Microbial diversity patterns in the root zone of two *Meconopsis* plants on the Qinghai-Tibet Plateau

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ABSTRACT

In the extreme alpine climate of the Qinghai-Tibet Plateau (QTP), plant growth and reproduction are limited by extremely cold temperatures, low soil moisture, and scarce nutrient availability. The root-associated microbiome indirectly promotes plant growth and plays a role in the fitness of plants on the QTP, particularly in Tibetan medicinal plants. Despite the importance of the root-associated microbiome, little is known about the root zone. This study used high-throughput sequencing to investigate two medicinal *Meconopsis* plants, *M. horridula* and *M. integrifolia*, to determine whether habitat or plant identity had a more significant impact on the microbial composition of the roots. The fungal sequences were obtained using ITS-1 and ITS-2, and bacterial sequences were obtained using 16S rRNA. Different microbial patterns were observed in the microbial compositions of fungi and bacteria in the root zones of two *Meconopsis* plants. In contrast to bacteria, which were not significantly impacted by plant identity or habitat, the fungi in the root zone were significantly impacted by plant identity, but not habitat. In addition, the synergistic effect was more significant than the antagonistic effect in the correlation between fungi and bacteria in the root zone soil. The fungal structure was influenced by total nitrogen and pH, whereas the structure of bacterial communities was influenced by soil moisture and organic matter. Plant identity had a greater influence on fungal structure than habitat in two *Meconopsis* plants. The dissimilarity of fungal communities suggests that more attention should be paid to fungi-plant interactions.

Subjects Microbiology, Molecular Biology, Mycology, Plant Science, Soil Science
Keywords Microbial community, Bacterial communities, Fungal communities, Soil properties, Community ecology, Microbial structure

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INTRODUCTION

The Qinghai-Tibet Plateau (QTP) is the largest ice storage area on earth, with low oxygen and harsh climatic conditions, and is known as the third pole of the earth (*Qiu, 2008*). Due to its unique geographical characteristics, there is a significant temperature difference between daytime and nighttime on the QTP (*Liu et al., 2013*). Plants on the QTP have evolved the morphological and physiological characteristics necessary to survive under

extreme cold and drought stress (*Sun et al., 2014*; *Boucher et al., 2016*; *Xu et al., 2021*). The root-associated microbiome also plays a role in the fitness of plants, especially in conditions of extreme cold and drought stress (*Acuña-Rodríguez et al., 2020*; *Trivedi et al., 2020*). The root microbiome is created from the surrounding bulk soil (*Yuan et al., 2015*), and can induce changes in the root exudates of plants suffering from poor nutrition, which then affects the structure of the rhizosphere microbiome. Following this pattern, plants produce beneficial microbes to indirectly promote their environmental tolerance, pathogen resistance, and fitness, which is of great significance to agricultural production (*Bulgarelli et al., 2013*; *Brunner et al., 2015*; *Bakker et al., 2018*; *Fitzpatrick et al., 2018*; *Hu et al., 2018*; *Yuan et al., 2018*; *Otto, 2021*). The root microbiome also influences the stabilization of soil organic carbon storage on the QTP over a long timescale, which is particularly important in the context of global warming (*Ruess et al., 2003*; *Moore et al., 2015*; *Tian et al., 2021*).

Bacteria and fungi make up the majority of soil microbial communities (*Fierer, 2017*). Biotic and abiotic factors, such as host genotype and soil type, influence the microbial composition of the roots (*Edwards et al., 2015*; *Agler et al., 2016*). Soil type is the main factor in the pH, temperature, moisture, and nutrient availability of the soil (*Bååth & Anderson, 2003*; *Pietri & Brookes, 2009*; *Li et al., 2016*; *Na et al., 2019*). The composition of the root microbiome is influenced by host-related genotype, growth stage, and microbial competition (*Chaparro, Badri & Vivanco, 2014*; *Castrillo et al., 2017*; *Dombrowski et al., 2017*; *Stringlis et al., 2018*). In *Boechera stricta* (Graham), host genotype had a greater impact on the leaf microbiome than the root microbiome, and the composition of the root microbiome was host-specific and changed with age (*Wagner et al., 2016*). A previous study found that bacterial communities showed significant differences due to geographical location, even among the same plant species (*Yamamoto et al., 2020*). Most research on the effects of plant identity or habitat on microbial communities focuses on endophytes and rhizosphere communities (*Glynou et al., 2016*; *Wippel et al., 2021*; *Zuo et al., 2021*; *Maciá-Vicente & Popa, 2022*), but the impact of plant identity or habitat on the root microbiome has received far less attention. The soil in the root zone is an important component of the soil-root system (*Shi et al., 2019*). This study of the root zone fills in a gap in the current research. A few studies have shown that plant identity and geographical location impact microbial structure. The tree oak clone DF159 was transplanted into various habitats to serve as an environmental measuring instrument, and it was found that environmental variables had a stronger impact than plant identity on the microbial communities of the root zone (*Habiyaremye et al., 2020*). *Bintarti et al. (2020)* reported that the microbial diversity in the root zone of apple trees was strongly influenced by geographical location. It is still unclear, however, whether plant identity or geographical factors have a greater impact on the microbial composition of the root zone of plants on the Qinghai-Tibet Plateau.

Meconopsis, of the Papaveraceae family, is mainly distributed in the Himalayas of China, except for one species that grows in Western Europe (*Wu, 1980*). Because of their resistance to cold temperatures and ultraviolet radiation, they grow at high altitudes and in low temperatures. *Meconopsis* is a traditional Tibetan medicinal plant that contains

bioactive compounds such as alkaloids, flavonoids, volatile oils, and triterpenes. Alkaloids and flavonoids are the main secondary metabolites of *Meconopsis* (*Zhang, Li & Zhou, 1997*; *Liu et al., 2014*; *Yun et al., 2015*). *Meconopsis horridula* Hook. f. & Thomson is used for its antitumor properties and *Meconopsis integrifolia* (Maxim.) Franch has hepatoprotective and antioxidant functions (*Zhou et al., 2013*; *Slaninová et al., 2014*; *Fan et al., 2015*). Most *Meconopsis* plants are currently threatened because of their low genetic diversity and genetic homogeneity of fixed alleles (*Sulaiman & Babu, 1996*; *Wang et al., 2021*). The cultivation and breeding of *Meconopsis* plants is still in the experimental stage. Elucidating the root microbial composition of *M. integrifolia* and *M. horridula* would provide data that would help support the cultivation and breeding of these Tibetan medicinal plants. A few studies have been published on the chemical composition and pharmacology of *M. horridula* and *M. integrifolia*, but the composition of the root microbiome of *Meconopsis* plants has not yet been studied.

This study used high-throughput sequencing to investigate the microbial community structures of the root zone of two *Meconopsis* plant species, *M. horridula* and *M. integrifolia*, both collected from Tibet, China. This study aimed to (i) investigate whether habitat or plant identity has a more significant impact on the microbial composition of the root zone, (ii) analyze whether there are differences in the composition of fungi and bacteria, and (iii) determine the factors that most impact the microbial composition of the root zone.

MATERIALS AND METHODS

Study area and sample collection

Mi La Mountain in Lhasa and the mountain near Dong De Cuo in Nagchu were chosen as study sites. Both *M. horridula* and *M. integrifolia* were collected from the grassy slopes of Mi La Mountain in Lhasa in October 2020, at an altitude of 4,886 m (M site; 29.82°N –92.36°E), and from the mountain near Dong De Cuo in Nagchu, Tibet, China, in July 2020, at an altitude of 4,872 m (D site; 30.99°N–92.94°E). The root zone soil of two plant species and bulk soil were collected from two sites with three replicates, including three root zone soil samples of *M. horridula*, three root zone soil samples of *M. integrifolia*, and three bulk soil samples from the M and D sites, respectively. A total of 18 soil samples were collected (Table 1). Root zone soil is loosely attached to the roots, whereas bulk soil is outside the root zone. For sampling, whole plants were dug up and the soil attached to the roots was shaken into a sterile bag. Bulk soil samples were collected 1 m away from the selected plants. The two species of *Meconopsis* sampled were located in the same habitat, and the replicates were approximately 50 m apart from one another. Each species was also taken within 500 m of another site, ensuring that the two species were obtained from the same habitat. All samples were stored in 95% alcohol at 4 °C for further experiments. The sampling for this scientific expedition was approved by the Forestry Department of the Tibet Region.

Table 1 Detailed information on the two sample sites.

Information of sample sites								
Sample site	**Altitude/m**	**Longitude (E)**	**Latitude (N)**	**Relative humidity (%)**	**Daytime temperature (°C)**	**Dew-point temperature (°C)**	**Illumination intensity (lx)**	**Samples**
Mountain nearby MILA Mountain (M)	4,886	92.36°	29.82°	38.2	20.9	7	1,222,496	*M. horridula* *M. integrifolia* Bulk soil
Mountain nearby DongDe Cuo (D)	4,872	92.94°	30.99°	54.5	21.8	13.4	160,064	*M. horridula* *M. integrifolia* Bulk soil

DNA extraction, PCR amplification, and sequencing

The total DNA of the root-soil bacteria and fungi was extracted using the SDS plus enzyme method. To characterize bacterial communities, primers 338F and 806R were used for the amplification of the hypervariable region V3-V4 in 16S rRNA (*Du et al., 2019*). The PCR for the bacterial communities used a 20 μL reaction system that included: 5 × Buffer, 4 μL; 2.5 mM dNTPs, 2 μL; Forward and Reverse Primer (5 μM), 0.8 μL; FastPfu polymerase, 0.4 μL; BSA, 0.2 μL; template, DNA 10 ng; and ddH_2O added to a total volume of 20 μL. Premier ITS-1 and ITS-2 were used to characterize the fungal communities (*Gardes & Bruns, 1993*; *Bergelson, Mittelstrass & Horton, 2019*), because they produce fewer non-fungal sequences than ITS3 or ITS4 (*Mello et al., 2011*). The PCR for the fungal communities also used a 20 μL reaction system that included: 10 × Buffer, 2 μL; 2.5 mM dNTPs, 2 μL; Forward and Reverse Primer (5 μM), 0.8 μL; rTaq polymerase, 0.2 μL; BSA, 0.2 μL; template, DNA 10 ng; and ddH_2O added to a total volume of 20 μL. The PCR parameters were: 95 °C for 3 min, 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 45 s for 25 cycles; 72 °C for 10 min, then the test was stopped at 10 °C. Nano Drop 2000 was used to test DNA purity and concentration, and agarose gel electrophoresis was used to check for PCR success. All the samples were sent to Shanghai Majorbio Bio-Pharm Technology Co., Ltd., and the sequencing results were obtained through the Illumina HiSeq platform.

Determination of soil properties

To measure soil properties, available kalium (AK), available phosphorus (AP), organic matter (OM), pH, soil moisture (SM), and total nitrogen (TN) were all measured. Total nitrogen was tested using the modified Kjeldahl Method (Chinese standard method HJ717-2014). Available phosphorus was obtained using the ascorbic acid colorimetric method (Chinese standard method HJ704–2014) (*Ren & Gao, 2022*). Available kalium was estimated using the NY/T889-2004 method, and the NY/T1121.6-2006 method was used for determining soil organic matter (*Lirong, 2019*). These four factors were measured by the Tibet BoYuan Environmental Testing Co., Ltd. Soil moisture was tested with the drying method: 5 g of soil was placed in a drying oven for 1 h and then weighed. The pH was tested using pH equipment (Sartorius PB-10), and the water-soil ratio was 1:1 (*Cao et al., 2022*).

Statistical analysis

A network analysis was performed on the OTU level using the CYTOSCAPE (CONET) software to estimate the correlation between the fungal and bacterial communities in the root zone. Pearson and Spearman correlations served as the foundation for both positive and negative correlations. A total of 100 randomization interactions were performed and the multiple test correction based on Benjamini-Hochberg was used. When the net sum of the relationships is positive, the impact is synergistic, and when it is negative, the impact of the relationship is antagonistic (*Fath & Patten, 1998*; *Cao et al., 2022*).

An alpha diversity analysis and Student's t test were employed to test the dissimilarity of the structure of the root zone microbiome between plant species and geographical locations. The alpha indexes, including community richness (Chao, coverage and ace) and diversity (Shannon and Simpson), were calculated using a mothur index analysis (version v.1.30.2, https://mothur.org/wiki/calculators/). Then the Shannon index for Student's t test (suitable for a small sample size, $n < 30$) was used to test between-group differences on the class level.

Based on the Bray-Curtis dissimilarity matrix, nonmetric multidimensional scaling (NMDS) was used to explore the similarities and differences in community composition among different grouped samples at the genus level. These grouped samples were then tested through Adonis and the weighted UniFrac distance was calculated with 999 permutations.

A total of five environmental factors were selected based on their *P* value for the canonical correlation analysis (CCA) or redundancy analysis (RDA) on the OTU level. The *P* value, which indicated the relevance of the CCA or RDA, was assessed by ANOVA. CCA and RDA assess the relationship between microbes and measured soil properties. DCA analysis results determined whether an RDA or CCA was used: if the result of the DCA analysis was greater than or equal to 3.5, a CCA was used; if the DCA analysis result was less than 3.5, an RDA was used (*Xiao et al., 2022*).

All the data were analyzed on the free online Majorbio Cloud Platform, except the results of the network analysis. The raw sequence data have been deposited into the NCBI Sequence Read Archive with accession numbers PRJNA814442 and PRJNA813297.

RESULTS

Analyses of sequencing data

The MiSeq sequencing analysis of 18 samples resulted in a total of 1,204,304 raw fungal reads and 893,452 raw bacterial reads. The average lengths for fungi and bacteria were 255 and 418 bp, respectively. The dilution curve analysis revealed clear asymptotes, indicating that the fungal and bacterial samples were nearly complete (Fig. 1).

Microbial composition across two plants

A total of 45 bacterial phyla were detected. The bacterial compositions of *M. horridula* and *M. integrifolia* across the two sites were similar. Actinobacteriota dominated the diversity of the two *Meconopsis* plants from the two different locations, with Proteobacteria following closely thereafter, and Firmicutes, Acidobacteriota, and Chloroflexi were also

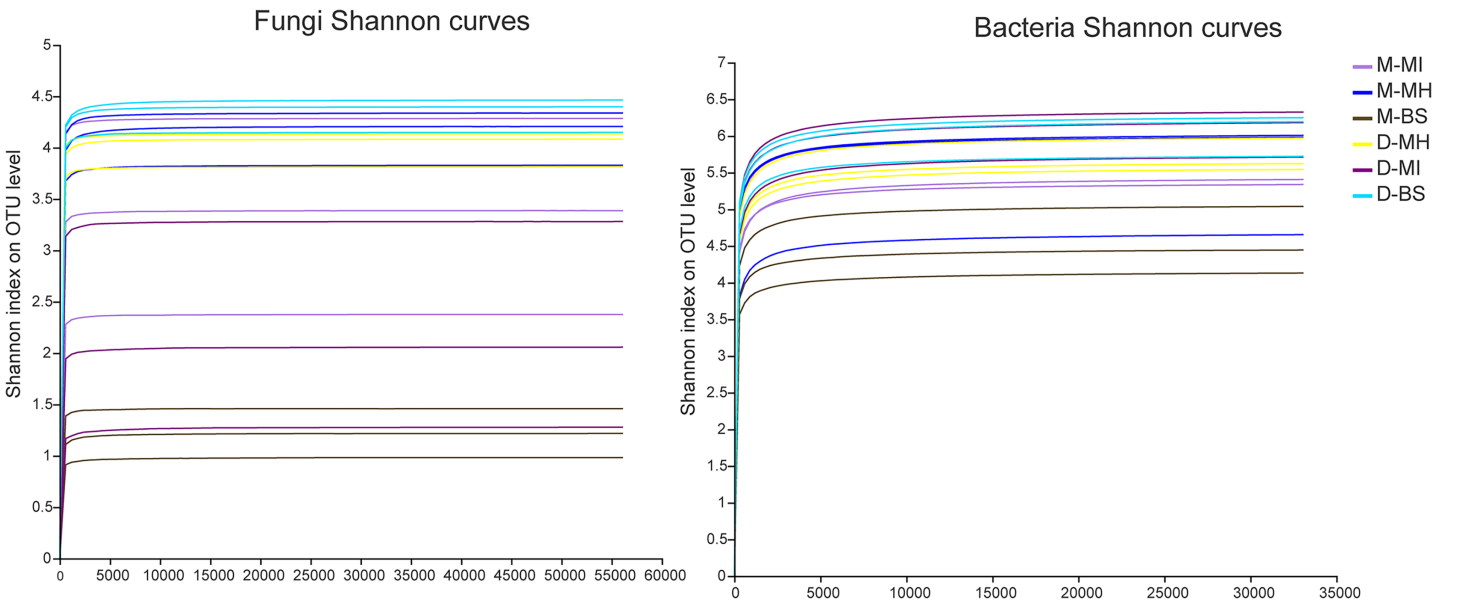

Figure 1 Shannon dilution curve. M, Mi La mountain; D, mountain near Dong De Cuo; MH, *Meconopsis horridula*; MI, *Meconopsis integrifolia*; BS, bulk soil.
Full-size DOI: 10.7717/peerj.15361/fig-1

abundant. The distribution of bacteria across the two plants was similar to that in bulk soil, although the abundance levels were different.

The ITS-1 and ITS-2 sequencing detected a total of 13 fungal phyla. The two plants had different fungal compositions. The phylum Ascomycota was the most numerous in *M. horridula*, accounting for more than half of the OTUs, followed by Basidiomycota and Mortierellomycota. In *M. integrifolia*, Basidiomycota was dominant, accounting for 57.28% of the total fungal composition at site M and 80.37% at site D. Of the fungi detected in the bulk soil, 91.83% were assigned to Basidiomycota at site M, and Ascomycota accounted for 70.24% at site D (Fig. 2).

The top 15 OTUs were selected to estimate the correlation between fungi and bacteria in the root microbiome. The results showed a positive correlation between fungi and bacteria in the root zone. A total of 30 nodes and 178 edges were identified for *M. horridula*, including 68 negative edges and 110 positive edges. For *M. integrifolia*, there were a total of 30 nodes and 296 edges, including 124 negative edges and 172 positive edges (Fig. 3). The synergistic effect was more significant than the antagonistic effect in the correlation of fungi and bacteria.

Effects of plant identity or habitat on fungi and bacteria

An alpha diversity analysis was used to test the similarity and dissimilarity of root zone microbial diversity to detect whether plant identity or habitat had a greater impact on the root microbial composition of plants on the Qinghai-Tibet Plateau. There were no significant differences observed in the bacterial or fungal communities of the same plant species collected from different habitats. There were also no significant differences in the bacterial diversity of the roots of *M. horridula* and *M. integrifolia* plants collected from the same area. However, there was a considerable difference in fungal Shannon diversity

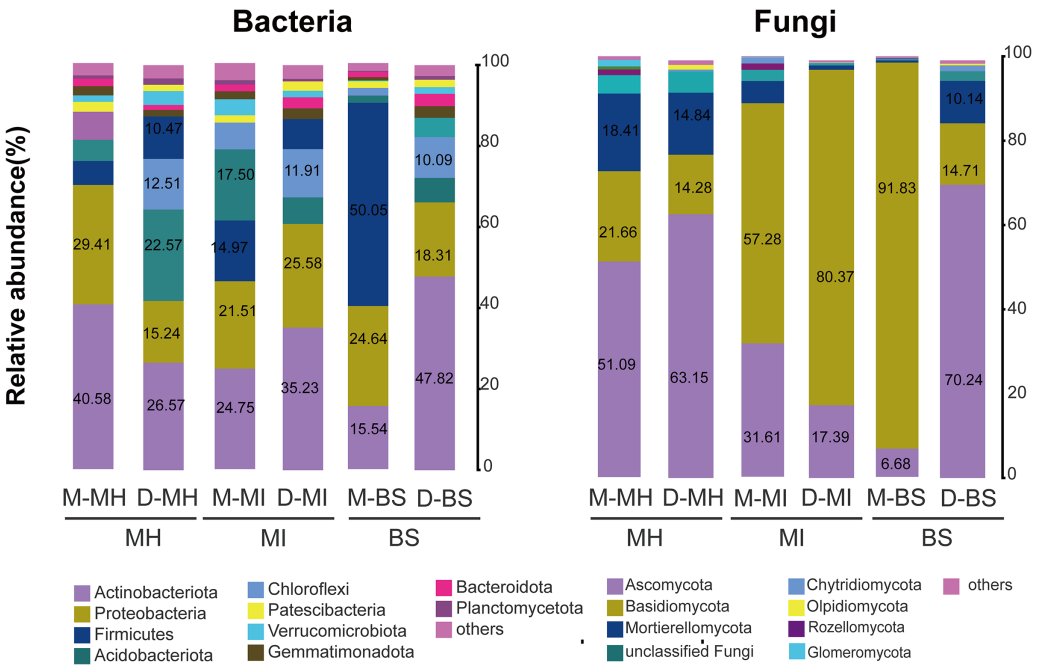

Figure 2 Relative abundance of fungi and bacteria at the phylum level. Fungi and bacteria with average relative abundance levels <0.01 were combined into the "Others" category. M, Mi La mountain; D, mountain near Dong De Cuo; MH, *Meconopsis horridula*; MI, *Meconopsis integrifolia*; BS, bulk soil.
Full-size DOI: 10.7717/peerj.15361/fig-2

between the two plants obtained from the same area. This finding indicates that plant identity has a stronger effect than habitat on fungi and that bacterial diversity was more similar than fungal diversity (Fig. 4).

To further investigate the similarity or dissimilarity of fungal and bacterial communities, an NMDS analysis was performed based on the Bray-Curtis dissimilarity matrix. The NMDS analysis of bacterial communities in *M. horridula* and *M. integrifolia* each revealed a cluster. The *M. horridula* from both locations formed a cluster in fungal communities, although relative plant species from the same habitat did not (Fig. 5). There were more similarities in bacterial diversity and more dissimilarities in the composition of fungi in the root zone of *Meconopsis* species. In summary, the fungal community compositions of root zones were significantly different between the two different plant species, but bacterial structure was similar in the two plants.

The relationship between microbes and soil properties

Study results (Table 2) showed different correlations between the root zone microbiome and soil properties in *Meconopsis* plants. The *P* value, as determined by ANOVA, demonstrated the relevance of the CCA or RDA. The relevance ranking for bacterial structure was: OM ($P = 0.003$) > SM ($P = 0.004$) > AP ($P = 0.115$) > pH ($P = 0.17$) > TN ($P = 0.376$) > AK ($P = 0.918$). The top five relevant factors, according to *P* value, were then used for the RDA. The relevance ranking for fungal structure was: pH ($P = 0.001$) > TN ($P = 0.001$) >AP ($P = 0.005$) > SM ($P = 0.006$) > OM ($P = 0.043$) > AK ($P = 0.045$). The top

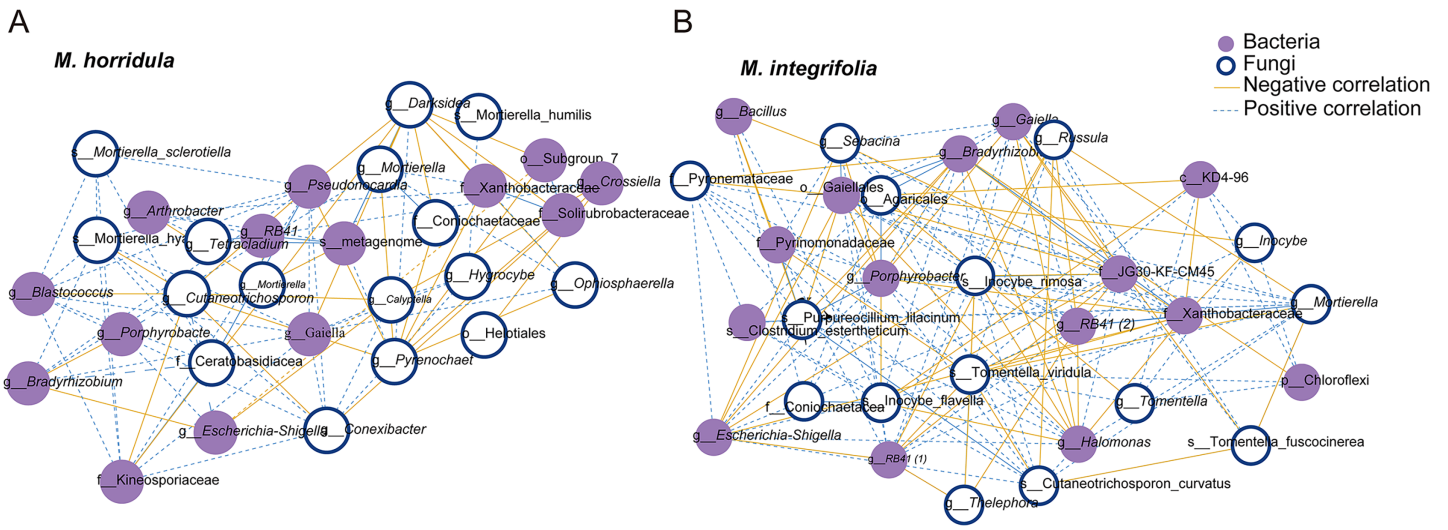

Figure 3 Network analysis of the correlation between bacteria and fungi. The top 15 fungal and bacterial OTUs were selected from *M. horridula* (A) and *M. integrifolia* (B). The nodes represent the bacterial (purple) and fungal (blue) OTUs. Edge color represents negative (yellow) and positive (blue) correlations.
Full-size DOI: 10.7717/peerj.15361/fig-3

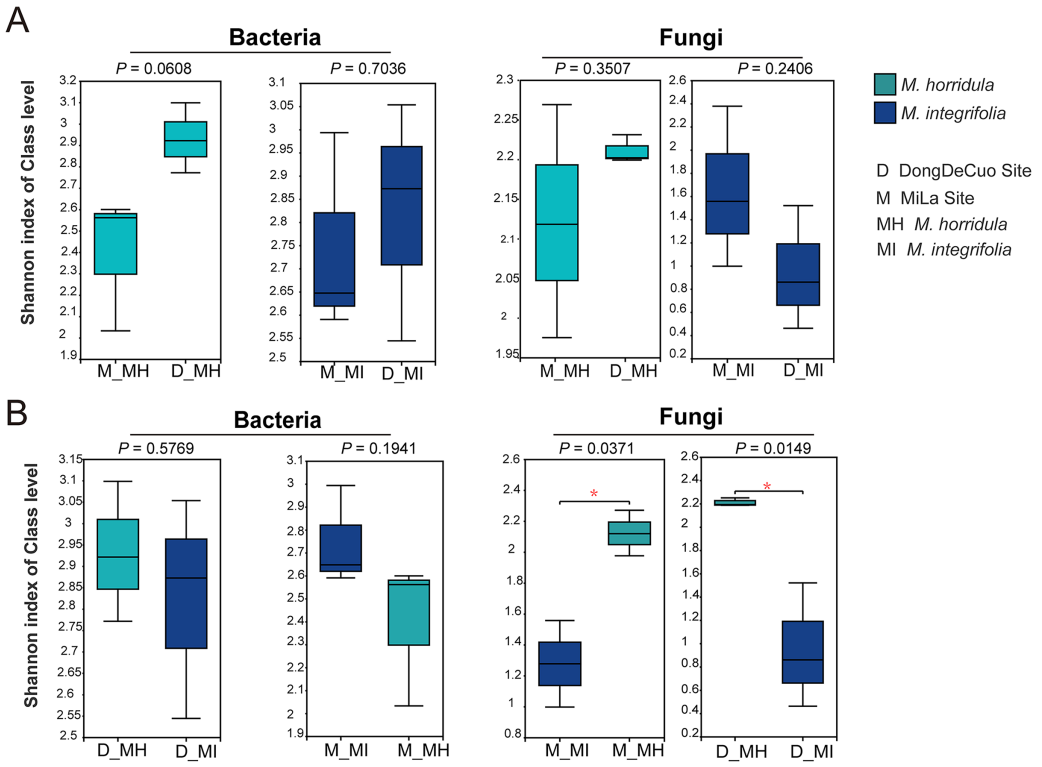

Figure 4 Alpha diversity analysis of the structure of bacterial or fungal diversity from two sister plants or two geographical locations based on Student's t-test. Asterisks indicate significant differences ($^{*}P < 0.05$). (A) The root zone microbiome of plants from different habitats at a class level and (B) Student's t-test analysis for the root zone microbiome of two plants in the same habitat. M, Mi La mountain; D, mountain near Dong De Cuo; MH, *Meconopsis horridula*; MI, *Meconopsis integrifolia*; BS, bulk soil.
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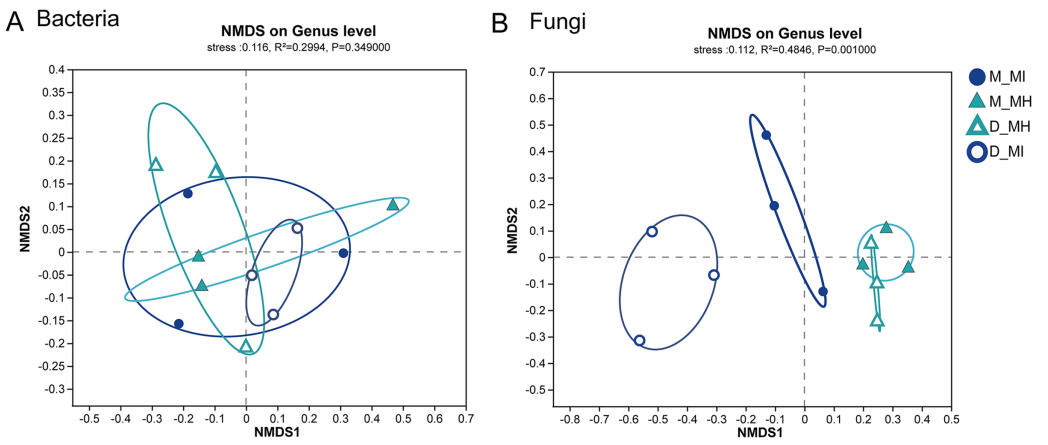

Figure 5 Nonmetric multidimensional scaling (NMDS) ordination of root zone bacterial and fungal communities across two host plants from different sites at a genus level. M, Mi La mountain; D, mountain near Dong De Cuo; MH, *Meconopsis horridula*; MI, *Meconopsis integrifolia*.
Full-size DOI: 10.7717/peerj.15361/fig-5

Table 2 Summary of the soil properties of each sample.

Site/*P* value	Sample	Soil moisture (%)	Organic matter/(mg/kg)	Available K/(mg/kg)	Available P/(mg/kg)	Total nitrogen/ (mg/kg)	pH
M site	*M. horridula*	13.4	42.5	18	9.6	2,430	8.13
M site	*M. integrifolia*	20.9	37.6	16	1.1	202	6.24
M site	Bulk soil	36.4	112	14	1.3	1,750	7.14
D site	*M. horridula*	27.7	66.2	14	1.5	2,880	6.94
D site	*M. integrifolia*	19.4	43.7	2	1.4	3,370	7.21
D site	Bulk soil	6.5	72.6	14	4.6	3,460	7.45
P value	(with Bacteria)	0.004	0.003	0.918	0.115	0.376	0.17
P value	(with Fungi)	0.006	0.043	0.045	0.005	0.001	0.001

five relevant factors, according to *P* value, were then used for the CCA (Fig. 6). These results demonstrated that soil moisture and organic matter had a major impact on bacterial structure, while pH and available nitrogen were more closely related to fungal structure.

DISCUSSION

The microbial community composition of the root zones of *Meconopsis* plants from the harsh environment of the QTP is comparable to root microorganisms in low-altitude regions, as shown in previous research. In our study, the structure of fungal communities detected by ITS-1 and ITS-2 in the root zone was influenced significantly by plant identity rather than habitat, but bacterial communities were not significantly affected by either plant identity or habitat. In agreement with numerous studies, the effect of plant identity on the microbiome was significant, especially in fungal communities, which have a

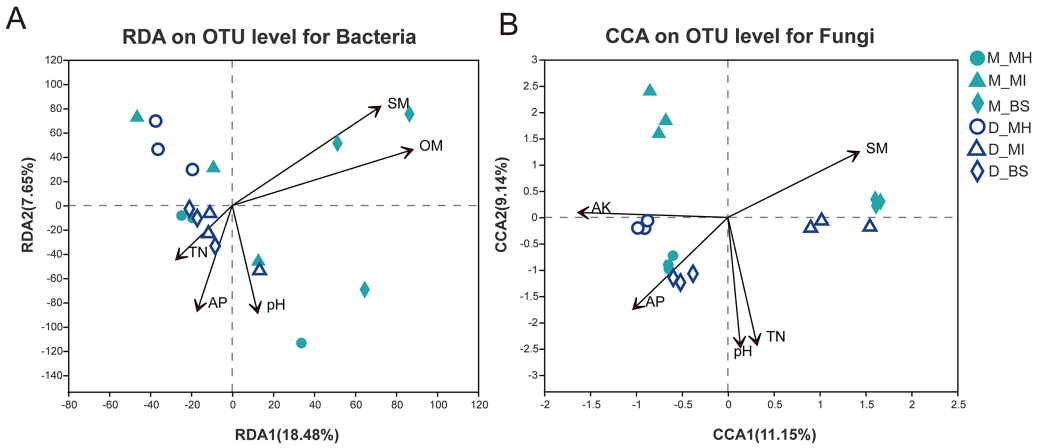

Figure 6 Redundancy analysis (RDA) for bacterial communities (A), Correspondence analysis (CCA) for fungal communities (B), and their interaction with soil properties. M, Mi La mountain; D, mountain near Dong De Cuo; MH, *Meconopsis horridula*; MI, *Meconopsis integrifolia*; BS, bulk soil.
Full-size DOI: 10.7717/peerj.15361/fig-6

stronger correlation with plant identity in contrast to other microbial communities (*Millard & Singh, 2010*; *Zinger et al., 2011*). Plant-fungal communities have a stronger impact on plant identity than plant-bacterial correlations (*Bergelson, Mittelstrass & Horton, 2019*). As the climate gets warmer on the QTP, the dissimilarity of root fungal communities between different plant species may increase due to potential changes in root exudates or disruptions in the adaptability of plants due to active selection by the host plant (*Jiang et al., 2021*). These findings suggest that fungi may be important to maintaining the stability of microbial communities.

There were significant differences in the root zone fungal composition of the two plant species, whereas bacterial composition was similar in the two plant species between the two sites. Two factors may explain this finding. First, fungi may significantly improve plant pathogens and environmental tolerance, particularly in harsh environments. This has been demonstrated in some studies of endophytes (*Rodriguez et al., 2008*; *Rodriguez, Woodward & Redman, 2010*). The root zone microbiome may play the same role as microbiota, where plants can communicate and recognize each other through plant-specialized metabolism, allowing the plants to modulate root-associated microbiota based on the plant's needs. In *Arabidopsis thaliana* (L.) Heynh, for example, triterpene compounds selectively affect root microbiome assembly (*Huang et al., 2019*). Second, the similarity of bacterial communities in roots allows plants to quickly colonize new habitats, improving their fitness. Fungi and bacteria play different roles in plant growth and stress tolerance. Previous studies have focused on the interaction between endophytic fungi and plants. The symbiosis of fungi as endophytes improves the stress tolerance of the environment and pathogens (*Márquez et al., 2007*; *Rodriguez et al., 2008*; *Rodriguez, Woodward & Redman, 2010*; *Fitzpatrick et al., 2018*). Some components of the root zone microbiome, such as ectomycorrhizal fungi, benefit plant growth (*Kumar & Atri, 2018*; *Guerrero-Galán, Calvo-Polanco & Zimmermann, 2019*, *Aryal, Meiners & Carlsward, 2021*), but the functions of other members of these microbial communities remain unclear. Investigating

the influence of root zone microbial communities enables the analysis of the impacts of plant identity and the geographical environment in manipulating the root-associated microbiome.

In contrast to our findings that fungal communities were impacted by plant identity, but not habitat, *Yao et al. (2013)* discovered that fungal communities in the root were influenced significantly by habitat. The composition of root-associated fungal communities differed across sampling sites, and these differences were linked to soil nutrient concentrations. Numerous studies have found that soil properties can influence microbial communities, but the impact varies depending on the habitat (*Li & Liu, 2019*; *Zhang et al., 2019*; *Wu et al., 2021*).

Total nitrogen and pH were the most significant factors affecting the structure of fungal communities in our study, whereas soil moisture and organic matter had the most significant impact on the structure of bacterial communities. The relationship between fungal richness and nitrogen concentration is strong (*Jiang et al., 2021*). In contrast to our findings, a previous study found that pH was positively related to the relative abundance and diversity of bacteria, but less related to fungal communities (*Cao et al., 2020*; *Rousk et al., 2010*). All of the samples in our study had alkaline pH values, and the pH effects may not be apparent with pH value changes as narrow as they were in our study, which may explain these differing results (*Fierer, 2017*).

The environment has been shown to impact the influence of soil properties on microbial communities. Moisture was more related to microbial composition in the warm dry sites and cool wet sites (*Brockett, Prescott & Grayston, 2012*). The impact of the interaction of soil properties on microbiome diversity and structure differed from the effect of a single factor (*He et al., 2016*). The interaction of factors, soil type, and nutrient conditions should all be considered when discussing the relationship between soil properties and the plant microbiome. On the QTP, there is currently no fixed index for evaluating soil nutrition. Due to differences in soil types and soil nutrition, it is impossible to determine which factors have a greater impact on microbial structure. More controlled experiments are required to further study the significance of environmental factors on plant-microbe interactions in this area.

Plants can function as ecosystem engineers, shaping the microbiome associated with their roots to their advantage (*Coyte, Schluter & Foster, 2015*). Co-occurrence networks can be used to study microbe–microbe interactions and to explain microbial community stability (*Barberán et al., 2012*; *de Vries et al., 2018*). The interaction of bacterial and fungal communities has an impact on the stability of these communities in harsh environments (*Coyte, Schluter & Foster, 2015*; *Jiao et al., 2022*). Negative feedback in microbial communities restrains positive feedback networks, which benefits the host plant and increases stability (*Coyte, Schluter & Foster, 2015*). However, in our study, the impact of positive feedback was stronger than the impact of negative feedback in the correlation of fungal and bacterial communities in the root zone microbiome of *Meconopsis* plants. This could be because of alpine habitat heterogeneity. Positive or negative interactions between

bacterial and fungal communities are a tradeoff, and synergistic interactions could be beneficial in some cases (*Mille-Lindblom, Fischer & Tranvik, 2006*).

In this study, Basidiomycota and Ascomycota were the dominant fungi in the root fungal communities, according to the ITS-1 and ITS2 sequencing results. Even though the ITS-1 and ITS-2 primers we used for fungi could offer adequate information for taxonomic assignment and are commonly used in amplicon pyrosequencing studies of fungal diversity (*Mello et al., 2011*; *Bergelson, Mittelstrass & Horton, 2019*), it is still unclear if they provide the best taxonomic resolution of the species (*Mello et al., 2011*). More ITS regions should be used in order to increase classification accuracy in future studies. The 16S rRNA results showed that Actinobacteriota, Proteobacteria, and Acidobacteriota were the dominant bacteria in the root bacterial communities of *Meconopsis* plants. Among the microbial communities we detected, the phyla Actinobacteriota and Proteobacteria were also the dominant bacteria of radiation-tolerant bacteria isolated from the soil in Tibet (*Rao et al., 2016*). In high alpine permafrost, Proteobacteria dominate the bacterial communities. To adapt to the environment, they minimize anaerobic metabolism and live ectosymbiotic lives (*Frey et al., 2016*). This could explain the predominance of Proteobacteria in the root zone of *Meconopsis* plants on the QTP. Certain bacteria may have a long-term association with plants (*Yeoh et al., 2016*). The phyla Basidiomycota and Ascomycota were found to be dominant in our study and are also the dominant fungi of *A. thaliana* plants (*Bergelson, Mittelstrass & Horton, 2019*). In other studies, the fungus Ascomycota is distributed over a large range of scales, and it is the dominant fungal phylum in Tibet (*Maestre et al., 2015*; *Prober et al., 2015*; *Yang et al., 2017*). A better understanding of the root microbial diversity and assembly characteristics of wild Tibetan medicinal plants could improve cultivation efforts of these plants and add to the knowledge of root zone soil microorganisms on the Qinghai-Tibet Plateau.

CONCLUSIONS

This study revealed that plant identity had a larger impact than habitat on the root zone fungal communities of two Tibetan medicinal plants, and that root bacterial communities did not differ between the two plant species or between the two sites. These findings demonstrate that the root zone microbial composition of fungi and bacteria respond differently to plant identity and habitat. We speculate that root fungal communities have a greater impact on plants than bacterial communities and that the fungi-plant relationship should be an area of future research. Furthermore, in the connection of fungi and bacteria, the synergistic effect was more significant than the antagonistic effect. Fungal community structures detected by ITS-1 and ITS-2 were influenced by available nitrogen and pH, whereas bacterial structures were significantly influenced by soil moisture and organic matter.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Shuting Chen conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Pengxi Cao analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Ting Li analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Yuyan Wang performed the experiments, prepared figures and/or tables, and approved the final draft.
- Xing Liu conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw sequence data are available at the NCBI Sequence Read Archive: PRJNA814442 and PRJNA813297.

Supplemental Information

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REFERENCES

Acuña-Rodríguez IS, Newsham KK, Gundel PE, Torres-Díaz C, Molina-Montenegro MA. 2020. Functional roles of microbial symbionts in plant cold tolerance. *Ecology Letters* **23(6)**:1034–1048 DOI 10.1111/ele.13502.

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLOS Biology* **14(1)**:e1002352 DOI 10.1371/journal.pbio.1002352.

Aryal P, Meiners SJ, Carlsward BS. 2021. Ectomycorrhizae determine chestnut seedling growth and drought response. *Agroforestry Systems* **95(7)**:1251–1260 DOI 10.1007/s10457-020-00488-4.

Bååth E, Anderson TH. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry* **35(7)**:955–963 DOI 10.1016/S0038-0717(03)00154-8.

Bakker PA, Pieterse CM, de Jonge R, Berendsen RL. 2018. The soil-borne legacy. *Cell* **172(6)**:1178–1180 DOI 10.1016/j.cell.2018.02.024.

Barberán A, Bates ST, Casamayor EO, Fierer N. 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. *The ISME Journal* **6(2)**:343–351 DOI 10.1038/ismej.2011.119.

Bergelson J, Mittelstrass J, Horton MW. 2019. Characterizing both bacteria and fungi improves understanding of the *Arabidopsis* root microbiome. *Scientific Reports* **9(1)**:24 DOI 10.1038/s41598-018-37208-z.

Bintarti AF, Wilson JK, Quintanilla-Tornel MA, Shade A. 2020. Biogeography and diversity of multi-trophic root zone microbiomes in Michigan apple orchards: analysis of rootstock, scion, and local growing region. *Phytobiomes Journal* **4(2)**:122–132 DOI 10.1094/PBIOMES-01-20-0007-R.

Boucher FC, Lavergne S, Basile M, Choler P, Aubert S. 2016. Evolution and biogeography of the cushion life form in angiosperms. *Perspectives in Plant Ecology, Evolution and Systematics* **20**:22–31 DOI 10.1016/j.ppees.2016.03.002.

Brockett BF, Prescott CE, Grayston SJ. 2012. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry* **44(1)**:9–20 DOI 10.1016/j.soilbio.2011.09.003.

Brunner I, Herzog C, Dawes MA, Arend M, Sperisen C. 2015. How tree roots respond to drought. *Frontiers in Plant Science* **6(e1000492)**:547 DOI 10.3389/fpls.2015.00547.

Bulgarelli D, Schlaeppi K, Spaepen S, Van Themaat EVL, Schulze-Lefert P. 2013. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* **64(1)**:807–838 DOI 10.1146/annurev-arplant-050312-120106.

Cao PX, Liu Y, Ma HM, Zhao N, Chen ST, Xu GQ, Liu X. 2022. Fungal diversity in the soil of the *Oxytropis glacialis* root system on the Qinghai-Tibet Plateau. *Frontiers in Microbiology* **13**:831783 DOI 10.3389/fmicb.2022.831783.

Cao PX, Liu YX, Xu GQ, Ji YL, Li JK, Li XY, Liu X. 2020. Bacterial diversity in the root system soil of *Oxytropis glacialis*. *Acta Ecologica Sinica* **40**:12.

Castrillo G, Teixeira PJPL, Paredes SH, Law TF, de Lorenzo L, Feltcher ME, Finkel OM, Breakfield NW, Mieczkowski P, Jones CD, Paz-Ares J, Dangl J. 2017. Root microbiota drive direct integration of phosphate stress and immunity. *Nature* **543(7646)**:513–518 DOI 10.1038/nature21417.

Chaparro JM, Badri DV, Vivanco JM. 2014. Rhizosphere microbiome assemblage is affected by plant development. *The ISME Journal* **8(4)**:790–803 DOI 10.1038/ismej.2013.196.

Coyte KZ, Schluter J, Foster KR. 2015. The ecology of the microbiome: networks, competition, and stability. *Science* **350(6261)**:663–666 DOI 10.1126/science.aad2602.

de Vries FT, Griffiths RI, Bailey M, Craig H, Girlanda M, Gweon HS, Hallin S, Kaisermann A, Keith AM, Kretzschmar M, Lemanceau P, Lumini E, Mason KE, Oliver A, Ostle N, Prosser JI, Thion C, Thomson B, Bardgett RD. 2018. Soil bacterial networks are less stable under drought than fungal networks. *Nature Communications* **9(1)**:3033 DOI 10.1038/s41467-018-05516-7.

Dombrowski N, Schlaeppi K, Agler MT, Hacquard S, Kemen E, Garrido-Oter R, Wunder J, Coupland G, Schulze-Lefert P. 2017. Root microbiota dynamics of perennial *Arabis alpina* are dependent on soil residence time but independent of flowering time. *The ISME Journal* **11(1)**:43–55 DOI 10.1038/ismej.2016.109.

Du R, Cao S, Li B, Zhang H, Li X, Zhang Q, Peng Y. 2019. Step-feeding organic carbon enhances high-strength nitrate and ammonia removal via DEAMOX process. *Chemical Engineering Journal* **360**:501–510 DOI 10.1016/j.cej.2018.12.011.

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA, Sundaresan V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences of the United States of America* **112(8)**:E911–E920 DOI 10.1073/pnas.1414592112.

Fan J, Wang Y, Wang X, Wang P, Tang W, Yuan W, Kong L, Liu Q. 2015. The antitumor activity of *Meconopsis horridula* Hook, a traditional Tibetan medical plant, in murine leukemia L1210 cells. *Cellular Physiology and Biochemistry* **37(3)**:1055–1065 DOI 10.1159/000430231.

Fath BD, Patten BC. 1998. Network synergism: emergence of positive relations in ecological systems. *Ecological Modelling* **107(2–3)**:127–143 DOI 10.1016/S0304-3800(97)00213-5.

Fierer N. 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology* **15(10)**:579–590 DOI 10.1038/nrmicro.2017.87.

Fitzpatrick CR, Copeland J, Wang PW, Guttman DS, Kotanen PM, Johnson MT. 2018. Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences of the United States of America* **115(6)**:E1157–E1165 DOI 10.1073/pnas.1717617115.

Frey B, Rime T, Phillips M, Stierli B, Hajdas I, Widmer F, Hartmann M. 2016. Microbial diversity in European alpine permafrost and active layers. *FEMS Microbiology Ecology* **9(3)**:fiw018 DOI 10.1093/femsec/fiw018.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2(2)**:113–118 DOI 10.1111/j.1365-294X.1993.tb00005.x.

Glynou K, Ali T, Buch AK, Kia SH, Ploch S, Xia X, Çelik A, Thines M, Maciá V, Jose G. 2016. The local environment determines the assembly of root endophytic fungi at a continental scale. *Environmental Microbiology* **18(8)**:2418–2434 DOI 10.1111/1462-2920.13112.

Guerrero-Galán C, Calvo-Polanco M, Zimmermann SD. 2019. Ectomycorrhizal symbiosis helps plants to challenge salt stress conditions. *Mycorrhiza* **29(4)**:291–301 DOI 10.1007/s00572-019-00894-2.

Habiyaremye JDD, Goldmann K, Reitz T, Herrmann S, Buscot F. 2020. Tree root zone microbiome: exploring the magnitude of environmental conditions and host tree impact. *Frontiers in Microbiology* **11**:749 DOI 10.3389/fmicb.2020.00749.

He D, Xiang X, He JS, Wang C, Cao G, Adams J, Chu H. 2016. Composition of the soil fungal community is more sensitive to phosphorus than nitrogen addition in the alpine meadow on the Qinghai-Tibetan Plateau. *Biology and Fertility of Soils* **52(8)**:1059–1072 DOI 10.1007/s00374-016-1142-4.

Hu L, Robert CA, Cadot S, Zhang X, Ye M, Li B, Manzo D, Chervet N, Steinger T, van der HM, Schlaeppi K, Erb M. 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications* **9(1)**:1–13 DOI 10.1038/s41467-018-05122-7.

Huang AC, Jiang T, Liu YX, Bai YC, Reed J, Qu B, Goossens A, Nützmann HW, Bai Y, Osbourn A. 2019. A specialized metabolic network selectively modulates *Arabidopsis* root microbiota. *Science* **364(6440)**:eaau6389 DOI 10.1126/science.aau6389.

Jiang S, Ling N, Ma Z, He X, He JS. 2021. Short-term warming increases root-associated fungal community dissimilarities among host plant species on the Qinghai-Tibetan Plateau. *Plant and Soil* **466(1–2)**:597–611 DOI 10.1007/s11104-021-05073-x.

Jiao S, Chu HY, Zhang BG, Wei XR, Chen WM, Wei GH. 2022. Linking soil fungi to bacterial community assembly in Arid Ecosystems. *iMeta* **1(1)**:e2 DOI 10.1002/imt2.2.

Kumar J, Atri NS. 2018. Studies on ectomycorrhiza: an appraisal. *The Botanical Review* **84(2)**:108–155 DOI 10.1007/s12229-017-9196-z.

Li Y, Lin Q, Wang S, Li X, Liu W, Luo C, Zhang Z, Zhu X, Jiang L, Li X. 2016. Soil bacterial community responses to warming and grazing in a Tibetan alpine meadow. *FEMS Microbiology Ecology* **92(1)**:fiv152 DOI 10.1093/femsec/fiv152.

Li W-H, Liu Q-Z. 2019. Changes in fungal community and diversity in strawberry rhizosphere soil after 12 years in the greenhouse. *Journal of Integrative Agriculture* **18(3)**:677–687 DOI 10.1016/S2095-3119(18)62003-9.

Lirong HE. 2019. Study on the impact of land consolidation project on soil quality in Bainijing Town, Dingbian County. *IOP Conference Series: Earth and Environmental Science* **330(3)**:032102 DOI 10.1088/1755-1315/330/3/032102IOP Publishing.

Liu K, Baskin JM, Baskin CC, Bu H, Du G, Ma M. 2013. Effect of diurnal fluctuating versus constant temperatures on germination of 445 species from the eastern Tibet Plateau. *PLOS ONE* **8(7)**:e69364 DOI 10.1371/journal.pone.0069364.

Liu J, Wu H, Zheng F, Liu W, Feng F, Xie N. 2014. Chemical constituents of *Meconopsis horridula* and their simultaneous quantification by high-performance liquid chromatography coupled with tandem mass spectrometry. *Journal of Separation Science* **37(18)**:2513–2522 DOI 10.1002/jssc.201400379.

Maciá-Vicente JG, Popa F. 2022. Local endemism and ecological generalism in the assembly of root-colonizing fungi. *Ecological Monographs* **92(1)**:e01489 DOI 10.1002/ecm.1489.

Maestre FT, Delgado-Baquerizo M, Jeffries TC, Eldridge DJ, Ochoa V, Gozalo B, Quero JL, García-Gómez M, Gallardo A, Ulrich W, Bowker MA, Arredondo T, Barraza-Zepeda C, Bran D, Florentino A, Gaitán J, Gutiérrez JR, Huber-Sannwald E, Jankju M, Mau RL, Miriti M, Naseri K, Ospina A, Stavi I, Wang D, Woods NN, Yuan X, Zaady E, Singh BK. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences of the United States of America* **112(51)**:15684–15689 DOI 10.1073/pnas.1516684112.

Márquez LM, Redman RS, Rodriguez RJ, Roossinck MJ. 2007. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science* **315(5811)**:513–515 DOI 10.1126/science.1136237.

Mello A, Napoli C, Murat C, Morin E, Marceddu G, Bonfante P. 2011. ITS-1 versus ITS-2 pyrosequencing: a comparison of fungal populations in truffle grounds. *Mycologia* **103(6)**:1184–1193 DOI 10.3852/11-027.

Millard P, Singh BK. 2010. Does grassland vegetation drive soil microbial diversity? *Nutrient Cycling in Agroecosystems* **88(2)**:147–158 DOI 10.1007/s10705-009-9314-3.

Mille-Lindblom C, Fischer HJ, Tranvik L. 2006. Antagonism between bacteria and fungi: substrate competition and a possible tradeoff between fungal growth and tolerance towards bacteria. *Oikos* **113(2)**:233–242 DOI 10.1111/j.2006.0030-1299.14337.x.

Moore JA, Jiang J, Patterson CM, Mayes MA, Wang G, Classen AT. 2015. Interactions among roots, mycorrhizas and free-living microbial communities differentially impact soil carbon processes. *Journal of Ecology* **103(6)**:1442–1453 DOI 10.1111/1365-2745.12484.

Na X, Yu H, Wang P, Zhu W, Niu Y, Huang J. 2019. Vegetation biomass and soil moisture coregulate bacterial community succession under altered precipitation regimes in a desert steppe in northwestern China. *Soil Biology and Biochemistry* **136**:107520 DOI 10.1016/j.soilbio.2019.107520.

Otto G. 2021. The root of nutrient stress resistance. *Nature Reviews Microbiology* **19(3)**:139 DOI 10.1038/s41579-021-00521-y.

Pietri J, Brookes PC. 2009. Substrate inputs and pH as factors controlling microbial biomass, activity and community structure in an arable soil. *Soil Biology & Biochemistry* **41(7)**:1396–1405 DOI 10.1016/j.soilbio.2009.03.017.

Prober SM, Leff JW, Bates ST, Borer ET, Firn J, Harpole WS, Lind EM, Seabloom EW, Adler PB, Bakker JD, Cleland EE, DeCrappeo NM, DeLorenze E, Hagenah N, Hautier Y, Hofmockel KS, Kirkman KP, Knops JMH, La P, Kimberly J, MacDougall AS, McCulley RL, Mitchell CE, Risch AC, Schuetz M, Stevens CJ, Williams RJ, Fierer N, Klironomos J. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology Letters* **18(1)**:85–95 DOI 10.1111/ele.12381.

Qiu J. 2008. China: the third pole. *Nature News* **454(7203)**:393–396 DOI 10.1038/454393a.

Rao S, Chan OW, Lacap-Bugler DC, Pointing SB. 2016. Radiation-tolerant bacteria isolated from high altitude soil in Tibet. *Indian Journal of Microbiology* **56(4)**:508–512 DOI 10.1007/s12088-016-0604-6.

Ren Z, Gao H. 2022. Abundant and rare soil fungi exhibit distinct succession patterns in the forefield of Dongkemadi glacier on the central Qinghai-Tibet Plateau. *Science of the Total Environment* **828**:154563 DOI 10.1016/j.scitotenv.2022.154563.

Rodriguez RJ, Henson J, Van VE, Hoy M, Wright L, Beckwith F, Kim Y, Redman RS. 2008. Stress tolerance in plants via habitat-adapted symbiosis. *The ISME Journal* **2(4)**:404–416 DOI 10.1038/ismej.2007.106.

Rodriguez RJ, Woodward C, Redman RS. 2010. Adaptation and survival of plants in high stress habitats via fungal endophyte conferred stress tolerance. In: *Symbioses and Stress*. Dordrecht: Springer, 461–476 DOI 10.1007/978-90-481-9449-0_23.

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal* **4(10)**:1340–1351 DOI 10.1038/ismej.2010.58.

Ruess RW, Hendrick RL, Burton AJ, Pregitzer KS, Sveinbjornssön B, Allen MF, Maurer GE. 2003. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. *Ecological Monographs* **73(4)**:643–662 DOI 10.1890/02-4032.

Shi W, Li M, Wei G, Tian R, Li C, Wang B, Lin R, Shi C, Chi X, Zhou B, Gao Z. 2019. The occurrence of potato common scab correlates with the community composition and function of the geocaulosphere soil microbiome. *Microbiome* **7(1)**:14 DOI 10.1186/s40168-019-0629-2.

Slaninová I, Pěnčíková K, Urbanová J, Slanina J, Táborská E. 2014. Antitumour activities of sanguinarine and related alkaloids. *Phytochemistry Reviews* **13(1)**:51–68 DOI 10.1007/s11101-013-9290-8.

Stringlis IA, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk MC, Berendsen RL, Bakker PAHM, Feussner I, Pieterse CMJ. 2018. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proceedings of the National Academy of Sciences of the United States of America* **115(22)**:E5213–E5222 DOI 10.1073/pnas.1722335115.

Sulaiman IM, Babu CR. 1996. Enzyme polymorphism analyses in three endangered species of Himalayan poppy, *Meconopsis* (*Papaveraceae*). *Genetic Resources and Crop Evolution* **43(4)**:351–356 DOI 10.1007/BF00132955.

Sun H, Niu Y, Chen YS, Song B, Liu C-Q, Peng D-L, Chen J-G, Yang Y. 2014. Survival and reproduction of plant species in the Qinghai-Tibet Plateau. *Journal of Systematics and Evolution* **52(3)**:378–396 DOI 10.1111/jse.12092.

Tian J, Zong N, Hartley IP, He N, Zhang J, Powlson D, Zhou J, Kuzyakov Y, Zhang F, Guirui Y, Dungait JA. 2021. Microbial metabolic response to winter warming stabilizes soil carbon. *Global Change Biology* **27(10)**:2011–2028 DOI 10.1111/gcb.15538.

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology* **18(11)**:607–621 DOI 10.1038/s41579-020-0412-1.

Wagner MR, Lundberg DS, Tijana G, Tringe SG, Dangl JL, Mitchell OT. 2016. Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nature Communications* **7(1)**:1–15 DOI 10.1038/ncomms12151.

Wang WT, Guo WY, Jarvie S, Svenning JC. 2021. The fate of Meconopsis species in the Tibeto-Himalayan region under future climate change. *Ecology and Evolution* **11(2)**:887–899 DOI 10.1002/ece3.7096.

Wippel K, Tao K, Niu Y, Zgadzaj R, Kiel N, Guan R, Dahms E, Zhang P, Jensen DB, Logemann E, Radutoiu S, Schulze-Lefert P, Garrido-Oter R. 2021. Host preference and invasiveness of commensal bacteria in the Lotus and *Arabidopsis* root microbiota. *Nature Microbiology* **6(9)**:1150–1162 DOI 10.1038/s41564-021-00941-9.

Wu CY. 1980. A study on the taxonoiviic system of the genus *Meconopsis*. *Acta Botanica Yunnanica*.

Wu X, Liu P, Wegner CE, Luo Y, Xiao KQ, Cui Z, Zhang F, Liesack W, Peng J. 2021. Deciphering microbial mechanisms underlying soil organic carbon storage in a wheat-maize rotation system. *Science of the Total Environment* **788(6)**:147798 DOI 10.1016/j.scitotenv.2021.147798.

Xiao D, He H, Yan X, Keita M, Díaz ND, Chen D, Yan X. 2022. Dataset of response characteristics of H2-producing bacteria consortium to β-Lactams, Aminoglycosides, Macrolides, Quinolones antibiotics. *Data in Brief* **43**:108354 DOI 10.1016/j.dib.2022.108354.

Xu B, Luo D, Li ZM, Sun H. 2021. Corrigendum to: evolutionary radiations of cushion plants on the Qinghai-Tibet Plateau: insights from molecular phylogenetic analysis of two subgenera of *Arenaria* and *Thylacospermum* (*Caryophyllaceae*). *Taxon* **70(3)**:687 DOI 10.1002/tax.12534.

Yamamoto K, Matsutani M, Shiwa Y, Ishige T, Sakamoto H, Saitoh H, Tsushima S. 2020. Comparative analysis of bacterial diversity and community structure in the rhizosphere and root

endosphere of two halophytes, *Salicornia europaea* and *Glaux maritima*, collected from two Brackish Lakes in Japan. *Microbes and Environments* **35(3)**:ME20072 DOI 10.1264/jsme2.ME20072.

Yang T, Adams JM, Shi Y, He JS, Jing X, Chen L, Tedersoo L, Chu H. 2017. Soil fungal diversity in natural grasslands of the Tibetan Plateau: associations with plant diversity and productivity. *New Phytologist* **215(2)**:756–765 DOI 10.1111/nph.14606.

Yao F, Vik U, Brysting AK, Carlsen T, Halvorsen R, Kauserud H. 2013. Substantial compositional turnover of fungal communities in an alpine ridge to snowbed gradient. *Molecular Ecology* **22(19)**:5040–5052 DOI 10.1111/mec.12437.

Yeoh YK, Paungfoo-Lonhienne C, Dennis PG, Robinson N, Ragan MA, Schmidt S, Hugenholtz P. 2016. The core root microbiome of sugarcanes cultivated under varying nitrogen fertilizer application. *Environmental Microbiology* **18(5)**:1338–1351 DOI 10.1111/1462-2920.12925.

Yuan J, Chaparro JM, Manter DK, Zhang R, Vivanco JM, Shen Q. 2015. Roots from distinct plant developmental stages are capable of rapidly selecting their own microbiome without the influence of environmental and soil edaphic factors. *Soil Biology and Biochemistry* **89(9)**:206–209 DOI 10.1016/j.soilbio.2015.07.009.

Yuan J, Zhao J, Wen T, Zhao M, Li R, Goossens P, Huang Q, Bai Y, Vivanco JM, Kowalchuk GA, Berendsen RL, Shen Q. 2018. Root exudates drive the soil-borne legacy of aboveground pathogen infection. *Microbiome* **6(1)**:1–12 DOI 10.1186/s40168-018-0537-x.

Yun WU, Liu YR, Han P, Yong Y, Qiang AZ. 2015. Pollination ecology of alpine herb *Meconopsis integrifolia* at different altitudes. *Chinese Journal of Plant Ecology* **39(1)**:1–13 DOI 10.17521/cjpe.2015.0001.

Zhang WW, Chong W, Rui X, Wang LJ. 2019. Effects of salinity on the soil microbial community and soil fertility. *Journal of Integrative Agriculture* **18(6)**:1360–1368 DOI 10.1016/S2095-3119(18)62077-5.

Zhang GL, Li BG, Zhou ZZ. 1997. Non-alkaloidal constituents from *Meconopsis punicea* Maxim. *Natural Product Research* **9**:4–6 DOI 10.16333/J.1001-6880.1997.02.002.

Zhou G, Chen Y, Liu S, Yao X, Wang Y. 2013. In vitro and in vivo hepatoprotective and antioxidant activity of ethanolic extract from *Meconopsis integrifolia* (maxim.) franch. *Journal of Ethnopharmacology* **148(6)**:664–670 DOI 10.1016/S2095-3119(18)62077-5.

Zinger L, Lejon DP, Baptist F, Bouasria A, Aubert S, Geremia RA, Choler P. 2011. Contrasting diversity patterns of crenarchaeal, bacterial and fungal soil communities in an alpine landscape. *PLOS ONE* **6(5)**:e19950 DOI 10.1371/journal.pone.0019950.

Zuo Y, Li X, Yang J, Liu J, Zhao L, He X. 2021. Fungal endophytic community and diversity associated with desert shrubs driven by plant identity and organ differentiation in extremely arid desert ecosystem. *Journal of Fungi* **7(7)**:578 DOI 10.3390/jof7070578.