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Evolutionary history and environmental filtering jointly structure ectomycorrhizal fungal communities across Pinaceae forests on the Qinghai-Tibetan Plateau

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Abstract

Plant–microbe interactions are fundamental to biodiversity maintenance and ecosystem functioning, and their assembly is shaped by a complex interplay of ecological and evolutionary processes. However, how these forces jointly influence ectomycorrhizal (EcM) fungal communities, especially those dominant in subalpine forests, remains poorly understood. To address this, we investigated EcM fungal communities associated with 11 species of Pinaceae (*Abies*, *Picea* and *Pinus*) across 195 monodominant stands in the subalpine forests of the Qinghai-Tibetan Plateau. We found that all pine species are consistently associated with a broad phylogenetic range of EcM fungal lineages, and that pine–EcM association networks exhibit low connectance, indicating low partner specificity. Variation in fungal community structure was significantly influenced by host identity, environmental factors and spatial distribution, but not by host phylogenetic relatedness. Notably, fungal taxa from three dominant lineages (*Sebacina*, *Russula* and *Inocybe*) were clustered phylogenetically with globally distributed Pinaceae-associated taxa, pointing to evolutionarily conserved symbiotic associations across biogeographic regions. Together, these results indicate that EcM fungal communities in subalpine Pinaceae forests are assembled through a combination of evolutionary conservatism and environmental filtering. The persistent association with key EcM fungi across Pinaceae species underscores their essential role in supporting tree physiology and forest ecosystem stability in subalpine environments.

Keywords: ectomycorrhiza, Pinaceae, community assembly, phylogeny, Qinghai-Tibetan Plateau

进化历史和环境过滤共同塑造青藏高原松科植物外生菌根真菌群落结构

摘要：植物-微生物互作在生物多样性与生态系统功能维持中扮演重要角色。然而，学术界仍不清楚环境与进化因素如何影响外生菌根(EcM)真菌群落组成与多样性。本研究以青藏高原亚高山森林11种冷杉属(*Abies*)、云杉属(*Picea*)和松属(*Pinus*)的松科植物为对象，系统分析了195个单优势种林分中的EcM真菌群落结构及其影响因素。结果表明：1)所有松科树种的EcM真菌均具有较高的系统发育多样性，且宿主植物与真菌间的共生网络连接度较低，表明该共生关系的专一性较弱。2)EcM真菌群落结构变异主要受宿主物种特性、环境因子和地理分布影响，而与宿主系统发育距离的相关性不明显。3)该区域的3大优势EcM真菌类群，即蜡壳菌属(*Sebacina*)、红菇属(*Russula*)和丝盖伞属(*Inocybe*)，在全球松科EcM真菌的系统发育树中并未形成独立分支，预示着松科植物与这些核心真菌的共生关系在不同生物地理区域具有进化保守性。上述研究结果表明，青藏高原亚高山森林中松科植物EcM真菌群落构建是进化发育保守性与环境过滤共同作用的结果，关键EcM真菌类群在维持松科树木生理过程及生态系统稳定性中起到核心作用。

关键词：外生菌根真菌，松科(Pinaceae)，群落构建，系统发育关系，青藏高原

INTRODUCTION

What governs the assembly of microbial communities is a fundamental question in microbial ecology, with profound relevance for understanding biodiversity maintenance and ecosystem functioning (Martiny *et al.* 2006; Stegen *et al.* 2013). While environmental filtering has traditionally been viewed as the major driver of microbial assembly (Baas-Becking's 'everything is everywhere, but the environment selects') (De Wit and Bouvier 2006), this paradigm poorly explains host-associated microbes, whose distributions are often constrained by host presence rather than abiotic conditions alone (Fontaine *et al.* 2011; Thompson 1999). In symbiotic associations, evolutionary history can rival or even outweigh ecological pressures in structuring microbial diversity and community composition (Hanson *et al.* 2012; Maherali and Klironomos 2007; Peay *et al.* 2016; Rezende *et al.* 2007). This is exemplified by phylosymbiosis, where microbial communities mirror host phylogenies, implying co-evolutionary processes in symbiotic relationships (Brooks *et al.* 2016). As such, the diversity and specificity of host–microbe interactions are increasingly understood as outcomes of both ecological and evolutionary processes acting in concert (Rezende *et al.* 2007; Thompson 2005). Disentangling these dual forces is key to understanding how host–microbe associations arise, persist and evolve across ecosystems, which is essential for predicting and preserving ecosystem function and stability in changing environments.

Mycorrhizal symbioses are among the most widespread and evolutionarily ancient forms of host–microbe interactions (Brundrett and Tedersoo 2018; Strullu-Derrien *et al.* 2018), linking plant roots with diverse fungal partners in a mutualistic exchange of carbon and soil nutrients (Smith and Read 2008). As one of the major mycorrhizal types, ectomycorrhizal (EcM) symbiosis underpins the functioning of many forest ecosystems in temperate, boreal and (sub-)alpine biomes, owing to their widespread partnerships with ecologically dominant woody trees (Brundrett and Tedersoo 2018; van der Heijden *et al.* 2015). EcM associations have arisen repeatedly and independently across both plant and fungal lineages (Hibbett *et al.* 2000; Hoeksema *et al.* 2018; Martin *et al.* 2016), a pattern that may have contributed to the pronounced lineage-specific variation in partner specificity observed in nature (Bruns *et al.* 2002; Molina and Horton 2015). Many EcM trees (e.g. *Pinus*, *Betula*, *Quercus*, *Eucalyptus*, *Salix*, *Populus*) associate with a broad diversity of fungal partners, which themselves often display wide host compatibility (Ishida *et al.* 2007; Tedersoo *et al.* 2013, 2024). In contrast, some EcM associations exhibit strikingly high specificity, exemplified by the Pinaceae–*Suillus* association, where certain *Suillus* strains depend exclusively on pine hosts (e.g. *Pinus*, *Larix*) for establishment and reproduction (Bogar *et al.* 2019; Liao *et al.* 2016; Lofgren *et al.* 2018). This generalist-to-specialist spectrum makes EcM symbioses an ideal system for exploring the evolutionary and ecological forces that shape variation in mutualistic interactions.

The relative roles of evolutionary constraints versus environmental filtering in shaping microbial community composition can be examined by integrating phylogenetic approaches with community ecology (Cavender-Bares *et al.* 2009; Webb *et al.* 2002). In EcM symbioses, host phylogeny often predicts fungal community composition across distantly related plant families (Bruns *et al.* 2002; Lofgren *et al.* 2018; Tedersoo *et al.* 2012), with evolutionary constraints sometimes rivaling or even surpassing ecological factors (Ishida *et al.* 2007; Tedersoo *et al.* 2024; Wang *et al.* 2019a, 2019b). However, evidence for such phylogenetic effects at finer taxonomic scales (genus/species) remains scarce, with only a few documented cases in *Alnus* (Põlme *et al.* 2013), Salicaceae (Tedersoo *et al.* 2013), Fagaceae (Wu *et al.* 2018) and Betulaceae (Wang *et al.* 2019a, 2019b). Current limitations stem from study designs that either compare distantly related hosts at single sites or focus on geographically isolated lineages, making it challenging to disentangle genuine phylogenetic signals from confounding factors such as host traits or spatial variation (Yang *et al.* 2023). Targeted sampling of closely related, co-occurring species within the same family across shared environmental gradients would thereby enable the separation of host phylogenetic effects from spatial and ecological influences.

The Pinaceae family, the oldest EcM gymnosperm lineage, shows remarkable ecological adaptability across temperate, boreal and subalpine biomes in the Northern Hemisphere (Ran *et al.* 2018). While mid-latitude mountains have served as evolutionary museums for pine diversity (Jin *et al.* 2021), studies of pine-associated EcM communities have focused mainly on temperate and boreal forests (Ishida *et al.* 2007; Jonsson *et al.* 1999; Koizumi and Nara 2020; Miyamoto *et al.* 2022; Tedersoo *et al.* 2024), leaving pine-dominated subalpine forests at mid-altitudes largely unexplored. The Qinghai-Tibetan Plateau—the world's highest and largest plateau—hosts multiple Pinaceae species forming extensive monodominant stands along subalpine elevational gradients (ca. 2000–4500 m) (Jin *et al.* 2021). Its extreme climates, sharp environmental gradients and diverse pine trees (Kou *et al.* 2024) provide an unparalleled natural laboratory to study how evolutionary and ecological forces shape pine–EcM associations. In these environments, EcM associations represent a key adaptation strategy for pines to overcome nitrogen limitation and cold stress (Ding *et al.* 2023; Guo *et al.* 2021; Kou *et al.* 2024). Moreover, unlike mixed forests with few co-occurring pine species (Adamo *et al.* 2021; Guo *et al.* 2022; Yoshida *et al.* 2014), the Plateau's mosaic of locally segregated pine species (monodominance) within a shared regional setting offers a unique opportunity to disentangle host versus environmental controls on EcM community assembly. Although elevation-driven temperature variation is a known driver of EcM assemblages here (Kou *et al.* 2024), the interplay between environmental factors, spatial distribution and host phylogeny remains poorly understood. Addressing these knowledge gaps is particularly urgent for the Qinghai-Tibetan Plateau, which functions as a critical ecological barrier in Asia that regulates regional and global climate, sustains unique subalpine biodiversity, and is highly vulnerable to rapid climate change (Latif *et al.* 2019; Lin *et al.* 2023; Yao *et al.* 2012).

In this study, we investigated EcM associations of Pinaceae trees in Qinghai-Tibetan subalpine forests to examine how host effect (i.e. species identity, phylogenetic relationships), environmental factors and spatial distribution shape the diversity, specificity and community structure of EcM fungal communities. Our field sampling encompassed the full elevational range of subalpine pine forests on the Plateau, sampling 195 monodominant stands across 11 species from three ecologically dominant Pinaceae genera (*Abies*, *Picea* and *Pinus*). Using high-throughput Illumina MiSeq sequencing of root-associated EcM fungi and integrating phylogenetic approaches with microbial community analysis, the ultimate goal of this study was to disentangle the interacting influences of evolutionary history and environmental filtering on EcM community assembly within subalpine pine forests. Specifically, we addressed two central questions: (i) How do fungal diversity and specificity vary across pine species? and (ii) What are the relative influences of host identity and phylogeny, spatial proximity and environmental factors on pine–EcM community assembly?

MATERIALS AND METHODS

Study site and sampling

The subalpine coniferous forests of the Qinghai-Tibetan Plateau are dominated by Pinaceae trees from three genera: *Pinus*, *Picea* and *Abies*. These monodominant forests span a broad altitudinal range (2000–4500 m). For this study, we selected 11 pine species, including five *Abies* species (*A. ernesti*, *A. fargesii*, *A. forrestii*, *A. georgei* var. *smithii* and *A. squamata*), three *Pinus* species (*P. armandii*, *P. densata*

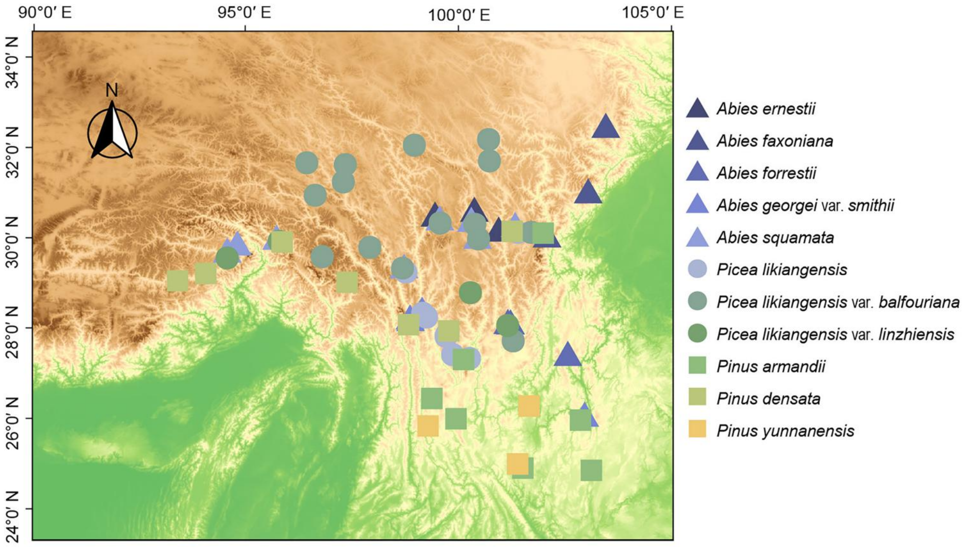

Figure 1: Distribution of sampling sites for the 11 studied Pinaceae species within the alpine forests of the Qinghai-Tibetan Plateau. Each species is represented by a unique color and symbol. The topographic map was retrieved from the '*elevatr*' R package (Hollister et al. 2025).

and *P. yunnanensis*) and three *Picea* species/subspecies (*P. likiangensis*, *P. likiangensis* var. *balfouriana* and *P. likiangensis* var. *linzhiensis*). To ensure species-specific root sampling, all fieldwork was conducted exclusively in monodominant forests of the respective species. A total of 65 sampling sites were established across the primary distribution ranges of these species (25°23′–32°19′ N, 93°23′–103°28′ E, Fig. 1).

At each site, three plots (30 m × 40 m) were established, spaced 50 m apart. Within each plot, five healthy, similarly sized target trees (with comparable diameters at breast height and at least 10 m apart) were selected. Roots were collected using the root-tracing method and the soil block method (Guo *et al.* 2008), ensuring that all sampled roots were correctly matched to the target individual (Ding *et al.* 2023). For each tree individual, intact roots with at least five branching orders were excavated from five soil blocks, ensuring consistency in root architecture across species. To reduce variability, we subsampled only the first two order roots (Pregitzer 2002) and pooled root tips from all five trees within each plot. While pooling may limit the resolution of individual-level variation, it offers a more representative profile of plot-specific EcM communities under shared habitat conditions. In parallel, five soil cores (0–20 cm depth) were randomly collected per plot using a soil auger (5 cm diameter × 20 cm length), following removal of surface litter. These soil cores were thoroughly mixed to produce one composite sample per plot, minimizing spatial heterogeneity. In total, 195 root and soil samples from the 11 selected Pinaceae species were collected across all 65 sites (Supplementary Tables S1 and S2). Once collected, all samples were immediately placed on ice, transported to the laboratory and stored in a refrigerator until further analysis.

Fungal ITS2 metabarcoding

After thorough rinsing with water and surface sterilization with bleach and ethanol, root tips were freeze-dried (using a P4K-S lyophilizer with a vacuum pump, Germany) and ground into a fine powder using a ball mill (Retsch, Germany). DNA was extracted from 50 mg of homogenized powder using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA). For PCR amplification, the fungal universal primer pair fITS7 and ITS4 (Ihrmark *et al.* 2012) was used. To ensure comprehensive detection of fungal diversity, triplicate PCR amplifications were performed for each DNA sample, and the resulting amplicons were pooled. The merged amplicons were then purified and indexed using the TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA). All samples were pooled in equimolar concentrations and sequenced on the Illumina MiSeq platform to generate 250-bp paired-end reads. Raw sequence reads are available under NCBI SRA BioProject accession PRJNA1002620.

After demultiplexing, raw sequencing reads were analyzed following the USEARCH v11 pipeline (Edgar *et al.* 2011) for fungal ITS amplicon analysis. Paired reads of each sample were merged and primers were trimmed using CUTADAPT 1.0 (Martin 2011); Low-quality sequences were filtered out with an error rate >1.0 (Edgar and Flyvbjerg 2015); Fungal operational taxonomic units (OTUs) were clustered at a 97% similarity threshold, and chimeric sequences were identified and removed using the UCHIME *de novo* algorithm (Edgar *et al.* 2011); Global singletons were excluded to improve diversity estimation accuracy (Waud *et al.* 2014); Taxonomic assignment of OTUs was performed using USEARCH against the UNITE reference database (https://unite.ut.ee/repository.php; Version 10.0 released on 19 February 2025). Fungal functional guilds were annotated using the FungalTraits database (Põlme *et al.* 2021). The quality-filtered sequencing data generated a total of 3434 OTUs for 195 root samples, of which 932 OTUs (45% of all filtered sequences) were assigned to the guild of EcM fungi belonging to 37 fungal families (Supplementary Tables S3 and S4). To ensure data quality, we excluded nine samples with fewer than 1000 EcM sequencing reads from subsequent analyses due to uneven read distribution across samples. Prior to fungal community analyses, the association matrix of pine trees and EcM OTUs based on absolute read abundances was normalized to relative abundances in R 4.4.3.

Environmental data compiling

Environmental data for each site included a comprehensive set of climatic and edaphic variables analyzed by Kou *et al.* (2024). Climatic variables included mean annual temperature (MAT) and mean annual precipitation (MAP). Using coordinates of the sampling sites in the ‘*rgbif*’ R packages (v3.8.1) (Chamberlain *et al.* 2025), MAT and MAP were retrieved from the WorldClim database (http://www.worldclim.org) with a spatial resolution of 2.5 arc-minutes. Homologized soil samples were used to measure edaphic variables, including pH, total carbon (TC), dissolved organic carbon (DOC), total nitrogen (TN), inorganic nitrogen (IN), total dissolved nitrogen (TDN), total phosphorus (TP) and available phosphorus (AP). The detailed procedures of soil measurements were previously described by Ding *et al.* (2023) and Kou *et al.* (2024). Briefly, soil pH was measured using a glass electrode; TC and TN were determined using a combustion-based elemental analyzer (Vario Macro cube, Elementar, Hanau, Germany); DOC and TDN were extracted by mixing 0.5 g of fresh soil with 50 mL of distilled water and measured using a TOC/TN analyzer (MultiN/C 2100, Analytik Jena AG, Germany); TP was analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-OES; Optima 5300 DV, Perkin Elmer, USA) after digestion with concentrated H_2SO_4-$HClO_4$; IN was measured colorimetrically with an AutoAnalyser III (SEAL Analytical, Germany) after extraction with 2 mol L^{-1} KCl; AP concentration was determined using the molybdenum-blue colorimetric method following extraction with 0.5 mol L^{-1} $NaHCO_3$.

Data analysis

Plant and fungal phylogeny

The phylogenetic relationships among 11 Pinaceae species were derived from the angiosperm megatree, which incorporates the comprehensive seed plant phylogeny published by Zanne *et al.* (2014). The three closely related species/subspecies of *Picea* were treated as polytomy because the reference phylogeny resolves relationships only at the species level. For EcM fungi, a maximum likelihood (ML) phylogeny was inferred using RAxML-NG v1.1.0 (Kozlov *et al.* 2019; via the CIPRES Science Gateway). Sequence alignment was performed with MAFFT v7 (Katoh and Standley 2013), and Mucoromycotina OTUs were designated as the outgroup. To constrain higher-level taxonomic relationships (phylum, order and family), a backbone tree was incorporated into the phylogenetic reconstruction. For relative time scaling, branch lengths of the fungal ML tree were converted to relative time units using the ‘*compute.brlen*’ function of the ‘*ape*’ R package (v5.8.1). This approach rescales the tree so that branch lengths represent relative divergence times rather than substitutions, thereby generating a time-calibrated fungal phylogeny. The resulting phylogeny is presented in Supplementary Table S5.

Fungal diversity

To assess differences in EcM fungal α-diversity across host plant species and genera, we calculated two complementary diversity metrics for each root sample: (i) species richness (SR; observed OTU count) and (ii) phylogenetic diversity (PD; Faith 1992). Faith's PD was computed using the time-calibrated fungal phylogeny to incorporate evolutionary relationships among OTUs. We employed Kruskal-Wallis rank-sum tests (non-parametric ANOVA equivalents) to evaluate significant differences in median SR and PD values among plant species/subspecies (11 Pinaceae taxa) and genera (*Abies*, *Pinus*, *Picea*). For significant tests ($P < 0.05$), we conducted *post hoc* comparisons using Dunn's tests.

Partner specificity

To quantify partner specificity at the species level, we employed normalized degree (ND) centrality to describe how specialized or generalized a plant or fungal species is within a bipartite plant–fungal network (Martín González *et al.* 2010). ND is calculated as $ND = k/K$, where k represents the observed number of unique interaction partners for a given species, and K denotes the total number of potential partners in the opposing trophic level (i.e. all fungal species for a given plant host, or all plant species for a given fungal symbiont; Gomes *et al.* 2022). This standardized metric ranges from 0 (high specificity; specialist) to 1 (low specificity; generalist), with intermediate values reflecting facultative specificity (preferential but non-exclusive partnerships). ND values were computed with the '*ND*' function of the '*bipartite*' R package (v2.18) (Dormann *et al.* 2008).

Fungal community structure

All fungal community analyses described herein were performed using the '*vegan*' R package (v2.6.10) (Oksanen *et al.* 2025), unless noted otherwise. To assess whether and to what extent variation in fungal community structure is explained by host plant effect (species identity, genus affiliation and phylogenetic relatedness), spatial factors and environmental conditions (climatic and edaphic factors) (β-diversity), we calculated the Bray–Curtis dissimilarity metric (Bray and Curtis 1957) based on the relative abundances of EcM OTUs. To account for spatial structure, we performed a Principal Coordinates of Neighbourhood Matrices (PCNM) analysis on the geographic coordinates (latitude and longitude) of the sampling sites. From this analysis, we retained only the eigenvectors associated with positive eigenvalues for use as spatial predictors in subsequent models. For environmental variables, the compiled climatic and edaphic factors were subjected to forward model selection using the '*ordiR2step*' function to identify significant predictors (MAP, MAT, TN, IN, TC, pH, AP, TP), which were subsequently used in community analyses to assess environmental effects (Supplementary Table S2).

After verifying variance homogeneity using the '*betadisper*' function, we performed a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) using the '*adonis2*' function (9999 permutations) to test the significance of each factor as well as their combined effects. Non-metric multidimensional scaling (NMDS) was used to visualize the variation in fungal community structure among plant genera. Using the '*varpart*' function for variation partitioning, we assessed the unique and shared contributions of environmental conditions and host plant effects to the variation in fungal community. Host plant effects were represented by a matrix of dummy variables corresponding to plant species identity.

To further test whether the overall phylogenetic relatedness of Pinaceae species influences their fungal community composition, we computed Mantel tests (9999 permutations) between the Pinaceae phylogenetic distance metric and the fungal Bray-Curtis dissimilarity metric. The Pinaceae phylogenetic distance metric was calculated using the '*cophenetic.phylo*' function and input into Mantel tests.

Phylogenetic analyses of global Pinaceae–EcM associations

Given the observed dominance of three EcM genera (i.e. *Sebacina*, *Russula* and *Inocybe*) across all studied Pinaceae species, we investigated whether these dominant fungal taxa exhibit phylogenetic clustering or divergence relative to populations from other biogeographic regions. To achieve this, we conducted a comprehensive phylogenetic analysis using both our dataset and globally distributed sequences. The global fungal ITS sequences of the three EcM genera were retrieved from the NCBI GenBank database following stringent quality criteria, including: explicit host association with *Picea*, *Abies* or *Pinus* species; clearly documented geographic origin; exclusion of cultivated or experimental specimens (e.g. from nurseries or pot studies); and restriction to forest ecosystems. The resulting dataset comprises sequences from diverse biogeographic regions across Europe, North America and Asia (Supplementary Table S6).

To determine whether fungal OTUs from pines in this study form phylogenetically distinct clusters, we performed phylogenetic analyses for each of the three focal genera (*Sebacina*, *Russula* and *Inocybe*) against a background of globally sourced reference sequences. Sequence alignment was conducted using MAFFT v7 (Katoh and Standley 2013) implemented in Geneious Prime platform (https://www.geneious.com/), followed by ML phylogenetic reconstruction with RAxML-NG (Kozlov *et al.* 2019) using 1000 bootstrap replicates. For phylogenetic inference, we used *Sebacina* sequences as the outgroup for *Russula* and *Inocybe* analyses, and vice versa. The resulting phylogenies were visualized and annotated using the '*ggtree*' R package (v3.14.0) (Yu *et al.* 2017), enabling interpretation of phylogenetic relationships between Qinghai-Tibetan and global fungal lineages.

RESULTS

Qinghai-Tibetan Pinaceae trees associated with a diverse array of EcM fungi

Across 195 root samples from 11 Pinaceae species, we identified 932 EcM OTUs, which represented a diverse taxonomic assemblage comprising 72 genera (36 families) from Basidiomycota and Ascomycota, as well as one genus from the order Endogonales of Mucoromycotina (Supplementary Table S3). Community composition was heavily skewed, with seven fungal genera collectively accounting for 73.2% of total EcM reads: *Sebacina* (20.6%), *Russula* (14.5%), *Inocybe* (9.3%), *Tomentella* (8.1%), *Hygrophorus* (7.9%), *Piloderma* (6.9%) and *Amphinema* (5.9%). Seven additional genera (*Amanita*, *Clavulina*, *Lactarius*, *Cortinarius*, *Suillus*, *Otidea* and *Pseudotomentella*) showed low abundances (1%–5% each), while the remaining 58 genera each represented <1% of reads. Notably, those high-abundant EcM genera were ubiquitously distributed across all plant species, with their relative abundances varying slightly between species (Fig. 2a). As a result, plant species and genera showed no significant differences in SR and PD of fungal communities (Fig. 2b, c).

Low specificity prevails in Pinaceae–EcM associations

Using the pine–EcM association matrix (Supplementary Table S4), we assessed the degree of partner specificity of pine species, as well as EcM OTUs. ND values indicated similarly moderate to low specificity across Pinaceae species (ND ~ 0.3, Fig. 3a), reflecting limited fungal partner selectivity. The most abundant fungal OTUs showed extremely low specificity (ND ~ 1, Fig. 3b), demonstrating broad host compatibility. Overall, Pinaceae–EcM networks exhibited high connectivity, involving phylogenetically diverse fungal taxa across multiple EcM lineages (Fig. 3c).

Host species identity and environment rather than host phylogeny shape EcM community structure

Our dispersion test showed no overdispersion among plant species ($P = 0.072$, pseudo-$F = 1.754$), excluding the influence of varying sample sizes on within-species variation. PERMANOVA analyses showed that host plant ($R^2 = 0.227$, pseudo-$F = 5.148$, $P < 0.001$), environmental factors ($R^2 = 0.199$, pseudo-$F = 3.590$, $P < 0.001$) and spatial distribution ($R^2 = 0.200$, pseudo-$F = 15.213$, $P < 0.001$) each significantly influenced EcM community structure, collectively explaining 43.8% of the total variation (Fig. 4a, b). Variation partitioning further confirmed these effects, attributing unique contributions of 4.7% to host plant identity, 2.7% to environmental factors and 3.2% to spatial effects (Fig. 4c).

A dbRDA model identified significant environmental variables (MAP, MAT, TN, IN, TC, pH, AP and TP) that together explained >13.1% of EcM community variation (Fig. 4d). Despite the strong host effect represented by plant species identity, Mantel tests detected no significant correlation between Pinaceae phylogenetic distance and EcM community dissimilarity (Pearson's $r = 0.018$, $P = 0.289$, Fig. 4e), demonstrating the lack of host phylogenetic signal in EcM assembly within Pinaceae.

Commonly detected EcM lineages exhibit globally widespread associations with pine trees

Given the consistent dominance of *Sebacina*, *Russula* and *Inocybe* across Pinaceae hosts on the Qinghai-Tibetan Plateau, we examined whether the observed OTUs from these genera were phylogenetically related to Pinaceae-associated sequences reported globally. Phylogenetic analyses showed that these OTUs clustered with conspecifics from diverse geographic regions, including Europe, North America and Asia (Fig. 5). These findings suggest a cosmopolitan distribution of these EcM groups with pine trees.

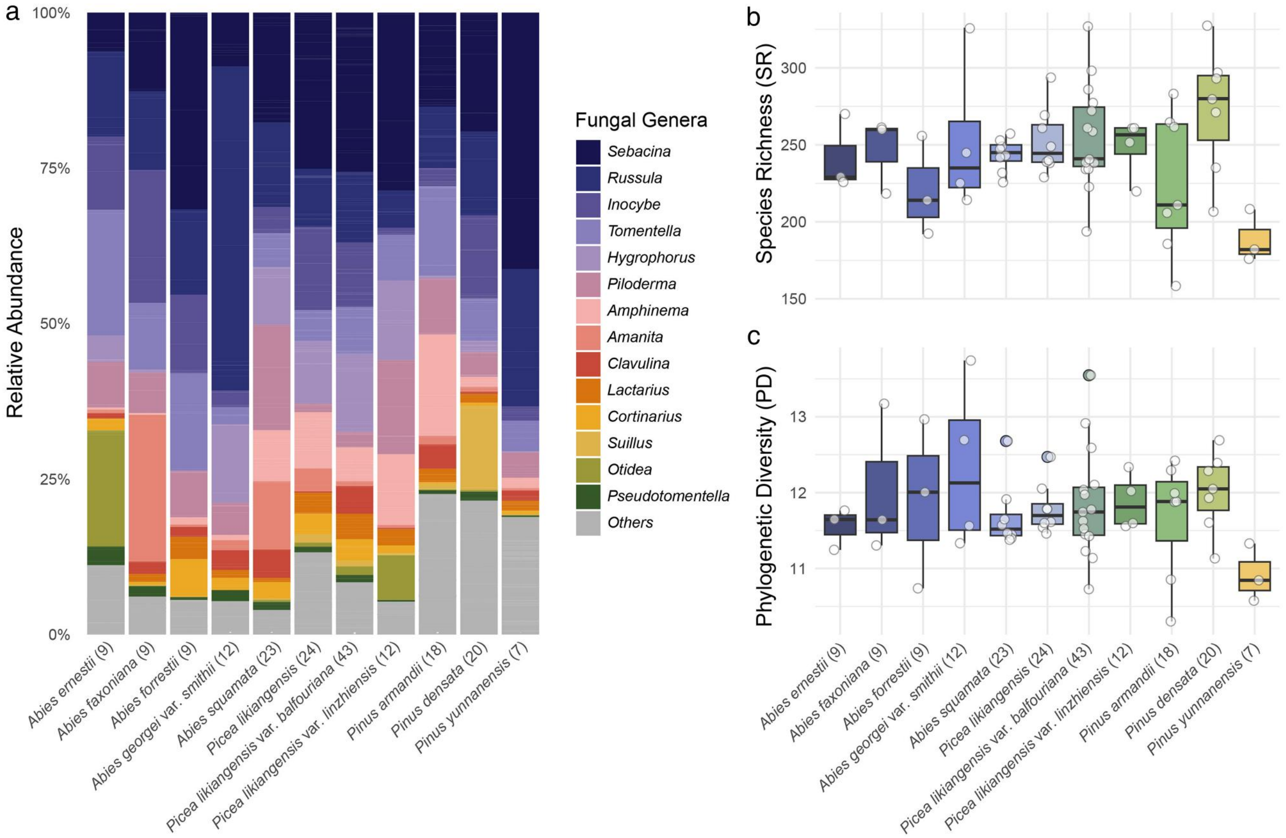

Figure 2: Ectomycorrhizal (EcM) fungal composition and diversity of Pinaceae trees. (a) Relative reads abundance of major fungal genera; ‘Others’ represents genera with low read abundances (in total <1% of all reads) (b) Species richness (SR); (c) Phylogenetic diversity (Faith’s PD).

DISCUSSION

Broad but selective Pinaceae–EcM associations in Qinghai-Tibetan subalpine forests

Our multisite analysis across 11 Pinaceae species on the Qinghai-Tibetan Plateau reveals that pine trees consistently associate with phylogenetically diverse EcM fungal communities, displaying high SR and PD (Fig. 2). These patterns match or exceed those reported for pine–EcM communities in other comparable regional studies (Adamo *et al.* 2021; Koizumi and Nara 2020; Yang *et al.* 2019) and are comparable with those observed in other EcM trees such as Betulaceae and Salicaceae that dominate in boreal and temperate forests (Tedersoo *et al.* 2012; Timling *et al.* 2012). The observed EcM fungal diversity associated with the studied pine trees may reflect a species–area relationship (Gaston 2000), whereby dominant trees maintain richer EcM communities on a global scale (Tedersoo *et al.* 2012).

Despite the broad partner breadth, our results also revealed a core set of fungal lineages (most prominently *Sebacina*, *Russula* and *Inocybe*, *Tomentella*, *Hygrophorus*, *Piloderma* and *Amphinema*) shared across Pinaceae hosts, albeit with variation in relative read abundance (Fig. 2). This pattern reflects a strategy of ‘selective generalism’ adapted to the nutrient-poor, seasonally variable conditions of subalpine forests on the Qinghai-Tibetan Plateau (Guo *et al.* 2021; Kou *et al.* 2024; Zhang *et al.* 2025). From the plants’ perspective, this strategy may offer baseline symbiotic functionality via reliable core partners, while maintaining flexibility through a diverse set of low-abundance associates that provide functional redundancy and adaptive capacity in response to environmental fluctuations (Matias *et al.* 2012; Wu *et al.* 2025; Yachi and Loreau 1999; Zhang *et al.* 2022). For the fungi, an effective ‘hub’ strategy, characterized by associations with multiple plant hosts (Fig. 3), is likely advantageous in spatially patchy environments (Montesinos-Navarro *et al.* 2012; Toju *et al.* 2014). Generalist fungi can mitigate host limitation risks in variable environments and enable their global dominance, a pattern commonly observed in arbuscular mycorrhizal fungi (Davison *et al.* 2015; Tedersoo *et al.* 2014).

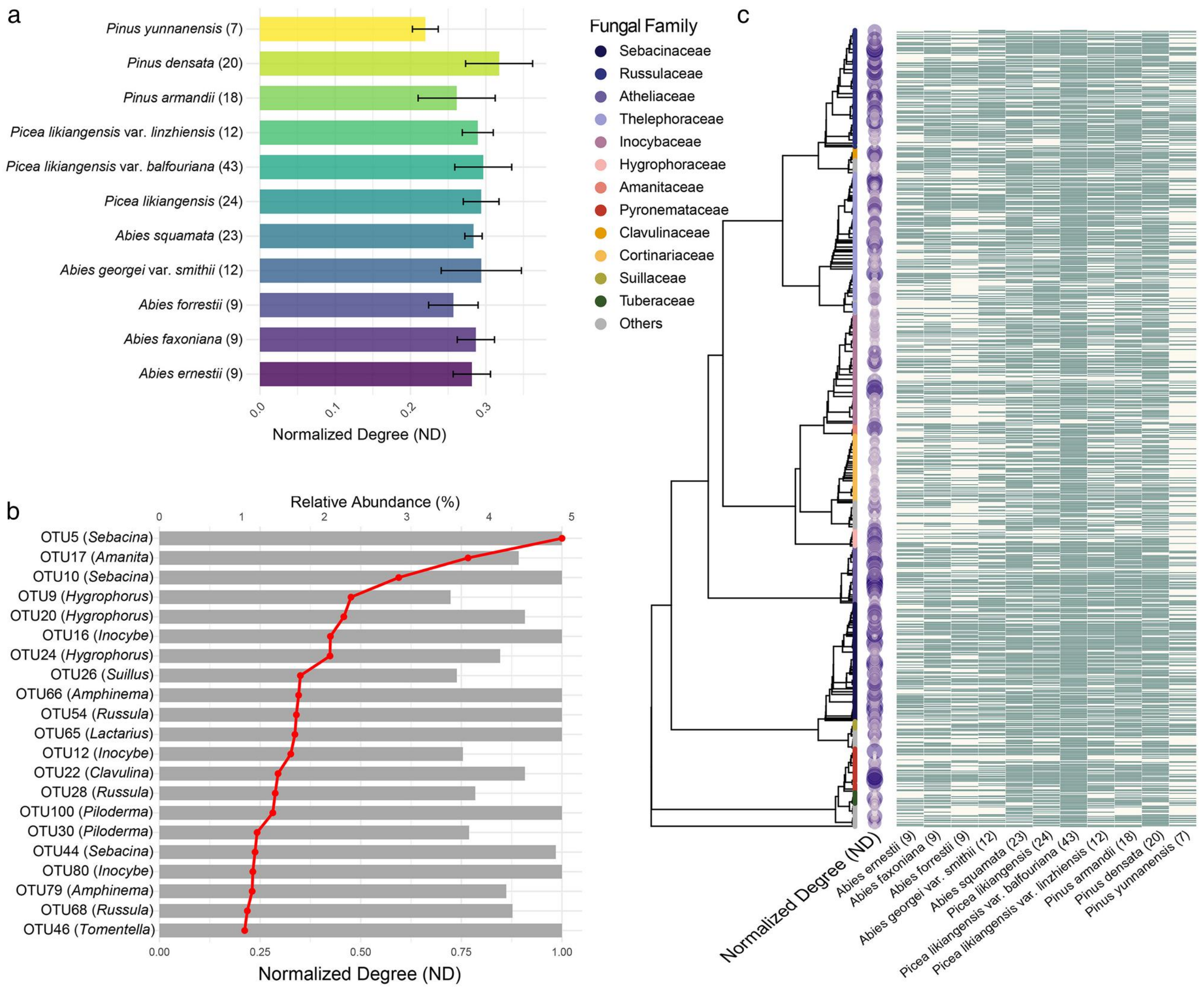

Figure 3: Network centrality in Pinaceae ectomycorrhizal (EcM) fungal associations. (a) Normalized degree (ND) centrality of host plant species; (b) ND centrality of keystone EcM fungal OTUs; (c) phylogenetic distribution of EcM fungal OTUs with tip points and labels colored by family; ND of each OTU is shown by point size (small to large). The presence–absence association matrix of fungal OTU and plant species is displayed on the right.

While classic ecological theory predicts a trade-off between partner breadth and partnership efficiency (Futuyma and Moreno 1988), our findings support that pine–EcM associations on the Qinghai-Tibetan Plateau are functionally redundant, offering mutual advantages for both partners under highly variable environmental conditions such as pronounced seasonal climate fluctuations and patchy nutrient distribution.

The absence of host phylogenetic signal in pine–EcM communities

In tightly coevolved symbiotic associations, host phylogenetic relatedness is often positively correlated with differences in microbial community composition (Hanson *et al.* 2012; Maherali and Klironomos 2007; Peay *et al.* 2016; Rezende *et al.* 2007). Among mycorrhizal symbioses, the pattern of phylogenetic signal is especially pronounced in orchid mycorrhizal interactions (Jacquemyn *et al.* 2011; Shefferson *et al.* 2007), though it appears weaker at the orchid family level (Wang *et al.* 2025). In EcM associations, host phylogenetic relationship has also been reported as one of the key drivers of fungal diversity, distribution and community assembly in *Alnus* (Põlme *et al.* 2013), Salicaceae (Tedersoo *et al.* 2013), Fagaceae (Wu *et al.* 2018) and Betulaceae (Wang *et al.* 2019a, 2019b). However, in this study, we detected no significant host phylogenetic signal in the EcM fungal communities associated with Pinaceae (Fig. 4). When interpreted alongside previous findings that Pinaceae

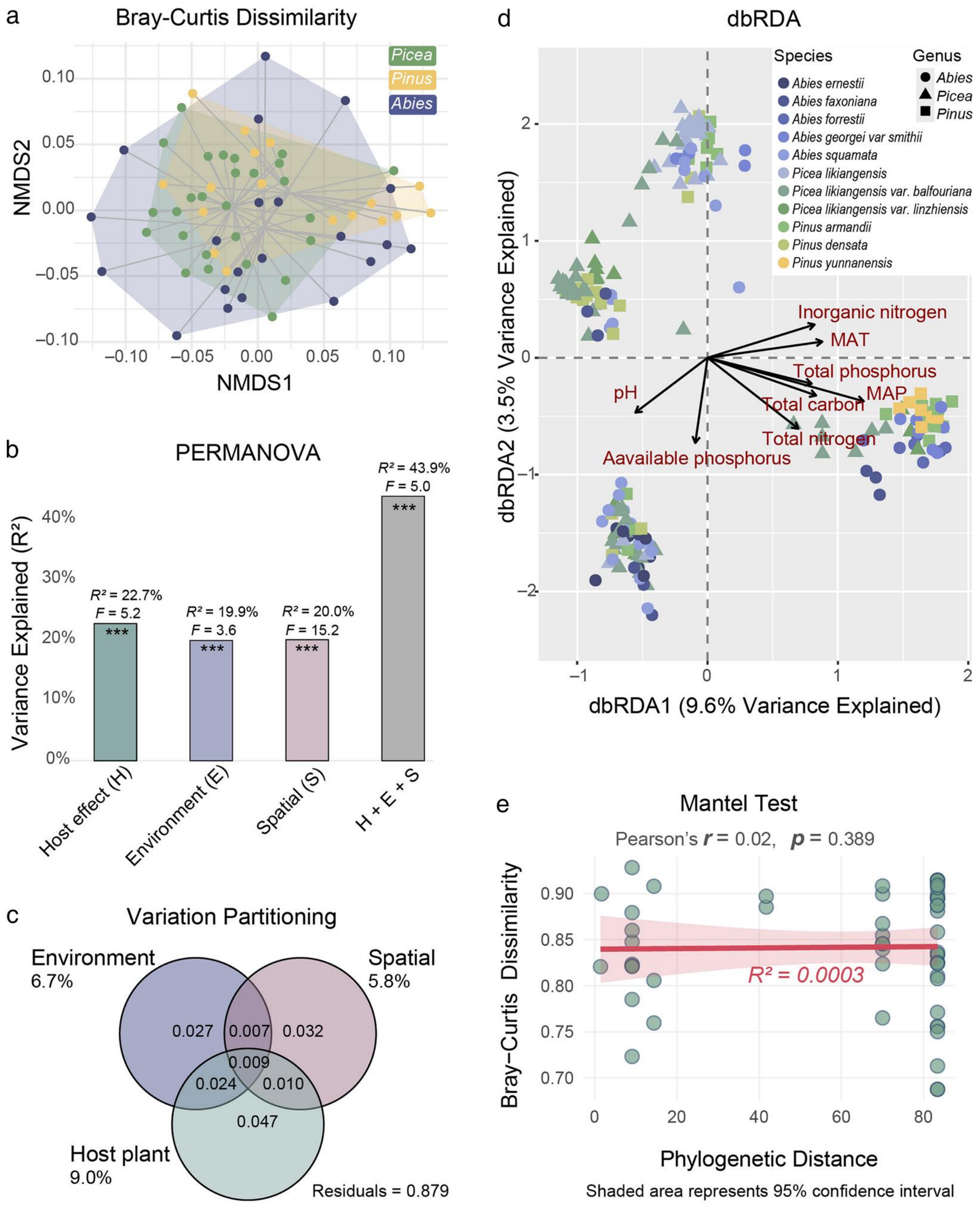

Figure 4: Drivers of ectomycorrhizal (EcM) fungal community assembly in Pinaceae trees. (a) Non-metricmultidimensional scaling (NMDS) ordination visualizing community dissimilarity patterns across plant species and genera; (b) Permutational multivariate analysis of variance (PERMANOVA) models showing explanatory power (R^2) of host plant species (H), environmental factors (E) and spatial distribution (S), as well as their combinations (H + E + S), with significance thresholds: ***$P < 0.001$. (c) Variation partitioning analysis quantifying unique/shared effects among host plant, environment and spatial distribution; (d) dbRDA analysis identifying climatic and edaphic variables that significantly accounted for the variation in EcM communities. (e) Mantel test on the correlation between plant phylogenetic distance and EcM fungal community dissimilarity.

host distinct EcM communities compared with other plant lineages (Ishida *et al.* 2007; Miyamoto *et al.* 2022; Tedersoo *et al.* 2024; Yang *et al.* 2019), our findings suggest that phylogenetic conservatism in EcM fungal assemblages is maintained primarily at or above the family level. This idea is further supported by the phylogenetic clustering of dominant EcM fungal groups between Qinghai-Tibetan pines and other Northern Hemisphere Pinaceae (Fig. 5), indicating that many of these fungi are conserved across broad evolutionary and biogeographic scales. Such an interpretation also aligns with global-scale

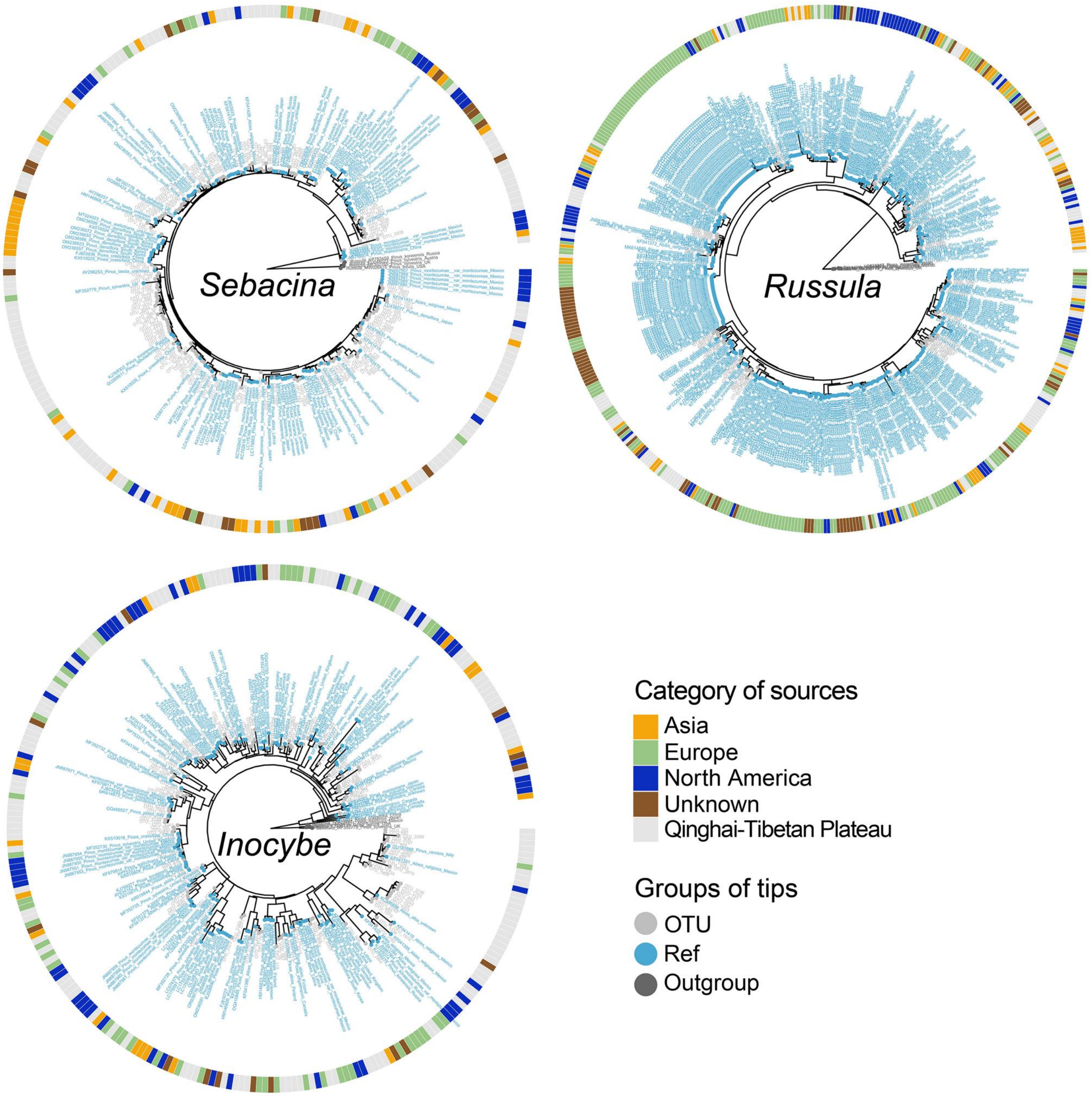

Figure 5: Geographic conservation patterns of three dominant ectomycorrhizal (EcM) fungal genera (*Sebacina*, *Russula* and *Inocybe*) associated with global Pinaceae hosts. Tip colors and labels indicate sequence origins: OTU (studied OTUs of Pinaceae species on the Qinghai-Tibetan Plateau), Ref (global fungal associates of Pinaceae species) and outgroup (sequences for confining the ingroup during phylogenetic inference). Outer ring colors represent geographic origins (Asia [excluding Qinghai-Tibetan Plateau], Europe, North America, unknown [geographic origins not reported in sequence annotations]) and Qinghai-Tibetan Plateau.

analyses showing that plant family affiliation imposes phylogenetic constraints on EcM fungal community composition (Tedersoo *et al.* 2012).

Furthermore, the lack of a detectable phylogenetic signal in pine–EcM associations could stem from the fact that eco-spatial factors and host evolutionary history jointly structure EcM communities (Martiny *et al.* 2006; Hanson *et al.* 2012; Peay *et al.* 2016), potentially masking phylogenetic patterns among closely related hosts, as has been observed in family-wide orchid mycorrhizal interactions (Wang *et al.* 2025). This is strongly supported by our findings that ecological and spatial factors exert strong effects on EcM community variation (Fig. 4).

An alternative explanation involves the limited evolutionary divergence among closely related pine species and subspecies, which may be insufficient to drive strong host-specific fungal associations (Ackerly 2009; Revell *et al.* 2008). To resolve these possibilities, we anticipate that future studies encompassing the full phylogenetic breadth of Pinaceae and its sister clades would enable a more rigorous evaluation of how the strength of host phylogenetic signals varies across taxonomic scales.

Evolutionary history and environmental filtering jointly structure pine–EcM associations at different scales

The absence of a clear host phylogenetic effect, coupled with strong influences of host species identity, environmental and spatial factors on EcM community composition (Fig. 4), collectively suggests a dual assembly process for EcM communities in Pinaceae. In this framework, evolutionary history constrains core EcM lineages at regional and higher taxonomic scales, while environmental filtering fine-tunes community composition at local and species-specific scales. At small scales, climatic factors (e.g. MAT, MAP) and a suite of edaphic variables (Fig. 4d) capture the pronounced environmental heterogeneity across the widely separated monodominant pine forests of the Qinghai-Tibetan Plateau (Ding *et al.* 2020; Kou *et al.* 2024), acting as key local filters on pine–EcM interactions. This pattern aligns with previous work showing that environmental filtering, mediated by species-specific traits and habitat preferences, determined the realized fungal assemblage (Wang *et al.* 2019a, 2019b). At large scales, the consistent and phylogenetically clustered occurrence of predominant EcM taxa (i.e. *Sebacina*, *Russula* and *Inocybe*) in pine forests of both the Qinghai-Tibetan Plateau and other biogeographic regions (Fig. 5) highlights the role of evolutionary history and biogeographic conservatism in structuring EcM communities (Bahram *et al.* 2013; Tedersoo *et al.* 2020). This scale-dependent perspective of pine–EcM associations is consistent with microbial biogeography, in which community composition reflects the combined effects of environmental conditions, dispersal limitation and evolutionary history (Hanson *et al.* 2012; Martiny *et al.* 2006; Peay *et al.* 2016).

Global analyses demonstrate that soil fungi in general exhibit biogeographic patterns closely paralleling those of plants and animals, with similar climatic and edaphic variables emerging as the strongest predictors of fungal richness and community composition worldwide (Tedersoo *et al.* 2014). This pattern underscores a broad ecological congruence of fungal assemblages. While Pinaceae are most abundant in cold or temperate climates of the Northern Hemisphere (Bahram *et al.* 2013; Geml *et al.* 2017; Tedersoo *et al.* 2012), they span an unusually wide array of biomes, ranging from Mediterranean to coastal and tropical montane forests (Adamo *et al.* 2021; Bahram *et al.* 2012; Geml *et al.* 2014, 2017). The persistence of their associations with a core set of predominant EcM taxa across such diverse environments suggests that these symbioses are maintained not solely by ecological congruence but also by multiple complementary mechanisms involving both plant and fungal ecology (Tedersoo *et al.* 2012). From the plant perspective, the Qinghai-Tibetan Plateau, harboring ancient pine lineages and extensive undisturbed forests (Jin *et al.* 2021), may have acted as a long-term refuge that preserved ancestral, stable pine–fungal associations. From the fungal perspective, the cosmopolitan distribution of these fungal genera (Bahram *et al.* 2013; Geml *et al.* 2017; Tedersoo *et al.* 2012) points to broad ecological tolerance and high dispersal capacity, likely conferring competitive advantages across diverse environments. Nevertheless, these findings challenge the prevailing expectation of high endemism in subalpine and alpine ecosystems, often observed in plants and animals (Kier *et al.* 2005; Steinbauer *et al.* 2016). Instead, they point to biogeographically conserved Pinaceae–EcM symbioses that transcend major spatial barriers, paralleling patterns observed in arbuscular mycorrhizal fungi (Davison *et al.* 2015). Global comparative studies across pine forests will be crucial to determine whether the Qinghai-Tibetan Plateau represents an exception or reflects a broader pattern of symbiotic conservation in subalpine conifer systems. Also, integrating ecological niche modeling, comparative genomics and reciprocal transplant experiments will be essential for disentangling the relative roles of biogeography, ecology and evolutionary history in the assembly and persistence of these associations.

CONCLUSIONS

Our regional-scale investigation of EcM communities in Qinghai-Tibetan Pinaceae forests reveals a selective generalist strategy, characterized by both high PD of fungal partners and the preferential retention of a few dominant lineages. This pattern reflects a dual assembly process: phylogenetic conservatism sustains stable associations across Pinaceae genera, while environmental filtering drives species-specific community differentiation at local scales. Although broader verification is needed, the widespread distribution of dominant fungal groups suggests biogeographic conservatism in the Pinaceae–EcM partnership. Together, this study reveals that EcM communities in alpine pine forests are shaped jointly by evolutionary history and environmental filtering at the regional scale, underscoring their importance for host tree physiology and ecosystem functions.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Summary of sampling information.

Table S2: Environmental variables of sampling plots.

Table S3: Taxonomic assignment of EcM fungal OTUs of Pinaceae trees.

Table S4: Association matrix of EcM fungal OTUs (rows) and plant root samples (columns) with read abundances.

Table S5: Time-calibrated phylogeny of EcM OTUs in Newick format.

Table S6: GenBank accessions of *Sebacina*, *Russula* and *Inocybe* extracted from roots of Pinaceae species (*Abies*, *Picea* and *Pinus*) across the globe.

Authors' Contributions

Deyi Wang (Conceptualization, Formal analysis, Methodology, Writing—original draft), Hans Jacquemyn (Methodology, Writing—review & editing), Guangru Wang (Methodology, Writing—review & editing), Yongping Kou (Data curation, Validation), Junxiang Ding (Data curation), Peipei Zhang (Writing—review & editing) and Huajun Yin (Funding acquisition, Project administration, Supervision, Writing—review & editing).

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