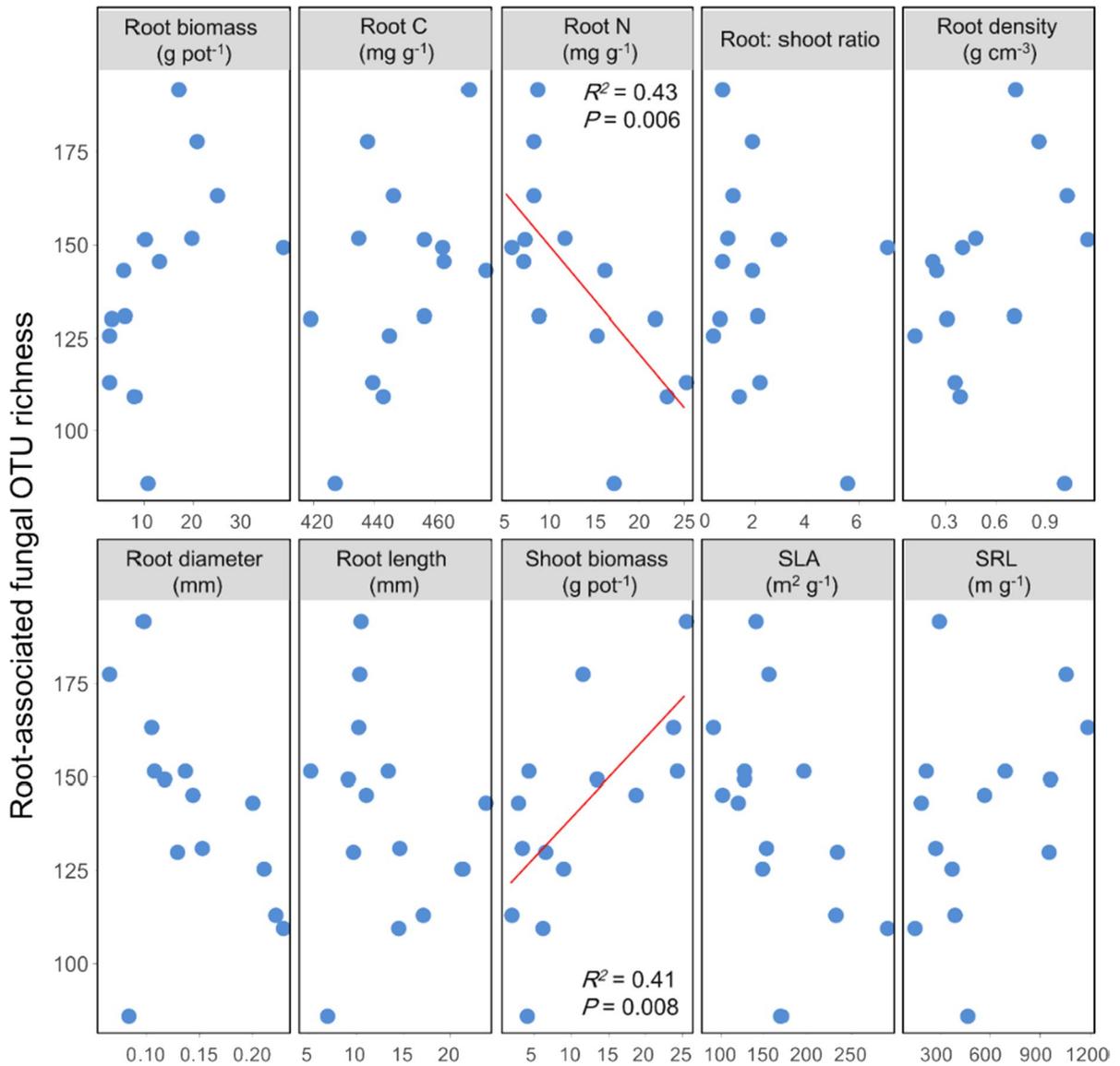
#### Relationship between root-associated fungi and plant traits

Although warming did not significantly influence (all  $P > 0.05$ ) the plant traits, the richness of root-associated fungi correlated positively with the shoot biomass, but negatively with root N concentration, regardless of treatment (Fig. 6). Multiple linear regression analyses showed that the root N concentration was the variable most closely related to fungal richness ( $t = -2.90$ ,  $P = 0.004$ ). Mantel tests also revealed that the root N concentration had the strongest effect on root-associated fungal communities (mantel  $r = 0.19$ ,

$P = 0.001$ ; Table 1). Besides, the root C concentration was important in shaping the root-associated fungal composition (mantel  $r = 0.14$ ,  $P = 0.001$ ). The richness of the different root-associated fungal trophic groups was correlated to different plant traits (Table S3). Only root N ( $t = -3.374$ ,  $P = 0.001$ ) was related to the richness of saprotrophic fungi. Meanwhile, the root C ( $t = 2.431$ ,  $P = 0.016$ ), root diameter ( $t = -2.582$ ,  $P = 0.011$ ), root length ( $t = 2.477$ ,  $P = 0.015$ ), and shoot biomass ( $t = 3.275$ ,  $P = 0.001$ ) were related to the richness of AM fungi. The root C ( $t = -2.925$ ,  $P = 0.004$ ), root length ( $t = 2.033$ ,  $P = 0.044$ ), and shoot biomass ( $t = -2.192$ ,  $P = 0.03$ ) were related to the richness of EcM fungi. However, none of the plant traits were related to the richness of plant pathogenic fungi (all  $P > 0.05$ ). Furthermore, the root N concentration was significantly related to the community structure of saprotrophic fungi, AM fungi, and plant pathogenic fungi, but not EcM fungi (Table S4).

#### Discussion

By examining the root-associated fungal community structure of 14 host plant species, we found



**Fig. 6** Linear regressions of root-associated fungal richness versus plant traits across 14 species. Regression relationships were tested using the mean data from different host plant spe-

cies. Significant linear relationships ( $P < 0.05$ ) are indicated with regression lines

that short-term warming does not affect the root-associated fungal diversity and community composition, which are strongly influenced by plant identity and root stoichiometry. On the other hand, we demonstrated that short-term climate warming significantly increases the dissimilarity of root-associated fungal community composition among different host plant species, suggesting the potential

application of temperature within predictive frameworks of plant–microbe interactions.

Climate warming slightly impacts root-associated fungal richness and community composition

The alpine grassland has a high diversity of root-associated fungi. We observed 1564 OTUs, with an

**Table 1** Mantel test analyses for the relationships of root-associated fungal community composition with plant parameters. Bonferroni adjusted *P*-value significance threshold was 0.005 (0.05/10=0.005) and significant correlations are highlighted in bold

Plant parameters	Mantel <i>r</i>	<i>P</i>
Root biomass (g pot <sup>-1</sup> )	0.084	0.034
Root C (mg g <sup>-1</sup> )	<b>0.140</b>	<b>0.001</b>
Root N (mg g <sup>-1</sup> )	<b>0.188</b>	<b>0.001</b>
Root density (g cm <sup>-3</sup> )	0.086	0.055
Root diameter (mm)	0.085	0.026
Root length (mm)	0.104	0.012
SRL (m g <sup>-1</sup> )	-0.060	0.901
Shoot biomass (g pot <sup>-1</sup> )	0.025	0.217
SLA (m <sup>2</sup> g <sup>-1</sup> )	0.024	0.257
Root: shoot ratio	0.129	0.020

SRL specific root length, SLA specific leaf area

average fungal richness (142) higher than other studies in Arctic ecosystems (Abrego et al. 2020a, b; Botnen et al. 2020) and similar to temperate forests (Nguyen et al. 2020). These results reveal a rich diversity of root-associated fungi on the Qinghai-Tibetan Plateau. Although various root-associated fungi have been identified in our study, separating fungal rDNA from the mixed DNAs extracted from the plant roots was challenging. Universal primers (e.g., ITS3/ITS4) that target all eukaryotes generate a high proportion of plant sequences, which was also the case in our study (Fig. S5). The lack of higher specificity of PCR primers potentially limits the overall assessment of root-associated fungal diversity. Thus, it is highly recommended to design and optimize more conservative primers to improve the detection of root-associated fungi in root samples.

Contrary to our expectation, we did not observe significant effects of warming on the root-associated fungal richness, which is also supported by other studies (Coince et al. 2014; Lorberau et al. 2017). Changes in root-associated fungal richness caused by warming are often attributed to shifts in carbon allocation to roots (Wang et al. 2017). In our case, the warming treatment did not increase the root biomass or root: shoot biomass ratio. Therefore, it is possible that the shorter period of warming or the tolerance to warming in plants could have no effect on root-associated fungal diversity. Nonetheless, given that the richness of root-associated fungi positively

correlated with the shoot biomass, long-term warming is expected to increase the root-associated fungal richness, since global warming tends to increase the production of plant biomass in cold ecosystems (Cornelissen et al. 2007). Conversely, given that soil fungi in the alpine grasslands of the Qinghai-Tibetan Plateau show insensitive responses to short-term warming (Che et al. 2019; Xiong et al. 2014; Zhang et al. 2016a, b), this region could have an inherently stable richness of root-associated fungi. The limitation of our study design is that we did not identify fungal communities from soil samples; therefore, we could not conclude whether the stable richness of root-associated fungi was related to soil fungi. Thus, a combined approach based on both root and soil DNA, would be appropriate to measure fungal communities in future research, especially in areas that have experienced long periods of warming.

The effects of increased temperature on root-associated fungal communities are variable and complicated, the response varies among study sites, the temperature range investigated and host plants (Fujimura et al. 2008; Lorberau et al. 2017; Semenova et al. 2015; Rasmussen et al. 2020). In the present study, warming imparted no significant effect on the root-associated fungal communities, even among specific fungal groups. Previous studies have reported that root-associated fungal community composition are often affected by soil and root factors (Jiang et al. 2018; Francioli et al. 2020). Our findings also support this because the changes in root-associated fungal community composition were strongly associated with the concentrations of root N and C. However, in our study, none of the plant traits that impact the composition of the root-associated fungal community was affected by the short warming period. Therefore, it is possible that the response of the host plant species to warming may not achieve the threshold to influence the composition of root-associated fungal communities. Delgado-Baquerizo et al. (2020) reported an overall increase in the relative abundance of soil-borne pathogens worldwide, which is not supported by our study. The average relative abundance of plant pathogens in our study (20.7%) was higher than that identified in that study (14.4%), and the dominant plant pathogen genera (*Ilyonectria*, *Fusarium*, *Ascochyta*, *Cephalosporium*) are also inconsistent with that reported by Delgado-Baquerizo et al. (*Alternaria*, *Fusarium*, *Venturia* and *Phoma*). As such, due

to the higher proportion and varying composition of putative fungal pathogens, short-term warming may not influence the relative abundance of specific root-associated plant pathogens on the Qinghai-Tibetan Plateau.

Interestingly, we found that climate warming reduced the root-associated fungal richness of *Potentilla fruticosa* and altered the root-associated fungal community composition of *Melissilus ruthenicus*. These specific results support our first hypothesis that the impacts of climate warming on root-associated fungi depend on the host plant species. Nevertheless, since our study only examined 14 host plant species, additional studies should include more plant species to draw a common conclusion about the effect of climate warming on root-associated fungi on the Qinghai-Tibetan Plateau. The morphological traits and tissue nutrients of alpine grassland vegetation differ significantly (Geng et al. 2014), which may cause varying responses of root-associated fungi to climate warming (Rudgers et al. 2020). However, the exact mechanisms underlying this species-specific response remain unknown and point to an area of needed future research.

#### Climate warming induces novel interactions between root-associated fungi and plants

Our results support that host plant species impart a substantial influence on root-associated microbes, which has also been reported previously (Fujimura and Egger 2012; Toju et al. 2013; Wang et al. 2019; Wehner et al. 2014). Differences in root-associated fungal communities may arise, in part, due to active selection by host plants (Maciá-Vicente et al. 2020). For example, plant species with thick and less branched roots may require more mycorrhizal fungi for nutrient absorption than species with a fibrous root system (Wilson and Hartnett 1998). Similarly, McCormick et al. (2018) reported that orchid populations and most mycoheterotrophic groups require the presence of orchid mycorrhizal fungi for germination and growth. This study also found that root-associated fungal community dissimilarities were correlated with host plant phylogenetic distances, indicating that host phylogeny is an essential factor in structuring the root-associated fungal community. This result concurs with previous findings by Wehner et al. (2014) and Koyama et al. (2019), who used data from 25

and 27 plant species, respectively. Besides, our findings reveal the host specialization of different root-associated fungal functional guilds. These results are further supported by previous findings based on an elevational gradient at a high arctic site (Abrego et al. 2020a, b), and provide additional evidence that plant pathogenic fungi are more specialized than symbiotic fungi on the Qinghai-Tibetan Plateau. However, high-throughput sequencing cannot reflect the absolute abundance of root-associated fungal taxa; further research should explore whether the root-associated fungal abundance or biomass also varies among different plant species.

Our results provide the first evidence that climate warming increases the dissimilarity of root-associated fungal communities among different plant species. Contrary to our expectation, the higher level of host specificity is not related to the changes in plant biomass. Although the exact mechanisms behind this variation still remain vague, the putative mechanism could involve shifts in plant root traits (Sweeney et al. 2020) or exudation patterns (Rodriguez et al. 2019). In our case, the root-associated fungal community composition was strongly associated with root N concentration (Table 1). Although climate warming might not affect the root N concentration, the quality or type of N-related resource exchanged between plants and root-associated fungi would be disturbed, inducing an increasing selective pressure on root-associated fungi (Hassani et al. 2018; Kinnunen-Grubb et al. 2020; Rudgers et al. 2020). In addition to root traits, root exudates have also been known as important factors that regulate the root-associated fungal community (Nguyen et al. 2020; Vives-Peris et al. 2020). Since numerous studies have reported a significant stimulation of the secretion of root exudates under experimental warming (Wang et al. 2021; Yin et al. 2013), the increase or change in root exudates may have contributed to this increased dissimilarity of root-associated fungal communities among different plant species. However, the dynamic root exudate chemistry and microbial substrate preferences under climate warming still merit further study. Another explanation for the more distinct root-associated fungal community composition may be related to the local adaptation of host plants. For thousands of years, plants and their associated fungi have co-evolved, leading to the adaptations to each other and the local environment (Thrall et al. 2007). As such,

environmental change (elevated temperature in our case) would disrupt the local adaptation of plants and change their selection for root-associated fungi. Fitzpatrick et al. (2018) found that plant species pairs with more distinct endophytic root bacteria communities have higher productivity than pairs with similar microbes. If root-associated fungi follow a similar trend, we can expect that plants select more dissimilar root-associated fungal communities to establish a more beneficial consortium that promotes their growth to adapt the new environment.

The results of this work indicate that climate warming could lead to a more divergent root-associated fungal community composition among different host plants despite the unremarkable changes in root-associated fungal community composition. This finding may partially explain the low to no host specificity of root-associated fungi in cold environments, especially in Arctic tundra or alpine ecosystems (Gao and Yang 2016; Walker et al. 2011), where plants colonize new habitats more rapidly with a lower selection of root-associated fungi (Botnen et al. 2014). However, the mechanisms for the selective recruitment of soil fungi by host plants are still obscure. Therefore, other relevant factors (e.g., light or nutrients) should also be evaluated to understand the relationship between plant and root-associated fungi fully. Moreover, the plant roots are also colonized by bacterial endophytes, which have beneficial functions for plant growth and health (Compant et al. 2021). Future experiments are encouraged to compare the host specificity of these bacterial endophytes. This will help improve our fundamental knowledge of the impact of climate change on the ecology of plant-soil microbe interactions.

## Conclusions

In summary, we have demonstrated that warming does not influence root-associated fungal richness and community composition but increases the dissimilarity of root-associated fungal communities among different host plant species. These results support the hypothesis (ii) that climate warming will increase host specificity of root-associated fungi due to potential changes in root exudates or disruption of local adaptation, but did not support the hypothesis (i) that increased temperature would lead to an increase

in root-associated fungal richness and alter the root-associated fungal community composition. Our findings provide evidence of the effect of temperature on the associations between host plant species and root-associated fungi from the extreme cold environments of the Qinghai-Tibetan Plateau. These results indicate that more diverse root-associated fungal communities among host plants may help plants with more flexibility to adapt to changing environmental conditions.

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