



Plant compartment niche is more important in structuring the fungal community associated with alpine herbs in the subnival belt of the Qiangyong glacier than plant species

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Abstract

The plant compartment niche (i.e., the host plant provides various microhabitats for the microbial community, such as the rhizosphere, root endosphere, leaf endosphere, and phylloplane) and plant species play a significant role in shaping the plant-associated microbial community assembly. However, in the mycobiome associated with alpine herbs in the subnival belt research, little work has been done to assess the contribution of plant compartment niches and plant species to fungal community variation and to reveal the plant compartment niche differentiation of fungal communities. In this study, we quantified the fungal communities associated with the rhizosphere soil, root endospheres, and leaf endospheres of three alpine herbs (*Rheum spiciforme*, *Eriophyton wallichii*, and *Rhodiola bupleuroides*) in the subnival belt of the Qiangyong glacier using high-throughput DNA sequencing. Our results revealed that the variation in diversity and composition of the fungal community was predominantly shaped by plant compartment niche rather than plant species. Rhizosphere soil exhibited the highest level of fungal diversity and niche breadth, while the lowest level was observed in the leaf endosphere. The fungal community composition significantly differed across different plant compartment niches. Fungal co-occurrence networks of the root endosphere and leaf endosphere were more complex and showed higher centrality and connectedness than the rhizosphere soil. Moreover, we also found that the deterministic process governed the fungal community assembly, and the host plant exerts stronger selection pressure on the leaf endophytes in comparison with the root endophytes. The root endophytes are the primary potential contributors to the leaf endophytes, compared with the fungal community associated with rhizosphere soil. Further, the *Pleosporaceae*, *Davidiellaceae*, and *Chaetomiaceae* were significantly enriched and overlapped in two plant compartment niches (root endosphere and leaf endosphere). Collectively, this study reveals that the variation in the diversity and composition of fungal communities associated with three alpine herbs were primarily affected by plant compartment niches rather than plant species. Additionally, this study also reveals that the diversity, composition, co-occurrence pattern, and assembly process of fungal communities associated with three alpine herbs exhibited plant compartment niche differentiation. These results provide a novel insight into the community assembly and ecological interactions of fungal communities associated with plants in harsh environments.

Keywords Fungal community · Alpine herbs · Subnival belt · Plant compartment niche · Plant species

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1 Introduction

The alpine subnival belt, which lies between the permanently snow-covered zone and the alpine meadow, is the terrestrial ecosystem inhabited by spermatophytes at the highest elevation (Körner 2003; Cun and Wang 2010). Plants growing in the subnival belt face harsh environmental stressors, including low atmospheric pressure and temperature, intense UV-B radiation, and the majority of nutrients are in plant-inaccessible forms (Heer and Körner 2002; Thomas et al. 2014; Given et al. 2020). Nevertheless, previous studies showed

that the subnival belt of the Qinghai-Tibetan Plateau harbors the richest alpine flora around the world (Wu 1988; Li 1993; Xu et al. 2013). Moreover, researchers have dedicated considerable effort in the past few decades to investigate plant biodiversity and unravel the mechanisms involved in plant adaptation and differentiation in the subnival belt (Li et al. 1985; Pauli et al. 1999; Yang et al. 2008; Noroozi et al. 2011; Xu et al. 2013; Peng et al. 2015; Niu and Sun 2018; Jiang et al. 2018). However, plants provide a multitude of compartment niches (e.g., root, stem, leaf, flower, and fruit) for the growth and proliferation of diverse microbes that in turn help to maintain plant growth, productivity, and fitness via nutrient acquisition, hormone production, and protection against biotic and abiotic stressors (Ritpitakphong et al. 2016; Hassani et al. 2018; Trivedi et al. 2020; Marian et al. 2022). Therefore, plants are not standalone entities but holobionts consisting of the hosts and their associated microbiome (Qian et al. 2019). Given the pivotal role of microbes in the health and adaptation of host plants, understanding the fundamental ecological processes governing the assembly of microbial communities associated with alpine plants in the subnival belt is critical for elucidating mechanisms of plant diversity maintenance and plant adaptation evolution.

Different plant compartments, such as rhizosphere, phyllosphere, and internal tissues, are colonized by a distinct microbial community (Uroz et al. 2010; Bulgarelli et al. 2012; Hamonts et al. 2017; Cregger et al. 2018; Xiong et al. 2020). For example, previous studies on microbial communities associated with *Cannabis sativa* (Wei et al. 2021), *Mussaenda kwangtungensis* (Qian et al. 2019), *Deschampsia antarctica* and *Colobanthus quitensis* (Zhang et al. 2020), and *Oxyria digyna* (Given et al. 2020) have demonstrated that there is a marked difference in diversity, composition, and co-occurrence patterns across different plant compartment niches. Soils provide a seed bank for plant-associated microbiomes (Vorholt 2012; Bulgarelli et al. 2012; Zarraonaindia et al. 2015), and plants can filter and choose niche-compliant microbiomes to occupy various compartments (Xiong et al. 2020). A growing body of evidence demonstrates the microbiome assembly along the soil–plant continuum (i.e., the microenvironment involved from soil to plant roots and above-ground portions, including bulk soil, rhizosphere soil, root rhizoplane, root endosphere, phylloplane, and leaf endosphere) is shaped predominantly by plant compartment niche (Coleman-Derr et al. 2016; Zheng and Gong 2019; Xiong et al. 2020; Zheng et al. 2021; Zhong et al. 2022; Guo et al. 2022; Huang et al. 2023). Additionally, plant species also played a crucial role in shaping the microbial community in terms of a specific compartment (Xiong et al. 2020; Laforest Lapointe et al. 2016; Walters et al. 2018). These studies highlight the decisive role of the host plant in shaping the assembly of microbial communities associated with plants. In comparison, in the microbial

communities associated with alpine herbs in the subnival belt research, existing literature mainly focuses on the effects of elevation, soil properties, and plant species on the diversity and composition of the rhizospheric microbial community (Miniaci et al. 2007; Teixeira et al. 2010; Nissinen et al. 2012; Fujimura and Egger 2012; Ciccazzo et al. 2014; Massaccesi et al. 2015; Mapelli et al. 2018). Little work has been conducted to explore the plant compartment niche differentiation, potential source and enrichment processes of the microbial communities associated with alpine plants in the subnival belt. Moreover, we still know little about the contribution of plant compartment niches and plant species to the assembly of microbial communities associated with alpine plants in the subnival belt along the soil–plant continuum.

Fungal communities associated with plants have a significant and pivotal impact on plant survival, health, productivity, and even the functioning of ecosystems (Berg et al. 2005; Rodriguez et al. 2009). Endophytic fungi can promote plant growth and resistance to biotic and abiotic stresses (Yao et al. 2019). Rhizosphere fungi also play a vital role in maintaining plant fitness and indirectly affect the composition and functioning of natural plant communities (Rudgers et al. 2012; Philippot et al. 2013). However, studies of the total communities of fungi associated with plants are few and far fewer than those of total communities of bacteria, in spite of the fact that, in terrestrial ecosystems, fungi account for more biomass than bacteria (Gao et al. 2020). Understanding the fundamental ecological processes of endophytic and rhizosphere fungal community assembly associated with alpine plants in the subnival belt is therefore would fill the gap in the knowledge of fungal community associated with plants, especially in harsh environments. Furthermore, this would provide vital information on elucidating mechanisms of plant diversity maintenance and plant adaptation evolution in the subnival belt ecosystem.

In the present work, we explore the fungal community diversity, composition, co-occurrence pattern, and assembly processes associated with the rhizosphere soil, root endospheres, and leaf endospheres of three alpine herbs (*Rheum spiciforme*, *Eriophyton wallichii* and *Rhodiola bupleuroides*) in the subnival belt of the Qiangyong glacier in Qinghai-Tibetan Plateau (Fig. 1c–e). The aim of the present study was to: 1) assess the contribution of plant compartment niches and plant species to the fungal community variation, 2) reveal the fungal community associated with alpine herbs exhibit compartment niche differentiation, and 3) disclose the potential source and enrichment processes of endophytic fungi associated with alpine herbs. We hypothesize that 1) the fungal community would primarily be structured by plant compartment niche rather than plant species, 2) the fungal community would differ significantly across the rhizosphere soil, root endosphere, and leaf endosphere, and

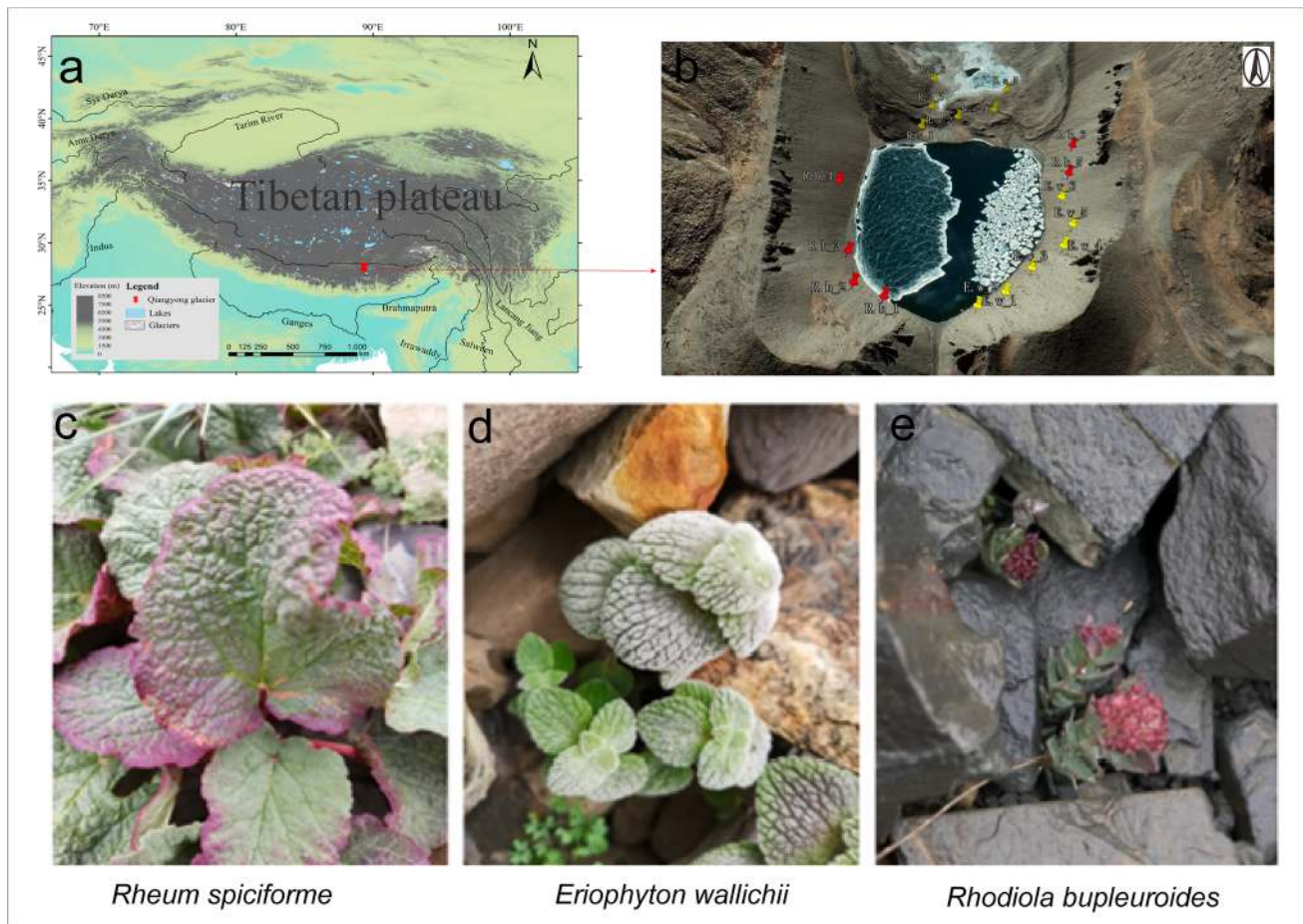


Fig. 1 The *Rheum spiciforme* (*R. spiciforme*), *Eriophyton wallichii* (*E. wallichii*) and *Rhodiola bupleuroides* (*R. bupleuroides*) in the subnival belt of the Qiangyong glacier. R.b_1-6: *Rhodiola bupleuroides*

samples; R.s_1-6: *Rheum spiciforme* samples; E.w_1-6: *Rheum spiciforme* samples

3) rhizosphere soil would be the seed bank of endophytic fungi, and some special taxa may be enriched in endosphere niches. *Rheum spiciforme* (*R. spiciforme*), *Eriophyton wallichii* (*E. wallichii*), and *Rhodiola bupleuroides* (*R. bupleuroides*) were species of perennial herb in the family *Polygonaceae*, *Lamiaceae*, and *Crassulaceae*, respectively. These plant species are sparsely occupying soils of deglaciated areas of the Qinghai-Tibetan Plateau. Thus, this study will advance our understanding in assembly principles and ecological interactions of fungal communities associated with alpine herbs in the subnival belt ecosystem.

2 Materials and methods

2.1 Study site and sampling

This study was conducted in the foreland of the Qiangyong glacier (28°53'N, 90°13'E), which is located in the southern part of the Qinghai-Tibetan Plateau (Fig. 1a, b). This

glacier has retreated at an average rate of 4 m per year between 1976 and 2006 (Yao et al. 2012). The annual air mean temperature and annual mean precipitation of this region is 2.4 °C and 370 mm, respectively (Gu et al. 2021). In August 2020, plant samples were collected from the region surrounding the Qiangyong Co Lake, which far away from the glacier terminus is about 1.3 km to 2.0 km (Fig. 1c, Table S1). In detail, we selected six healthy individuals for each species (i.e., *R. spiciforme*, *E. wallichii*, and *R. bupleuroides*) at least 20 m away from each other to ensure sample independence. Whole plants with intact root systems were excavated using sterilized shovels, shaking off the loosely bound rhizosphere soil. Thereafter, the leaves and roots were collected from one plant, respectively placed in DNA-free sterile plastic tubes, and labeled. All collected samples were frozen (-20 °C) immediately until arrival at the laboratory. To eliminate cross-contamination, the sample collection tools were washed with distilled water and wiped with 70% (vol/vol) ethanol during sampling.

2.2 DNA extraction, amplification and Illumina sequencing

According to the method described by Yao et al (2019) with some modification, surface sterilization was performed to obtain fungal communities in the leaf endosphere (Fig. S1). In brief, the leaves were washed with sterile cooled TE (Tris–EDTA, 10 mM Tris, 1 mM EDTA, pH 7.5) buffer using ultrasonic cleaner and vortex shaker. Successively, the leaves were disinfected by consecutive immersion for 1 min in 80% (vol/vol), 5 min in 3.25% (vol/vol) sodium hypochlorite, and 30 s in 80% ethanol. Finally, the leaves were rinsed in sterile distilled water three sequential times, followed by dried with sterile absorbent paper. To validate the effectiveness of surface disinfection, 100 µL of the third rinse was poured into potato dextrose agar (PDA) plates and cultured in the dark for 14 d at 28 °C to check for the appearance of colonies. To explore the fungal communities in the rhizosphere soil and root endosphere, the fibrous roots (diameter < 2 mm) separated from the taproot using sterilized scissors, and then root samples were placed into 50 mL sterile centrifuge tubes and were washed with PBS buffer (10 mM, pH 7.4) on a shaking Table (150 rpm) for 1 h, followed by the fibrous roots were separated from suspension (Fig. S2). The soil particles directly dislodged from the fibrous root were defined as the rhizosphere soil, which was pelleted by centrifugation (10,000×g for 10 min) in a 50 ml sterile centrifuge tube (Fig. S2). The root samples were then separated, surface-sterilized, and verified as described above for leaf samples (Fig. S2). In total, 54 samples (3 plant species×6 individual plants×3 plant compartment niches) were used in this study. All the samples were stored at -80 °C until required for DNA extraction.

The roots and leaves were aseptically cut, and freeze-dried in liquid nitrogen, followed by homogenized with a mortar and pestle under aseptic conditions. Samples of the homogenized tissues (3 g) and rhizosphere soils were used for DNA extraction with DNeasy[®] PowerSoil[®] Kit (Mo Bio Laboratories, Carlsbad, CA, United States) according to manufacturer instructions. The quantity and concentration of DNA extracts were determined by NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States). The extracted DNA was subjected to fungal ITS2 region amplification using primers fITS7(GTGARTCATCGAATCTTTG) and ITS4(TCCTCCGCTTATTGATATATGC). PCR was performed in a 20 µL reaction solution consisting of 4 µL 5×FastPfu Buffer, 2.5 mM dNTPs (2 µL), 5 µM of each primer (0.8 µL), 0.4 µL FastPfu Polymerase, 0.2 µL BSA, and 10 ng of template DNA. The PCR thermal profile was as follows: initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. PCR products were cleaned using AxyPrep

DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) and quantified using Quantus[™] Fluorometer (Promega, United States). The sequencing library was constructed by the addition of an Illumina sequencing adaptor to the product using the NEXTFLEX[®] Rapid DNA-Seq Kit according to the manufacturer's protocol. Libraries were sequenced on the Illumina MiSeq PE 250 platform with a Paired-End protocol at the Majorbio Bio-Pharm Technology, Co., Ltd. (Shanghai, China). The ITS rRNA sequences were submitted to NCBI Sequence Read Archive (BioProject accession number: PRJNA1061354).

2.3 Bioinformatic analysis

We trimmed and filtered the adapters, primer sites, and low-quality ends of raw sequences using Trimmomatic-0.39, and the overlapping paired-end reads were merged to a single sequence by performing the 'fastq_mergepairs' command in VSEARCH (2.17.1). The 'derep_fulllength', 'cluster_unoise', and 'uchime3_denovo' commands in VSEARCH were performed to detect and remove the sequences of redundant and chimera. The sequences were binned into operational taxonomic units (OTUs) with a 97% threshold of sequence similarity by employing the Uclust algorithm. After then, the RDP Classifier (Wang et al. 2007) was used to classify the taxonomic identity of each OTU, which was determined based on a BLAST search against the UNITE reference database.

2.4 Statistical analyses

All the analyses were implemented in R (version 4.0.3), apart from the visualization of fungi community co-occurrence network was implemented in Gephi (version 0.9.2) and the PAST software (version 4.03) was used to perform SIMPER (similarity percentage) analysis. The Two-way ANOVA with non-parametric tests was used to assess the effects of plant compartment niche, plant species and their interactions on the alpha diversity index and relative abundance of different taxonomic groups. We tested the difference in the fungal community composition among various plant compartment niches by conducting three different permutation tests including permutational multivariate analysis of variance (ADONIS), analysis of similarity (ANOSIM), and multiple response permutation procedure (MRPP). The differences in the fungal community composition were visualized by principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarities matrices using the "Vegan" package in R, and the Two-way ANOVA with non-parametric test was used to evaluate the contribution of the plant compartment niche, plant species and their interactions the fungal community composition variation. The sources of fungal communities in the root endosphere and leaf endosphere were

estimated by using Source Tracker (version 1.0) based on the Bayesian approach (Knights et al. 2011). The “EdgeR” package in R was used to perform differential abundance analysis, and the ‘depleted index (DI)’ value was used to estimate fungal selection processes from rhizosphere soil to other plant compartment niches (Xiong et al. 2020). Co-occurrence networks were constructed by calculating Spearman’s rank coefficients (r) between OTUs across different plant compartments. We considered a valid relationship to be statistically robust ($r > 0.7$) and statistically significant ($P < 0.01$). Node topology features including node degree, closeness centrality, and transitivity were calculated by using the “igraph” package in R, and the Wilcoxon test was used to evaluate differences. In addition, 10,000 Erdős-Rényi model random networks (Erdos and Rényi, 1960) were constructed for each plant compartment niche. To better understand the community assembly mechanisms controlling the fungal diversity, composition, and structure patterns, the null model-based modified stochastic ratio (MST) was calculated with Bray–Curtis metrics. MST was developed with 50% as the boundary point between more deterministic ($< 50\%$) and more stochastic ($> 50\%$) assembly (Guo et al. 2021). The “niche breadth” was calculated by the approach described by Levins (1968) to quantify habitat specialization.

3 Results

3.1 The effect of plant compartment niches and plant species on fungal community diversity and composition

There was a highly significant plant compartment niche effect on the Shannon diversity and Chao1 richness ($P_{\text{plant compartment niche}} < 0.001$) (Table S2, Fig. 2a, b). The plant species caused a significant on the Chao 1 richness ($P_{\text{plant species}} < 0.05$) (Table S2, Fig. 2b) Figs. 3 and 4. The rhizosphere soil of the three alpine herbs had the maximum number of shared OTUs (381), while the minimum number of OTUs (2) was shared by each plant compartment niche of the three alpine herbs (Fig. 5b). The numbers of unique OTUs gradually decreased along the rhizosphere soil-root endosphere-leaf endosphere, regardless of the plant species (*R. spiciforme*: 239–12–1, *E. wallichii*: 338–57–39, *R. bupleuroides*: 190–17–3) (Fig. 5b). The PCoA (Principal Coordinates Analysis) revealed that there is a clear separation of fungal community among different plant compartment niches (Fig. 5a, Table S4). Moreover, the primary source of the variation in fungal community

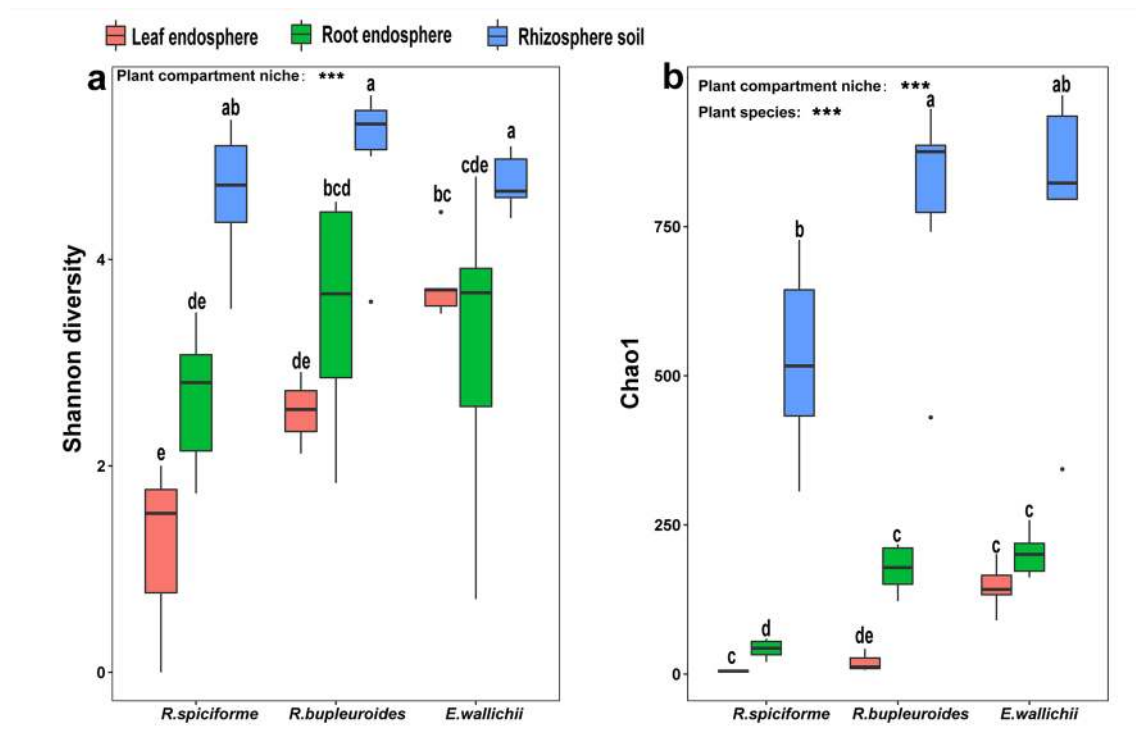


Fig. 2 Alpha diversity of fungal communities in the leaf endosphere, root endosphere, and rhizosphere soil. *R. spiciforme*, *Rheum spiciforme*; *E. wallichii*, *Eriophyton wallichii*; *R. bupleuroides*, *Rhodiola bupleuroides*. Results of the effects of the plant compartment

niche, plant species and their interactions are indicated above panel (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Bars without shared letters indicate significant differences at $P < 0.05$ tested by the Kruskal–Wallis method

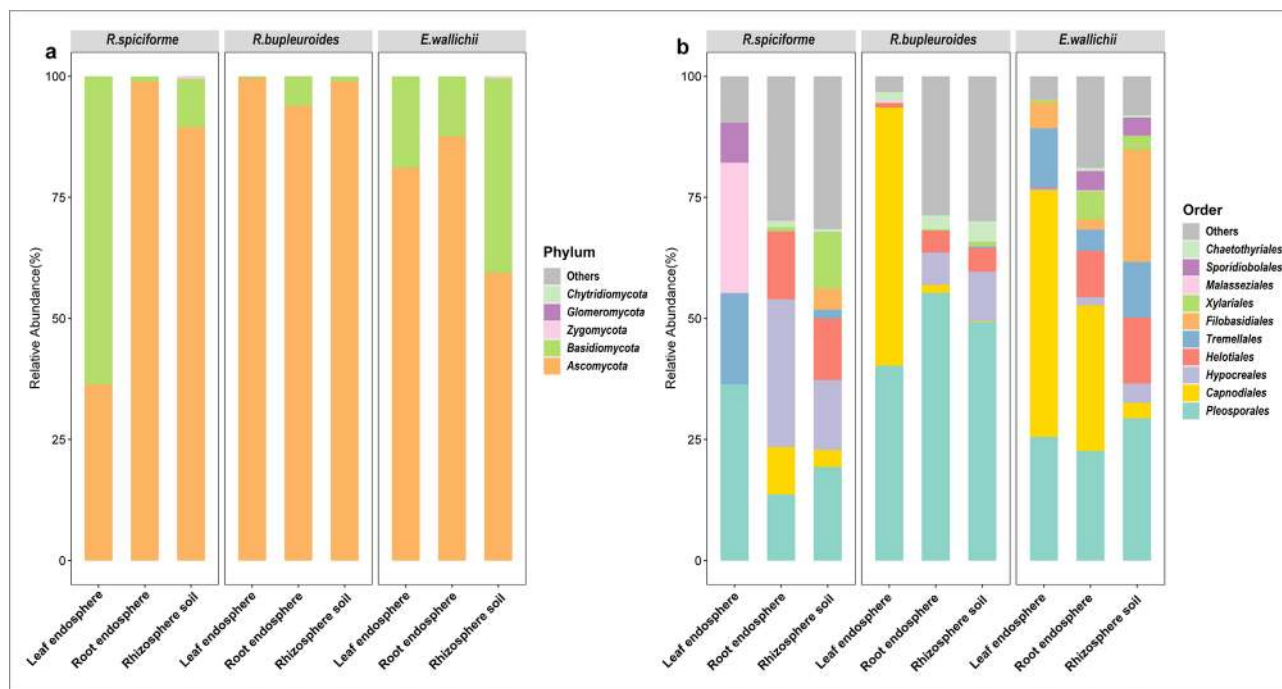


Fig. 3 Taxonomic composition of the fungal communities associated with *R. spiciforme* (*Rheum spiciforme*), *E. wallichii* (*Eriophyton wallichii*) and *R. bupleuroides* (*Rhodiola bupleuroides*) at the phylum and order level, respectively

composition was the plant compartment niche ($R^2 = 0.18$, $P = 0.001$) in comparison with the plant species ($R^2 = 0.11$, $P = 0.001$), and the interaction effect of plant compartment niche and plant species ($R^2 = 0.14$, $P = 0.001$). Based on the SIMPER analysis results, we identified 12 OTUs (contribution to dissimilarity $> 2\%$) that contributed to the variation in fungal community composition (Fig. 6). These 12 OTUs mainly belonged to the members of phylum Ascomycota and Basidiomycota, and explained 40.48% of the total variation in fungal community composition. Both the plant compartment niche and the plant species had a significant effect on the variation in the relative abundance of the Ascomycota ($P < 0.001$), while the variation in the relative abundance of the Basidiomycota was significantly influenced by the plant species (Table S2, Fig. 4a, b). At the order level, the top 8 orders, including Capnodiales, Hypocreales, Helotiales, Tremellales, Filobasidiales, Xylariales, Malasseziales, and Chaetothyriales, were significantly affected by plant compartment niche (Table S3, Fig. 4d, e, f, g, h, i, j, m).

3.2 Differences in fungal diversity, composition, co-occurrence networks, niche breadths, and assembly processes among the rhizosphere soil, root endosphere, and leaf endosphere

The Shannon diversity and Chao1 richness of fungal communities in rhizosphere soil were significantly higher than

those in root endosphere and leaf endosphere ($P < 0.05$) (Fig. 2a, b), and the lowest level of fungal diversity was found in the leaf endosphere. The fungal communities associated with rhizosphere soil, root endosphere and leaf endosphere of the *R. spiciforme*, *E. wallichii* and *R. bupleuroides* were dominated by the phylum Ascomycota and Basidiomycota (Fig. 3a). At the order level, the Pleosporales were commonly found in each plant compartment niche of the three herbs (Fig. 3b). The community composition in leaf endosphere exhibited quite different from rhizosphere soil and root endosphere, especially the *R. spiciforme*. For instance, the Malasseziales and Sporidiobolales exclusively existed in the leaf endosphere of the *R. spiciforme* (Fig. 3b). However, the community composition in rhizosphere soil and root endosphere exhibited more similarities. For example, the rhizosphere soil and root endosphere shared the taxa belonging to the Capnodiales, Hypocreales, Helotiales, and Xylariales (Fig. 3b).

The results showed that the rhizosphere soil network included 1155 nodes and 7698 edges, the root endosphere network consisted of 339 nodes and 6530 edges, and the network of the leaf endosphere only had 103 nodes and 1589 edges (Table 1). Moreover, the values of the average clustering coefficient, average path length, and modularity of each plant compartment niche Erdős-Rényi random network exhibited lower than those of the respective empirical network, indicating that the empirical network was non-random and modular structure (Table 1). In addition, the root

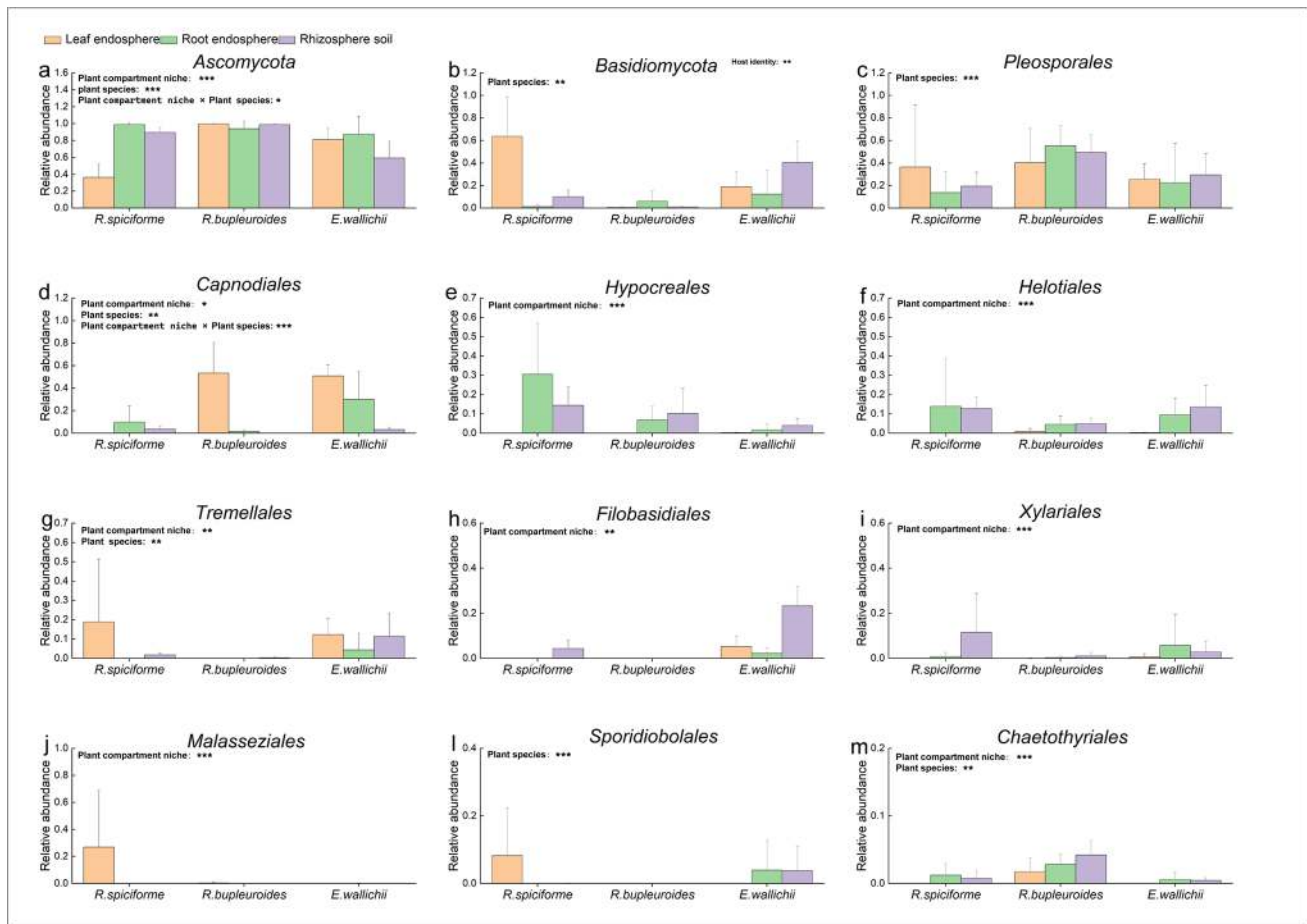


Fig. 4 Mean ($\pm$ SE) relative abundance of main fungal taxonomic groups at the phylum (a, b) and order (c-m) level. Results of the effects of plant compartmental niche, plant species and their inter-

actions are indicated above each panel (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). *R. spiciforme*, *Rheum spiciforme*; *E. wallichii*, *Eriophyton wallichii*; *R. bupleuroides*, *Rhodiola bupleuroides*

endosphere (38.53) and leaf endosphere (30.85) networks have higher average degree values than the rhizosphere soil networks (13.33) (Table 1). It indicated that the network of endophytic fungi is more complex than the rhizosphere. In contrast, the modularity of the rhizosphere soil (0.60) network is higher than that of the root endosphere (0.37) and leaf endosphere (0.37) networks (Table 1). The node degrees, closeness centrality, and eigenvector centrality are significantly larger for the root endosphere and leaf endosphere networks than for the rhizosphere soil network (Fig. S3B), suggesting higher centrality and connectedness in the endophyte networks. The taxonomic composition of the fungal co-occurrence network is similar among the rhizosphere soil, root endosphere, and leaf endosphere, with most nodes belonging to members of the *Ascomycota* and *Basidiomycota* (Fig. S3A).

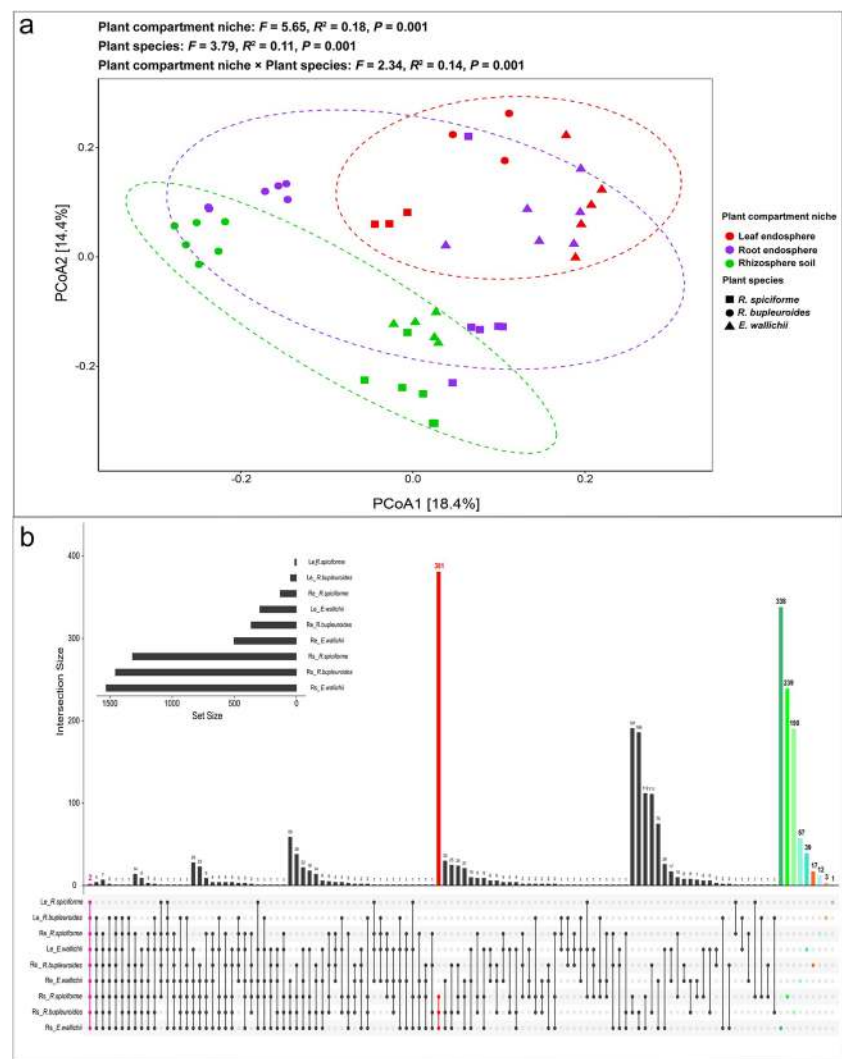
The MST (modified stochastic ratio) calculated for each plant compartment niche was all lower than 50% (Fig. 7a), suggesting that deterministic processes were dominant in structuring the fungal community. Additionally, the

fungal taxa in the rhizosphere soil possessed a wider niche breadth than those in the root and leaf endosphere (Fig. 7b), indicating that the endophytic fungi were specialists (Xu et al. 2020).

3.3 Potential source and enrichment processes of endophytic fungi community

A total of 21.6% of the fungi in the root endosphere originated from the rhizosphere soil (Fig. 8a). The fungal communities associated with the leaf endosphere had 31.8% and 7.9%, respectively, sourced from root endosphere and rhizosphere soil (Fig. 8a). To better understand fungal selection processes from rhizosphere soil to other plant compartment niches, the “Depleted index” (DI) defined by Xiong et al (2020) was used to evaluate the effect degree of each enrichment process. The higher DI value represents a greater depleted effect between root endosphere/leaf endosphere and rhizosphere soil. We found that the highest DI values recorded in the leaf endosphere (Fig. 8c). It is worth noting

Fig. 5 Principal coordinate analysis (PCoA) based on fungal OTUs data (a). Ellipses show 95% confidence intervals. Results of the effects of plant compartment niche, plant species and their interactions are indicated above panel. The Upset diagram shows unique and shared OTUs among different plant compartment niches of *R. spiciforme* (*Rheum spiciforme*), *E. wallichii*, (*Eriophyton wallichii*), and *R. bupleuroides* (*Rhodiola bupleuroides*) (b). Le_*R. spiciforme*, leaf endosphere of the *Rheum spiciforme*; Re_*R. spiciforme*, root endosphere of the *Rheum spiciforme*; Rs_*R. spiciforme*, rhizospheric soil of the *Rheum spiciforme*; Le_*E. wallichii*, leaf endosphere of the *Eriophyton wallichii*; Re_*E. wallichii*, root endosphere of the *Eriophyton wallichii*; Rs_*E. wallichii*, rhizospheric soil of the *Eriophyton wallichii*; Le_*R. bupleuroides*, leaf endosphere of the *Rhodiola bupleuroides*; Re_*R. bupleuroides*, root endosphere of the *Rhodiola bupleuroides*; Rs_*R. bupleuroides*, rhizospheric soil of the *Rhodiola bupleuroides*



that we found that the *Pleosporaceae*, *Davidiellaceae*, and *Chaetomiaceae* were significantly enriched and overlapped in two plant compartment niches (root endosphere and leaf endosphere), while the *Sarcinomyces*, *Tetracladium* and *Rhizoctonia* were significantly filtered (Fig. 8b).

4 Discussion

4.1 Plant compartment niches play a decisive role in shaping the diversity and composition of fungal community

In this study, we found that the effect of plant compartment niche on fungal diversity and composition was significant and the effect is larger in effect size than other effects (i.e., plant species, and plant compartment niches $\times$ plant species). Corresponding to our results, studies on crops (e.g., wheat, barley, and maize) demonstrated that fungal communities

associated with plants were shaped predominantly by plant compartment niches rather than by environmental factors (Xiong et al. 2021). These findings are supported by previous studies showing that different plant compartment niches are colonized by distinct fungal communities (Yu et al. 2018; Gao et al. 2020; Zuo et al. 2021). Further, prior studies on desert shrubs and mangrove forests showed that host species had a greater effect on fungal communities associated with plants (Zuo et al. 2021; Yao et al. 2019). Similarly, we found that the plant species had a significant effect on the diversity and composition of fungal communities associated with alpine herbs. In conclusion, all these findings highlight the decisive role of the host plant in shaping the assembly of microbial communities associated with plants.

The coevolutionary theory framework suggests that plants utilize signal molecules to attract specific microbiota, and subsequently apply selection pressure through their immune systems and provision of specialized nutrients and habitats (Martin et al. 2017; Foster et al. 2017; Cordovez et al.

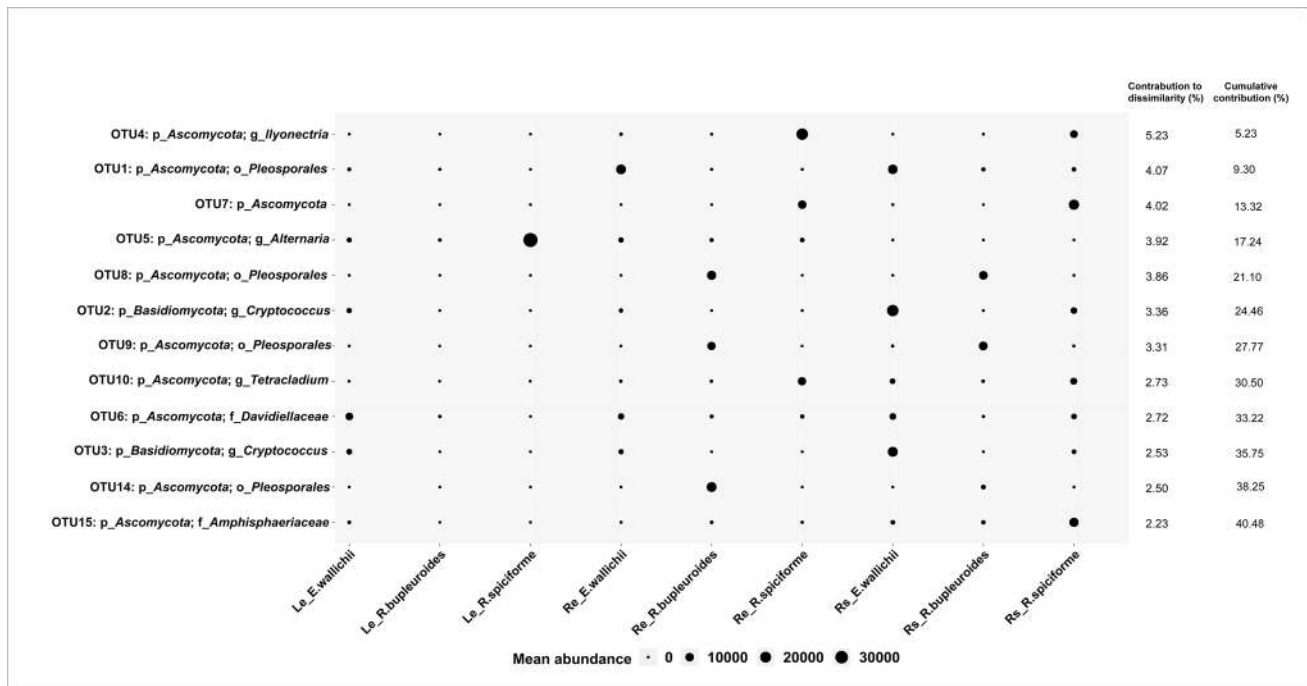


Fig. 6 The relative contribution of 12 representatives OTUs to community composition dissimilarities. The diameters of circles indicate the mean abundances of OTUs. Le_ *R. spiciforme*, leaf endosphere of the *Rheum spiciforme*; Re_ *R. spiciforme*, root endosphere of the *Rheum spiciforme*; Rs_ *R. spiciforme*, rhizospheric soil of the *Rheum spiciforme*; Le_ *E. wallichii*, leaf endosphere of the *Eriophyton wallichii*; Re_ *E. wallichii*, root endosphere of the *Eriophyton wallichii*; Rs_ *E. wallichii*, rhizospheric soil of the *Eriophyton wallichii*; Le_ *R. bupleuroides*, leaf endosphere of the *Rhodiola bupleuroides*; Re_ *R. bupleuroides*, root endosphere of the *Rhodiola bupleuroides*; Rs_ *R. bupleuroides*, rhizospheric soil of the *Rhodiola bupleuroides*

Table 1 Network properties of fungal community in the rhizosphere soil, root endosphere, and leaf endosphere

Network properties	Rhizosphere soil	Root endosphere	Leaf endosphere
Empirical networks			
Edges	7698	6530	1589
Nodes	1155	339	103
Average clustering coefficient	0.57	0.76	0.71
Average path length	6.43	2.19	2.00
Modularity	0.60	0.37	0.13
graph.density	0.01	0.11	0.30
Diameter	18.44	4.71	4.25
Average degree	13.33	38.53	30.85
Random networks			
Average path length	2.98 ± 0.001	1.89 ± 0.0006	1.69 ± 0.00009
Average clustering coefficient	0.01 ± 0.0006	0.11 ± 0.001	0.30 ± 0.003
Modularity	0.23 ± 0.003	0.12 ± 0.003	0.12 ± 0.005

2019). This process enables plants to attract and select beneficial microbiomes (Martin et al. 2017; Foster et al. 2017; Cordovez et al. 2019). Microbes that can identify the signal molecules and colonize specific niches within the compartment are selectively enriched, while others are eliminated through filtration (Hacquard et al. 2015; Müller et al. 2016). The plants growing in the subnival belt are

subjected to harsh environmental stress (e.g., strong annual fluctuations in temperature and precipitation, high-intensity ultraviolet radiation, and most nutrients are in plant-inaccessible forms) (Heer and Körner 2002; Thomas et al. 2014; Given et al. 2020), which will directly or indirectly regulate the plant metabolites. Thus, it is reasonable to assume that the higher compartment niche effect imposed

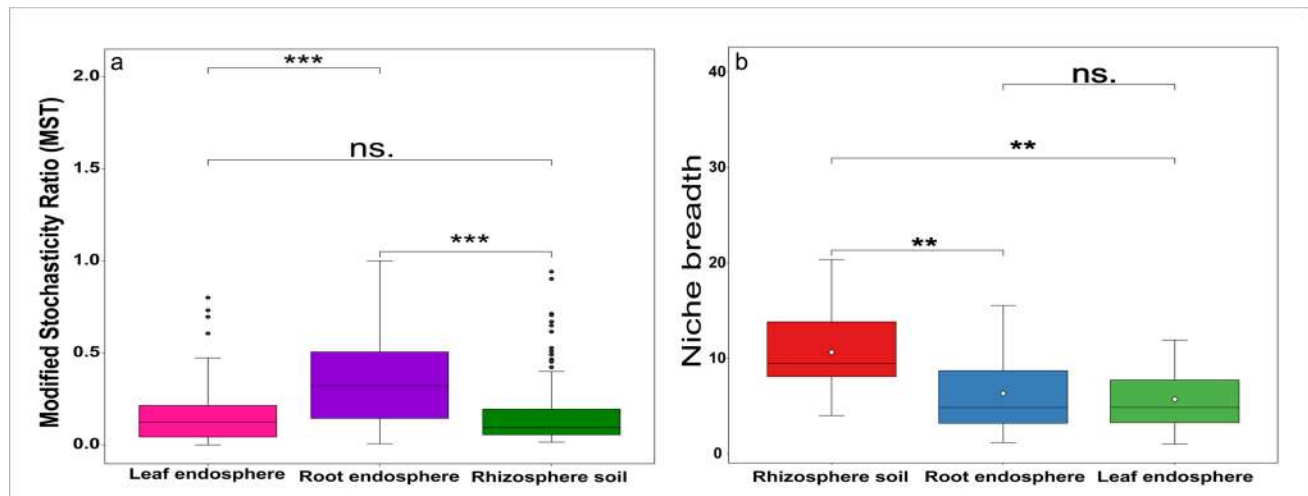


Fig. 7 Delineation of the assembly processes underlying the fungal community assembly associated with rhizosphere soil, root endosphere, and leaf endosphere (a). Comparison of mean habitat niche

breadth for all taxa among rhizosphere soil, root endosphere, and leaf endosphere (b). Asterisks indicate significant differences based on Wilcoxon Test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

by the alpine herbs on their associated fungal community is closely related to the adaptability of the plant to a harsh environment. For example, the fungal community enriched in the rhizosphere can provide mineral nutrients to their host plants, and the fungi inhabited in the plant endosphere can decompose cellulose in senescent leaves and young litters (Vacher et al. 2016). It is somewhat surprising that previous studies on microbial communities associated with the *Agave*, *Cycas panzhihuaensis*, wheat/barley/maize, grass species (*Stipa* spp., *Reaumuria* Linn, *Agropyron cristatum*, *Artemisia sacrorum*), *Pueraria phaseoloides*, and Apple also found that plant compartment niches are the primary driver in the assembly process of microbial communities (Coleman-Derr et al. 2016; Zheng and Gong 2019; Xiong et al. 2020; Zheng et al. 2021; Zhong et al. 2022; Guo et al. 2022; Huang et al. 2023). In comparison with the subnival belt, the sites of these studies are a suitable environment for plant growth. However, the host plant still exhibited a higher selection effect to their associated fungi. Although we still cannot quantify the differences in plant compartment niche effect between alpine herbs in the subnival belt and other plants, these findings clearly suggest that plant compartment niches play an essential role in plant-associated fungal community assembly processes, regardless of growth environment conditions and plant species.

4.2 Diversity, composition, and network of fungal community exhibit plant compartment niche differentiation

We found that the rhizosphere soil exhibits the highest levels of fungal diversity, while the lowest levels are observed in the root endosphere and leaf endosphere. Similar results

were found for the bacterial microbiome of Antarctic vascular plants (*Deschampsia antarctica* and *Colobanthus quitensis*) (Zhang et al. 2020) and the fungal community associated with *Mussaenda kwangtungensis* (Qian et al. 2019). The rhizosphere is an interface of plant and soil, which provides a suitable microhabitat for the growth and propagation of microbial communities (Buée et al. 2009). In comparison, the microbial communities in the root endosphere and leaf endosphere were selected by the plant via intra-tissue nutrients and host immunity responses (Yao et al. 2019, 2020). Thus, plants presumably exert more influence on the endophytic fungi than the epiphytic fungi (Yao et al. 2019), which was supported by the results of Xiong et al. (2020) who demonstrated that host plants exhibited a stronger selection effect on endophytic microbes. We also found that fungal communities associated with the endosphere of root and leaf were subjected to host plant selection pressure. Therefore, the lower diversity of endophytic fungi than that in rhizosphere soil should be a combined result of both the abiotic environmental filtration and the host plant selection. Further, microorganisms that reside in leaf environments are consistently subjected to severe conditions, including exposure to ultraviolet radiation, limited nutrient availability, and significant temperature fluctuations during the diurnal cycle (Remus-Emsermann and Schlechter 2018). The existence of these environmental filters indicates that the diversity of endophytic fungi in leaf habitats is likely to be lower compared with that found in belowground habitats.

We found that the fungal community composition associated with rhizosphere soil, root endosphere, and leaf endosphere had a well-defined separation. Similar results with *Agave*, *Salix*, *Cactaceae*, *Betula*, *kwangtungensis*, and 13 species of subtropical trees were reported in previous

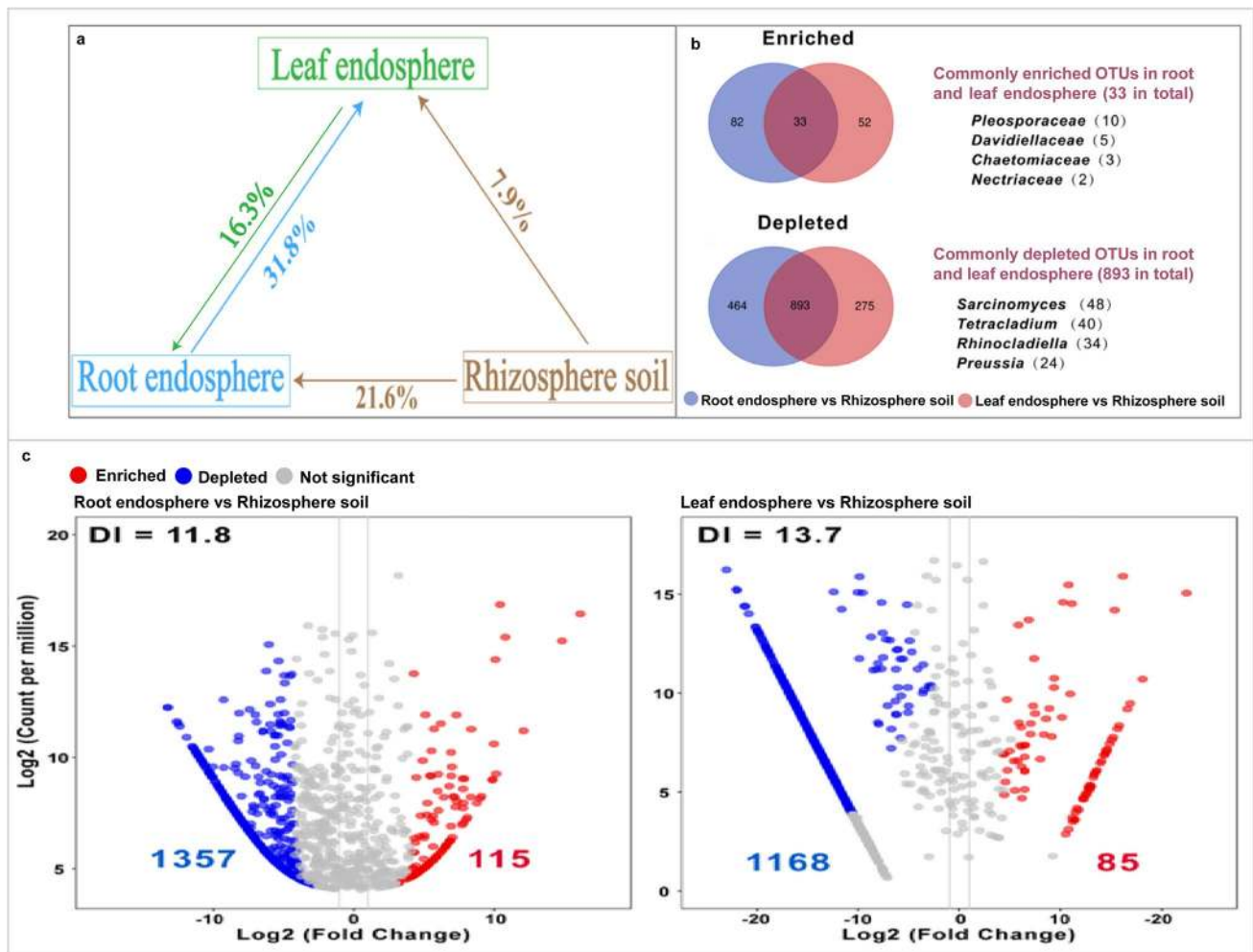


Fig. 8 Source model showing the potential sources of fungal communities associated with rhizosphere soil, root endosphere, and leaf endosphere (a). Venn diagrams showing the shared and specific fungal OTUs in different plant compartment niches within the significantly enriched OTUs and depleted OTUs (b). For these shared differentially enriched OTUs, only the top 4 taxonomies were shown. The volcano plot illustrating the enrichment and depletion patterns of the fungal microbiomes in root endosphere and leaf endosphere compared with

rhizosphere soil (c). Each red point represents an individual enriched OTU, while each blue point represents an individual depleted OTU. The “Depleted index” (DI = The numbers of depleted OTUs / The numbers of enriched OTUs) defined by Xiong et al (2020) was used to understand fungal selection processes from rhizosphere soil to other plant compartment niches. The higher DI value represents a greater depleted effect between root endosphere/leaf endosphere and rhizosphere soil

studies (Coleman-Derr et al. 2016; Tardif et al. 2016; Fonseca-García et al. 2016; Yang et al. 2016, 2022; Qian et al. 2019). This result may be explained by the fact that different plant compartment niche has different structure and exposure to different environmental conditions, which directly or indirectly induce each plant compartment niche to possess unique physicochemical properties (Fitzpatrick et al. 2020), and thus may result in host plant selective recruitment of compartmental niche-compliant microbial communities (Trivedi et al. 2020; Sun et al. 2021). The fungal community in the rhizosphere soil, root endosphere, and leaf endosphere was mainly comprised of *Ascomycota* and *Basidiomycota* taxa. Similar results were found for the fungal community in rhizosphere soil of alpine shrub (*Rhododendron nitidulum*)

(Xie et al. 2023), alpine cushion plant (*Thylacospermum caespitosum*) (Wang et al. 2020), and Vascular Plants in the high Arctic zone (Zhang and Yao 2015). Further, Bertini et al (2022) obtained 132 fungal strains, which mainly belong to the members of *Basidiomycota*, from the leaves of the Antarctic angiosperm *Colobanthus quitensis*. Previous studies on the alpine shrub (*Rhododendrons*) showed that this plant forms specific symbiotic associations with ericoid mycorrhiza from *Ascomycota* and *Basidiomycota* (Wang et al. 2017). The members of *Ascomycota*, such as the fungal endophyte *Phomopsis liquidambari* can enhance plant nitrogen utilization efficiency under low nitrogen conditions (Yang et al. 2014), the fungal endophyte *Fusarium* sp. can improve the plant utilization rate of

insoluble phosphorus (Nieva et al. 2019), and the fungal endophyte *Fusarium tricinctum* and *Alternaria alternata* promote the growth of rice roots and above-ground part by secreting growth hormones (Khan et al. 2015). The root endophytic basidiomycete *Piriformospora indica* has been shown to increase resistance against biotic stress and tolerance to abiotic stress in many plants (Baltruschat et al. 2008). Therefore, whether alpine herbs in the subnival belt can establish a symbiotic association with *Ascomycota* and *Basidiomycota* to ensure nutrient acquisition and health under an oligotrophic environment needs further exploration. Notably, the results of our study showed that the members of the order *Pleosporales* are universally present in each compartment niche of the three alpine herbs, and this taxon explained 13.74% of the total variation in fungal community composition. We also found that *Pleosporaceae* was significantly enriched in the endosphere of root and leaf. These results indicate that *Pleosporales* could colonize a wide range of compartment niches of three alpine herbs and play a crucial role in the composition variation of the fungal community associated with three alpine herbs. Prior sequence-based studies on plant endophytes in high Arctic zones (Zhang and Yao 2015) and arid desert ecosystems (Zuo et al. 2021) also found that the taxon of *Pleosporales* is the most represented order. Moreover, most of the endophytic fungi isolated from desert plants, belong to the order *Pleosporales* (Xie et al. 2017; Hou et al. 2019; Zuo et al. 2020). Taken together, it is reasonable to assume that the members of the *Pleosporales* provide important ecosystem services to the plant in a harsh environment. For example, the dark-septate fungi within the *Pleosporales* may form nutrient bridges between soil and plant (Green et al. 2008). Previous studies showed that the *Hypocreales* and *Helotiales* were the most abundant orders in the rhizosphere soil of the periglacial plant (*Ranunculus glacialis*) (Praeg et al. 2019). Our results showed that the *Hypocreales* and *Helotiales* are mainly distributed in the endosphere of the root and leaf. Such inconsistency may be ascribed to the differences in plant identity and environmental condition, as the climate and host-related factors might shape the fungal communities associated with the plant (Zhang and Yao 2015).

The complicated ecological relationships among microbial taxa can be represented in co-occurrence networks (Zhong et al. 2022). A more complex microbial co-occurrence network exhibited stronger resistance to biotic and abiotic stress through enhanced connections and resource transfer efficiency between members, while a simple network structure is vulnerable to environmental disturbance (Morriën et al. 2017; Yao et al. 2019; Wang et al. 2018). The high modularity of the co-occurrence network indicates a more heterogeneous and disconnected substrate architecture (Qian et al. 2019). In this study, we found that co-occurrence networks of fungal communities associated with

alpine herbs differ significantly between the rhizosphere soil, root endosphere, and leaf endosphere. This finding could have been generated because each plant compartment is a unique ecological niche (Qian et al. 2019). Notably, the co-occurrence networks of the fungal community in the leaf endosphere exhibited higher complex, centrality, and connectedness structures than those in the rhizosphere soil. One possible explanation for this structure characteristic is that the fungal community in the leaf endosphere was highly influenced by external environmental factors. This could have led to an improvement in the effectiveness of exchanging information, energy, and resources among different taxonomic groups, ultimately helping to mitigate the negative impacts of environmental disturbances. However, our result disagrees with the result of Qian et al (2019), who found that the fungal co-occurrence network in the rhizosphere of tropical plants is more complex than that in the phyllosphere. Previous studies demonstrated that within each plant compartment niche, mycobiomes are mainly influenced by plant species (Xiong et al. 2021). The interspecies relationships in fungal communities could be enhanced by abiotic stress (He et al. 2017). Therefore, this inconsistency could be a result of the difference in host plant species and growth environment conditions.

4.3 Potential sources and enrichment process of endophytic fungal community

Investigating the possible sources and filter processes of the fungal community associated with alpine plants in the subnival belt would provide a novel insight into understanding the interaction among soil, plants, and microorganisms. Previous studies demonstrated that soils are a major reservoir for plant-associated microbiomes (Bai et al. 2015; Zarraonaindia et al. 2015; Wagner et al. 2016), but we know little about the potential sources of fungal communities associated with alpine herbs in the subnival belt. Compared with crops (wheat, barley, and maize) (Xiong et al. 2021), we found that the contribution of rhizosphere soil to endophytic fungi in alpine herbs was lower. For example, we found that 21.6% of the fungi in the root endosphere originated from the rhizosphere soil, while at least 65% of fungi in the root endosphere of crops were sourced from soils (Xiong et al. 2021). Seeds are the most direct vehicle that a parent plant might use to transmit microbes to their offspring. Johnston-Monje et al (2021) found members of the *Hypocreales* and *Pleosporales*, such as *Fusarium* and *Alternaria*, are seed-transmitted fungi. We also found that the *Pleosporales* significantly enriched the endosphere of root and leaf. Hence, we suspected that seed-borne fungi may be the dominant source of fungi in alpine herbs in this study. The primary source of fungi in the leaf endosphere is fungi in the root endosphere in this study, indicating that the

fungi in the rhizosphere can be transported to plant above-ground compartments via internal plant tissue transmission (Vandenkoornhuyse et al. 2015; Johnston-Monje et al. 2021). Additionally, our found indicate that host plants exert significant selection pressure on endophytes. For instance, the members of the genus *Tetracladium* and *Rhinoctadiella* were significantly depleted in the endosphere of root and leaf. It is somewhat surprising that Grudzinska-Sterno et al (2016) showed that *Tetracladium* is one of the dominant endophytes of winter wheat, and Sati and Pant (2020) isolated *Tetracladium* species from the root endosphere and found that this taxon can promote plant growth. Yang et al (2013) obtained *Rhinoctadiella* sp. from the inner tissue of *Narcissus* sp. In theory, the fungi successfully colonized in plant inner tissue should possess two specific traits 1) high activity of cell-wall-degrading enzymes and 2) breaking through the innate immune system of the host plant (Patra et al. 2023). Hence, it is reasonable to assume that the alpine herbs examined in this study exhibit a selective barrier against some fungal taxa entering their internal environment, which could be attributed to their immune response. The capacity of the plant to be sensitive to some species could be one of the ways it adapts to harsh environments.

5 Conclusion

The conclusion of this study could be summarized as follows: (1) at the plant level, the variation in fungal community diversity and composition of the alpine herbs (*R. spiciforme*, *E. wallichii* and *R. bupleuroides*) in the subnival belt of Qiangyong glacier are predominantly shaped by plant compartment niche rather than plant species; (2) the fungal diversity, composition, niche breadth, and topological properties of the co-occurrence network varied significantly across different plant compartment niches. Notably, endophytic fungi showed lower taxonomic diversity, niche breadth, and a more complex and connected network, than the rhizosphere soil mycobiome did; (3) the endophytic fungi could select a part of the taxa from the nearby seed bank, with root endophytes as the main potential sources of leaf endophytes; and (4) the endophytic fungi subjected to host selection pressure, especially for leaf endophytes, and some specific microbial taxa like *Pleosporaceae*, *Davidiellaceae*, and *Chaetomiaceae* are enriched in the endosphere of root and leaf. These findings significantly advance the current understanding of the fungal community assembly and ecological interaction along a soil–plant continuum in the subnival belt, and highlight the importance of the host selection effect.

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Author contributions Wangchen Sonam and Yongqin Liu conceptualized and planned the study. Wangchen Sonam performed the fieldwork, analyzed the data, and prepared the manuscript. Yongqin Liu reversed the manuscript. All authors were involved in revising the manuscript critically.

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Data Availability The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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