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ARTICLE

Phylogenetic imprint of woody plants on the soil mycobiome in natural mountain forests of eastern China

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Abstract

Recent studies have detected strong phylogenetic signals in tree–fungus associations for diseased leaves and mycorrhizal symbioses. However, the extent of plant phylogenetic constraints on the free-living soil mycobiome remains unknown, especially at broad geographic scales. Here, 343 soil samples were collected adjacent to individual tree trunks, representing 58 woody plant species located in five mountain forests of eastern China. Integrating plant species identity and phylogenetic information, we aimed to unravel the relative contributions of phylogenetic relationships among tree species, abiotic environmental filtering, and geographic isolation to the geographic distribution of soil mycobiome. We found that the community dissimilarities of total fungi and each dominant guild (viz. saprotrophs, plant pathogens, and ectomycorrhizal fungi) significantly increased with increasing plant phylogenetic distance. Plant phylogenetic eigenvectors explained 11.4% of the variation in community composition, whereas environmental and spatial factors explained 24.1% and 7.2% of the variation, respectively. The communities of ectomycorrhizal fungi and plant pathogens were relatively more strongly affected by plant phylogeny than those of saprotrophs (13.7% and 10.4% vs. 8.5%). Overall, our results demonstrate how plant phylogeny, environment, and geographic space contribute to forest soil fungal distributions and suggest that the influence of plant phylogeny on fungal association may differ by guilds.

Introduction

The tight link between woody plants and fungi has broad ecological and evolutionary implications, impacting ecosystem functions and services in natural forests. Mycorrhizal associations play pivotal roles in resource exchange between symbiotic fungi and host trees [1, 2] and between neighboring trees [3, 4], regulating nutrient cycling and carbon flow belowground in forests. Infections of pathogenic fungi alter the structure of tree communities with "negative density dependence" and thereby maintain the coexistence and high diversity of trees in subtropical and tropical forests [5–8]. In addition, a great quantity of saprotrophic fungi, as the main force in decomposition of dead plant biomass (e.g., litters and woods), guarantees the sustainability of forest material circulation [9, 10]. Therefore, disentangling the relationship between woody plants

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and their associated fungi will greatly improve our predictive ability regarding aspects of forest health, nutrient dynamics, and ecosystem stability in a time of rapid global change [11, 12].

Phylogenetic analysis is an effective approach for studying community ecology in the light of evolutionary perspectives [13, 14] and has been recently employed to elucidate cryptic and complex plant–microbe interactions [15, 16]. Gilbert and Webb [17] found that the likelihood that a pathogenic fungus successfully infected two tree species decreased with increasing plant phylogenetic distance in a tropical forest. Liu et al. [18] observed a phylogenetically congruent pattern between woody plants and foliar/soil-borne pathogenic fungi; that is, congeneric host trees were usually infected by the same or closely related fungi. Likewise, strong plant phylogenetic signal was also detected in symbioses involving woody plants, arbuscular mycorrhizal (AM), and ectomycorrhizal (EcM) fungi [19–22], with closely related woody plants interacting with closely related fungal partners. However, the above studies mainly focused on biotrophic fungal groups and largely considered local geographic scales. It is unclear whether there is a similarly strong phylogenetic signal in the relationship between woody plants and the free-living mycobiome in the neighboring soils of plants, especially at broad geographic scales.

At local scales, the community composition of soil fungi was strongly correlated with the distribution of aboveground trees in studies of tropical, subtropical, and temperate forests [23–25]. Dominant trees or tree species identity were also reported to strongly affect the community composition of soil fungi [25–27]. The quality and quantity of compounds in plant residues and root exudates may partly contribute to the strong associations between plants and soil fungi through the establishment of belowground resource heterogeneity [28–30]. However, a few empirical studies have examined the effect of plant phylogeny on the soil mycobiome, and they have produced conflicting results. For instance, Barberan et al. [31] observed a strong correlation between soil fungal community composition and phylogenetic structure of trees even after controlling for the variation of soil conditions, whereas Leff et al. [32] failed to find any significant correlations between soil fungal community composition and plant phylogenetic structure in either a mesocosm experiment or a field survey.

Such an inconsistency might results from the different study scale and biotic/abiotic heterogeneity among different local sites under study. With an increase in geographic scale, the linkage between plants aboveground and the soil mycobiome may be masked by large-scale forces, such as geographic isolation and climatic drivers [33–35]. In addition, soil physicochemical properties affect soil fungal distributions at nearly all spatial scales [25, 33, 34, 36–38]. Our recent work demonstrated that the relative effects of soil properties and geographic distance on soil fungal communities changed gradually with increasing distance from wheat roots in the North China Plain [39], highlighting the complex interactive effects among plant, soil, and space on fungal communities. However, the holistic interactions of soil fungal communities with soil, climate, space, and plant (phylogeny and species identity) remain largely unknown, particularly in much less disturbed and heterogeneous forest ecosystems.

The natural forests in eastern China comprise relics of once widespread Tertiary mesophytic forests that spanned the Northern Hemisphere [40] and serve as both a floristic museum and an evolutionary cradle for woody plants [41]. Woody plants are primary producers in this forest zone, transporting a large proportion of fixed photosynthetic carbon to the neighboring soil. Here, we used woody plant individuals as our sampling units, collecting surface soil adjacent to each woody plant individual across five mountain forests in eastern China. We test the following hypotheses: (1) soil fungal communities are strongly influenced by plant phylogeny and plant species identity, even after accounting for abiotic environmental filtering, and geographic isolation; (2) plant phylogenetic effects on the neighboring soil mycobiome differ by fungal guild. Specifically, considering the strong phylogenetic linkages between trees and biotrophic fungi [17, 21] and the possible functional redundancy of saprotrophs [42], woody plant phylogeny may exert greater influences on the communities of EcM fungi and plant pathogens relative to that of free-living saprotrophs.

Materials and methods

Soil sampling and site characteristics

This study was conducted across five mountain forests in eastern China based on a uniform sampling protocol (see below; Fig. 1, Table S1). From north to south, the locations include Changbai Mountain (CB), Dongling Mountain (DL), Shennongjia Mountain (SNJ), Tianmu Mountain (TM) and Gutian Mountain (GT). All sampling occurred during the growing season of each forest, i.e., all leaves were green and non-senescent. We sampled soil associated with individual tree species that all belonged to disjunct tree genera found in eastern Asia and eastern North America [43]. After locating individuals of the targeted tree species, we measured the diameter at breast height (DBH) and collected soil cores with the trunk as center and the DBH as radius for sampling soil in four directions. All sampled trees were at least 15 meters apart to avoid spatial autocorrelation [44].

Soil cores of 3.5 cm diameter were collected to 10 cm depth after removal of the litter layer. Four cores from each

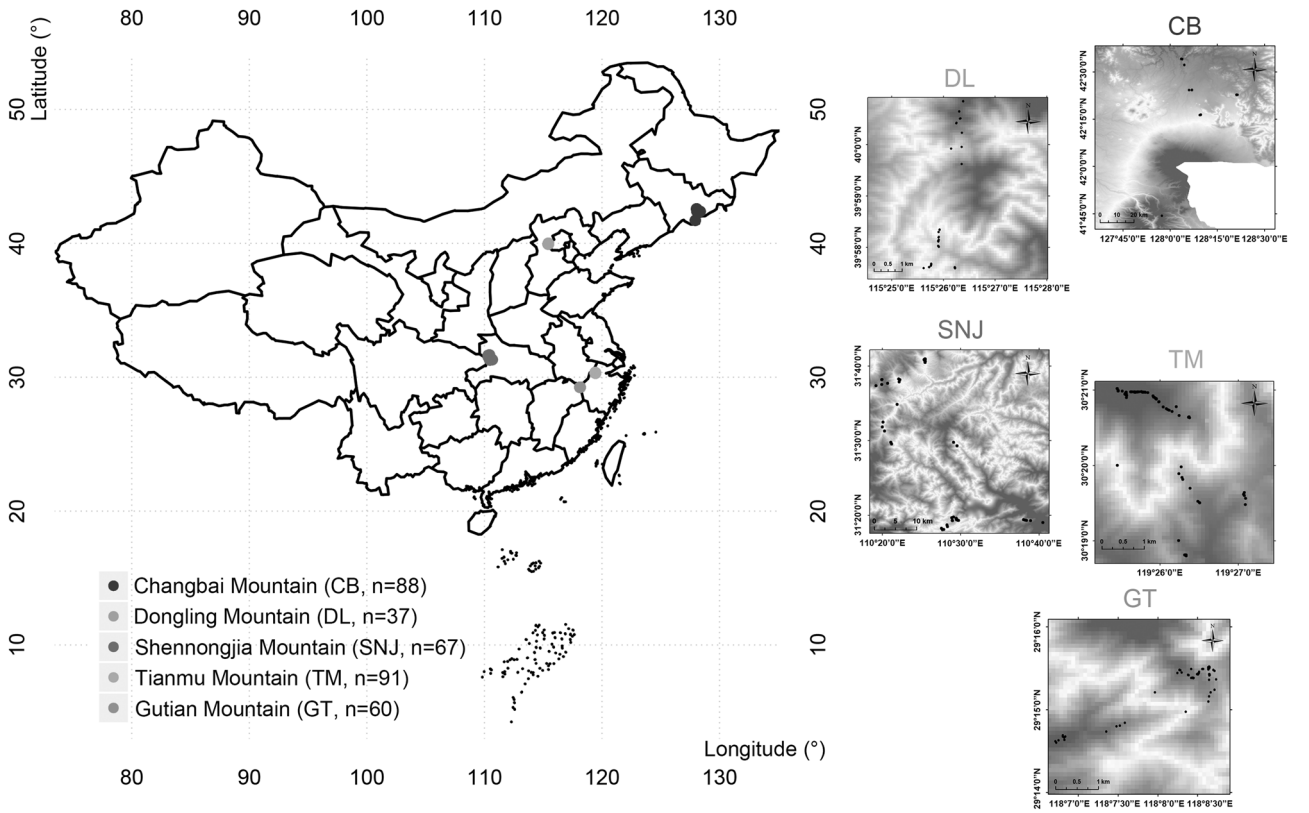


Fig. 1 Sampling map of five mountain forests in eastern China. Because of the establishment of nature reserve, these areas suffer from few human disturbances. The subfigures on the right show the distribution of sampling points (black) in each mountain forest. The hot-cold colors represent the elevational gradient: red means higher elevation, while blue means lower elevation

tree were combined and placed into a sterilized polyethylene bag as a single composite sample. The samples were brought back to the laboratory among ice bags within 12 h. After sieving through 2-mm mesh, each soil sample was divided into two subsamples: one was stored at 4 °C to determine the soil properties, and the other was stored at -40 °C for subsequent DNA extraction. In total, we collected 343 soil samples under 58 woody plant species belonging to 20 genera and 14 families (Fig. 2).

Tree traits and plant phylogeny reconstruction

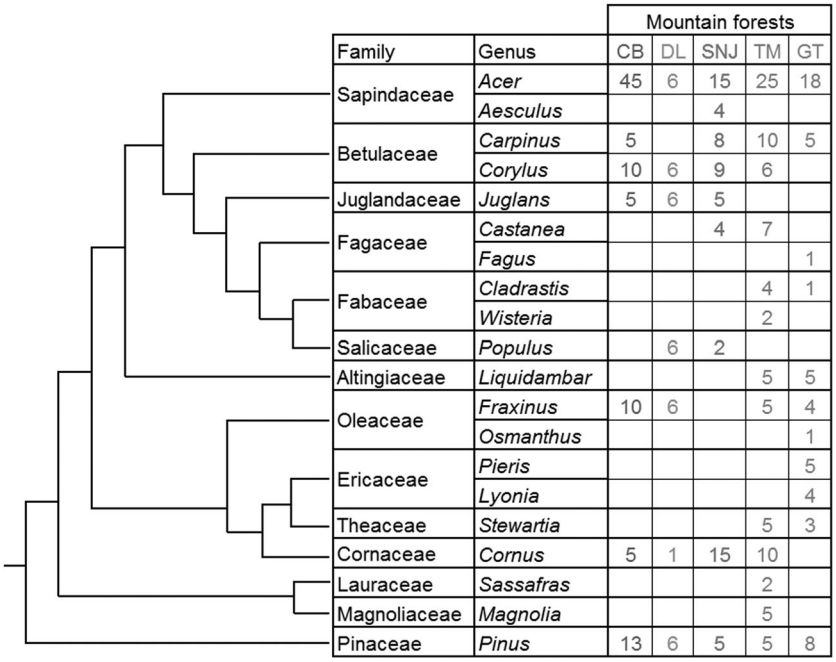
Corresponding to each sample, the basic traits of each tree were recorded in the field, including height, crown diameter and DBH. Mycorrhizal types of trees were assigned by experts' knowledge and previous studies [45]. The species identification was made by plant taxonomists; family names follow the most updated classification of the Angiosperm Phylogeny Group [46]. The above information is given in Table S2.

To compare and evaluate the robustness of our results, we generated phylogenetic trees using two different approaches: (1) we used Phylomatic v3 (http://phylodiversity.net/phylomatic/) to obtain a phylogeny for our sampled woody plants based on the Zanne et al. [47] phylogeny as the backbone; (2) we mined the GenBank database (Release 224; February 15, 2018) and obtained five widely sequenced loci (*atpB*, *rbcL*, *matK*, *matR* and ITS) using the PHyLogeny Assembly with Databases pipeline (PHLAWD, version 3.4a, https://github.com/blackrim/phlawd) [48]; then maximum likelihood (ML) analyses (to obtain the best ML tree) for the concatenated alignment was conducted using RAxML v8.2.10 [49] with 1000 bootstrap replicates under the GTRGAMMA model and constrained under the topology of the Open Tree of Life [50].

Both phylogenetic trees were in close agreement with relationships among families obtained in other recent phylogenetic analyses [e.g., 46, 51, 52]. The diagrams of phylogenetic trees were generated by MEGA6 [53] [Fig. S1–S2], and the phylogenetic distance between each sample was calculated by the function *cophenetic* in the R package "picante" [54].

Measurement of soil properties

We measured 17 soil variables, including soil moisture (SM), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), pH, total carbon (TC), total nitrogen (TN), C:N ratio, aluminum, calcium, copper, iron,



Family	Genus	Mountain forests				
		CB	DL	SNJ	TM	GT
Sapindaceae	*Acer*	45	6	15	25	18
	Aesculus			4		
Betulaceae	*Carpinus*	5		8	10	5
	Corylus	10	6	9	6	
Juglandaceae	*Juglans*	5	6	5		
Fagaceae	*Castanea*			4	7	
	Fagus					1
Fabaceae	*Cladrastis*				4	1
	Wisteria				2	
Salicaceae	*Populus*		6	2		
Altingiaceae	*Liquidambar*				5	5
Oleaceae	*Fraxinus*	10	6		5	4
	Osmanthus					1
Ericaceae	*Pieris*					5
	Lyonia					4
Theaceae	*Stewartia*				5	3
Cornaceae	*Cornus*	5	1	15	10	
Lauraceae	*Sassafras*				2	
Magnoliaceae	*Magnolia*				5	
Pinaceae	*Pinus*	13	6	5	5	8

Fig. 2 Diagram of plant and soil sampling. Relationships among the sampled plant lineages (families/genus) are indicated in a cladogram on the left panel. The numbers of soil samples corresponding to each genus from each mountain forest site are tabulated on the right panel and colored by sampling sites as Fig. 1

potassium, magnesium, manganese and phosphorus. SM was measured gravimetrically. DOC was measured using a liquid carbon and nitrogen analyzer Vario TOC cube (Elementar, Hanau, Germany). DON, NH_4^+-N and NO_3^--N were measured using the San++ continuous flow analyzer (Skalar, Breda, Netherlands). Soil pH was measured using a Thermo Orion-868 pH meter (Thermo Orion Co., Waltham, MA, USA) in a boiled deionized water system. TC and TN were determined with a carbon–hydrogen–nitrogen (CHN) elemental analyzer (2400 II CHN elemental analyzer; PerkinElmer, Boston, MA, USA). The other soil elements were measured with an ICP Optima 8000 (Perkin-Elmer, Waltham, MA, USA). Soil properties are shown in Table S3.

Collection of other environmental and geographic data

We recorded latitude, longitude, elevation, and slope of focal trees in the field. Mean annual temperature (MAT) and mean annual precipitation (MAP) were compiled from the WorldClim version 2 (www.worldclim.org) at 30 arc-second resolution. Annual potential evapotranspiration (PET) was obtained from the Global-PET database (https://cgiarcsi.community/data/global-aridity-and-pet-database/) at 30 arc-second resolution. The climatic and geographic data are shown in Table S4.

DNA extraction, PCR, and MiSeq sequencing

Total DNA from each sample was extracted under sterile conditions from 0.5 g of soil by using a FastDNA® Spin kit (Bio 101, Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted DNA was amplified by targeting the fungal Internal Transcribed Spacer 1 (ITS1) of the rDNA region using the primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA) [55] and ITS2 (5'-GCTGCGTTCTTCATCGATGC) [56] equipped with unique identifier tags. PCR reactions (30 µl) included 15 µl Phusion Master Mix (New England Biolabs, USA), 1.5 µl each of 2 µM forward and reverse primers, 10 µl DNA template (10 ng μl^{-1}), and 2 µl H_2O. PCR conditions were 1 min at 98 °C; 30 cycles of (10 s at 98 °C; 30 s at 52 °C; 30 s at 72 °C); 5 min at 72 °C. For each sample, we conducted PCR in three independent tubes and mixed them to represent that sample. The PCR products from all samples were normalized to equimolar amounts before sequencing and were sequenced on the Illumina MiSeq platform PE250 (Illumina, Inc., San Diego, CA, USA). The sequence data were submitted to the European Nucleotide Archive under the accession number ERP104329.

Bioinformatics

Beginning with the raw data on the Illumina sequencer, we first merged the paired-end reads using FLASH [57]. Qiime 1.9.0 [58] and Cutadapt 1.9.1 (https://doi.org/10.14806/ej.17.1.200) were applied for quality filtering, trimming and chimera removal. We obtained 12,158,131 reads after quality filtering (parameters: minlength = 240, maxambigs = 0, and phred quality threshold = 30). We then removed the flanking small ribosomal subunit (SSU) and 5.8 S genes by using ITSx 1.0.11 (http://microbiology.se/software/itsx/)

[59] and deleted the putative chimeric sequences by using a combination of de novo and reference-based chimera checking [60]. The remaining sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold based on the Usearch algorithm [61]. Singletons were also removed during the Usearch clustering process [62, 63]. We used the BLAST method in the *assign_taxonomy.py* script to assign a name to each fungal OTU and followed the e-value thresholds proposed by Tedersoo et al. [34]. The UNITE v.7.1 (http://unite.ut.ee) release for Qiime served as the reference database for fungal taxonomy [64].

After removing the non-fungal sequences, we obtained 11,548,916 fungal sequences covering 24,890 OTUs (minimum 6820; maximum 83,479; mean 33,670 sequences per sample). Seven fungal functional guilds (i.e., animal pathogens, EcM fungi, ericoid mycorrhizal (ErM) fungi, mycoparasites, plant endophytes, plant pathogens and saprotrophs) were identified according to Tedersoo et al. [34] and Nguyen et al. [65] (detail in Fig. S3). To eliminate the effects of different sequencing depth on the analyses, the data set was rarefied to 6820 sequences per sample (the minimum sequence number among 343 samples), leaving 20,685 fungal OTUs.

Statistical analyses

The observed OTUs were used to represent the taxonomic richness of soil fungi. Significant differences in richness at multiple plant taxonomic levels (from phylum to species) were tested using the Independent *t* test or Kruskal–Wallis test in the R package "stats" [66]. Post-hoc Kruskal–Wallis tests were used to compare fungal richness between pairwise plant families and genera by using the function *kruskalmc* in the R package "pgirmess" [67]. Ordinary least squares (OLS) multiple regression models were used to identify the best set of predictors of fungal richness. All the variables were centralized and standardized before conducting the multiple regression (average = 0 and SD = 1). Akaike's information criterion (AIC) and adjusted R-squares (R^2_{adj}) were used to determine the best OLS multiple regression models, and the criterion (variance inflation factor, VIF < 3) was used to eliminate multicollinear variables. AIC and VIF were calculated by the "MASS" [68] and "car" packages [69] of R, respectively.

For the analyses of fungal community composition, the rarefied OTU table was first Hellinger-transformed, and Bray–Curtis dissimilarity was calculated by the function *vegdist* in "vegan" [70]. To measure the relative effects of abiotic environmental filtering, geographic isolation and plant phylogeny on soil fungal distributions, distance-based linear model multivariate analysis (DISTLM) [71] and variation partitioning analysis (VPA) were used, which included 17 soil variables, 3 climatic variables, 4 spatial vectors and 49 plant phylogenetic eigenvectors. DISTLM was implemented with the computer program DISTLM forward [72]. VPA was performed with the *varpart* function in "vegan", which was based on redundancy analysis. Before VPA, the variables were selected by the marginal tests of DISTLM. Here, spatial vectors were represented by the first four principal coordinates of neighbor matrices (PCNM) vectors with positive spatial autocorrelation (Moran I = positive, Fig. S4) [73], and phylogenetic eigenvectors were derived from the phylogenetic distance matrices by using the function *pcnm* in "vegan" and then forward-selected ($\alpha = 0.05$) in the R package "packfor" [74]. The PCNM thresholds of spatial distance and plant phylogenetic distance were 1230.095 and 704.4688, respectively. In addition, plant species identity was transformed to dummy variables and also added into multivariate models (DISTLM and VPA) to compare the extent of plant species effect and phylogeny effect.

To examine the effects of multiple plant taxonomic levels on community composition, we conducted a nested permutational multivariate analysis of variance (PERMANOVA). Canonical analysis of principal coordinates (CAP) was performed to show the differentiation of soil fungal communities among plant families after controlling for the mountain-site effects, which was implemented by the function *capscale* in "vegan". Significant differences in community composition between pairwise plant families were also tested by PERMANOVA. To test the effects of plant phylogenetic distance, Mantel tests were used to examine the correlation between fungal community dissimilarity matrix (Bray–Curtis distance) and plant phylogenetic distance matrix. Then the relationship between pairwise community dissimilarity distances and logarithm-transformed phylogenetic distances was tested by linear regressions. The linearization of matrices was conducted with PASSaGE2 (www.passagesoftware.net). All tests of plant phylogenetic effect on fungal community composition were also implemented for the representative functional guilds (viz. saprotrophs, plant pathogens and EcM fungi) and by using two approaches of phylogenetic tree construction (viz. Phylomatic and RAxML). To confirm that the difference in the effect of plant phylogeny on different functional guilds was non-random and significant, we used a bootstrapping method to formulate 29 rarefied OTU tables for each functional guild and tested their DISTLM results using the Games–Howell test in SPSS STATISTICS 20.0 for windows (IBM-SPSS, Chicago, IL, USA). In addition, we tested the plant phylogeny effect on soil fungal community composition only within angiosperms using DISTLM.

To measure the effects of geographic isolation, nonmetric multidimensional scaling analyses (NMDS) were performed with the function *metaMDS* in "vegan", and the function *ordiellipse* was employed to fit the 95% confidence ellipses by five sites onto the NMDS ordination. The

significance of distance decay models was tested for the whole region and for each site separately [75, 76]. To visualize the effects of abiotic environmental filtering, the function *bioenv* in "vegan" was used to select the best set of environmental predictors and fitted them onto the NMDS ordination.

Results

Data characteristics

In total, we observed 24,890 fungal OTUs in 343 soil samples. Among them, 11,609 OTUs were assigned to seven functional guilds, which accounted for 62.5% of the sequences (Fig. S3). Soil fungal communities were dominated by Mortierellomycotina and Agaricomycetes, which accounted for 31.6% and 29.5% of the sequences, respectively (Fig. S5). In terms of the functional composition of fungal communities, saprotrophs (43.0%), EcM fungi (14.0%) and plant pathogens (1.2%) were the representative and dominant functional guilds (Fig. S3).

Taxonomic richness

The fungal richness of each sample varied from 113 to 761, and significantly differed at multiple plant taxonomic levels (Table S5). The post-hoc Kruskal–Wallis test showed that the mean fungal richness associated with *Juglans* (Juglandaceae) was significantly greater than that in *Corylus* (Betulaceae), *Magnolia* (Magnoliaceae), and *Pinus* (Pinaceae) ($P_{adj} < 0.05$, Fig. S6–S7), but all other pairwise comparisons between families and genera were not statistically significant.

Fungal richness responded significantly to several soil parameters, including DOC, NO_3^--N, pH, TC, iron, magnesium and manganese, as well as the climatic variable PET. These collectively explained 18.3% of the variation in fungal richness in the OLS multiple regression model (AIC = -60.6, $R^2_{adj} = 0.183$, Table S6). Further, when we incorporated plant phylogenetic eigenvectors and spatial vectors (SPCNM1-SPCNM4) into the OLS multiple regression model, this full model strongly improved the fit and explanatory power (AIC = -89.9, $R^2_{adj} = 0.261$, Table S7), accounting for additional 7.8% variation in fungal richness. The full model included five significant plant phylogenetic eigenvectors as the predictors of soil fungal richness.

Community composition

The best multivariate model (DISTLM) showed that the variation in soil fungal community composition was attributed to the combined effects of edaphic, climatic and spatial variables as well as plant phylogeny (Table S8). All predictor variables taken together explained 42.6% of the variation in fungal composition. Specifically, edaphic (pH, NH_4^+-N, NO_3^--N, phosphorus, DON, C:N ratio, potassium, TN, TC, manganese, magnesium, calcium, iron, SM, copper), climatic (MAP, PET, and MAT), and spatial factors (SPCNM1-SPCNM3) explained 18.9%, 5.2% and 7.2% of the variation in fungal composition, respectively, whereas plant phylogenetic eigenvectors explained 11.4% of the variation. VPA also showed that edaphic variables, climatic variables, spatial factors and plant phylogeny explained 17.0%, 10.5%, 11.0% and 15.8% of the variation in fungal community composition, respectively, but the majority of the variation (16.7%) resulted from the interaction of more than two factors (Fig. S8).

With increasing plant phylogenetic distance, the community dissimilarities of total fungi and each dominant guild (viz. saprotrophs, plant pathogens and EcM fungi) significantly increased (Fig. 3), and such a trend along plant phylogenetic gradients was stronger for biotrophic fungi (i.e., EcM fungi and plant pathogens) relative to that of free-living saprotrophs. DISTLM further revealed that plant phylogenetic eigenvectors explained 13.7%, 10.4% and 8.5% of the variation in community composition of EcM fungi, plant pathogens and saprotrophs, respectively (Table S9). Of note, the two different approaches of phylogenetic tree construction employed here did not influence the observation of plant phylogeny effect on community composition of total fungi and each functional guild (Fig. 3, S8–S10; Tables S8–S11). Based on the bootstrapping method, we found that the effects of plant phylogeny on EcM fungi and plant pathogens were significantly larger than that on saprotrophs ($P < 0.001$, Table S12). In addition, within the angiosperm-only data subset, plant phylogeny revealed somewhat weaker but significant effect on soil fungal community composition, explaining 8.3%, 6.2%, 5.9% and 4.7% of the variation in total fungi, EcM fungi, plant pathogens and saprotrophs, respectively (Tables S13 −S14).

Abiotic environmental filtering (mainly including soil pH MAP, and MAT) was still the primary driving force in soil fungal distributions (Fig. S11). In addition, soil fungal communities were significantly differentiated among mountain forest sites (Fig. S12), and the community similarities of soil fungi significantly decreased with the increasing geographic distance for the whole region and within each mountain forest (Fig. S13), indicating the hierarchical role of spatial distance in discontinuous forest ecosystems.

The fungal community composition at the levels of both fungal class and functional guild substantially differed among 14 analyzed plant families (Fig. S14). For example, the relative abundance of ErM fungi amounted to 6.0% for

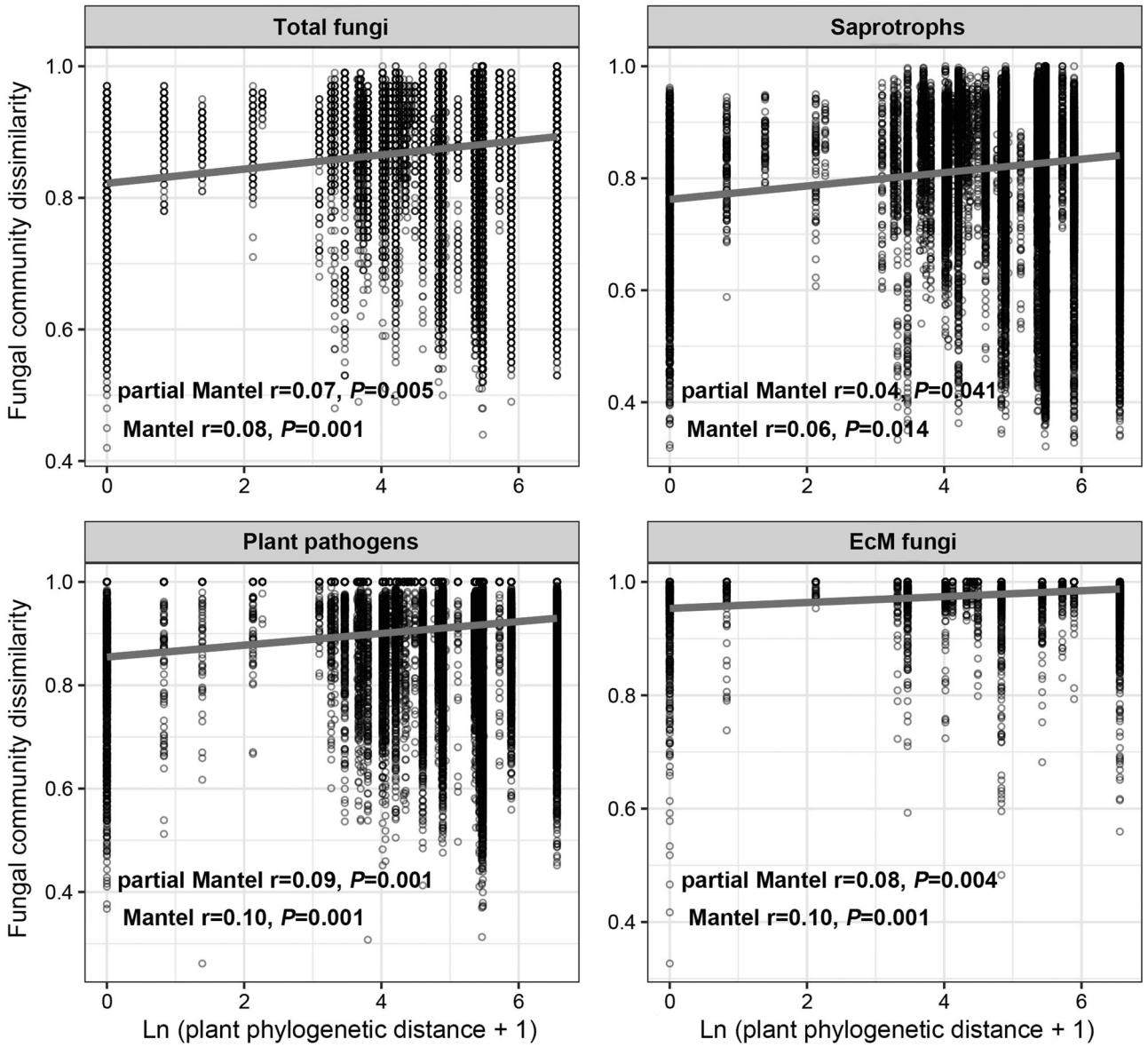


Fig. 3 The positive relationships between soil fungal community dissimilarities and plant interspecific phylogenetic distances for total fungi and each representative functional guild. The results of Mantel tests and partial Mantel tests are shown in diagrams

Ericaceae with comparison to an average of 0.5% for other plant families. The relative abundance of Dothideomycetes presented 6.2% for Juglandaceae in contrast to an average of 1.4% for other plant families. The fungal communities were significantly differentiated among a number of plant families, when we controlled for the effect of mountain forest sites by using CAP ($F = 1.77$, $df = 11$, $P < 0.001$, Fig. S15). Such a difference was also supported by the pairwise comparison using PERMANOVA (Table S15). Furthermore, the nested analysis showed that plant phylum (0.7%), order (6.1%), family (2.0%) and genus (2.5%) all explained significant amounts of variation in fungal community composition, but the majority (24.1%) of variation in community composition occurred at the species level of plants (Table S16).

When replacing plant phylogenetic eigenvectors with plant species (as dummy variables) in the multivariate analyses (viz. VPA and DISTLM), very similar explanatory power was provided by species identity as by phylogeny: species identity explained 11.5% of the variation in DISTLM (Table S17) and 16.8% of the variation in VPA (Fig. S16). Further, when synchronously incorporating plant species identity and phylogenetic eigenvectors into VPA, 15.8%–16.7% of the variation in community composition was attributed to the shared effects of plant species and plant phylogeny, and plant species identity also had the

unique effect on fungal community composition (0.4%–1.0%, Fig. S17–S18). These results indicated plant species effect and plant phylogeny effect are confounding.

Discussion

Effects of edaphic variables and plant phylogeny on fungal richness

The best OLS multiple regression model showed that the explained variation of soil fungal richness was mainly attributed to the effects of edaphic variables (DOC, NO_3^--N, pH, TC, iron, magnesium and manganese) and plant phylogenetic eigenvectors (PPCNM2, PPCNM14, PPCNM40, PPCNM49 and PPCNM55) (Table S7). Similarly, Tedersoo et al. [22] found that Salicaceae plant phylogeny explained 75% of the variation in its EcM fungal richness. More studies demonstrated the strong influences of edaphic variables on fungal richness, such as pH [34, 77], calcium [21], carbon [78], C/N ratio and phosphorus [38]. The effects of plant functional traits, such as the contents of rhizospheric exudates and litter chemistry [29, 79], need to be addressed in future studies to be able to disentangle the plant-related effects. In addition, more complicated models (e.g., nonlinear equations) may increase the explanatory power of variables.

Effects of plant phylogeny and species identity on community composition

Consistent with the first hypothesis, soil fungal communities were strongly influenced by plant phylogeny and plant species identity, even after accounting for abiotic environmental filtering and geographic distance. The effects of phylogenetic eigenvectors were evident in different statistical models (e.g., DISTLM and VPA) and consistent regardless of using different approaches of phylogenetic tree construction. In DISTLM and VPA, plant phylogenetic eigenvectors explained 11.4% and 15.8% of the variation in fungal community composition (Table S8, Fig. S8), and plant species identity (as dummy variables) explained 11.5% and 16.8% of the variation in the alternative multivariate models, respectively (Table S17, Fig. S16).

Previously, Tedersoo et al. [22] found that plant phylogenetic eigenvectors explained 20% of the variation in EcM fungal communities of Salicaceae in Estonia, and Põlme et al. [21] reported that host phylogenetic eigenvectors explained 43% of the variation in biogeography of EcM fungi associated with *Alnus* species at the global scale. The broader phylogenetic scale in our study is likely to yield stronger phylogenetic signals on the associations between woody plants and associated fungal communities. When we truncated the phylogenetic tree to angiosperms only, the power of the phylogenetic signal decreased (11.4% vs. 8.3% in Table S8 and S13, respectively), although it was still substantial. Based on 248 woody and palm species within a 50-ha tropical forest plot, Barberan et al. [31] also observed a strong correlation between soil fungal community composition and phylogenetic distance of aboveground plants after controlling for the variance in soil properties (partial Mantel $r = 0.18$). In our study, the dissimilarities in soil fungal community significantly increased with the increasing woody plant phylogenetic distance (Fig. 3), and there was a significant correlation between soil fungal community composition and plant phylogenetic distance after controlling for the environmental drivers (partial Mantel $r = 0.07$).

Although the influence of plant phylogeny on soil fungal communities was not as strong in our study as that reported in some studies for biotrophic fungal groups [18, 21, 22] and in analyses at local scales [31], the significant effect of plant phylogeny on soil fungal community composition is nonetheless of great ecological and evolutionary importance. The finding reflects the pronounced role of trees as superorganisms in the forest ecosystem and highlights that the evolutionary association between woody plants and fungi may be broadened to both saprophytic and biotrophic groups in the neighboring soil around trees [31, 80], even at a broad spatial scale.

In this study, plant phylogenetic eigenvectors explained significantly greater variation in community composition of EcM fungi and plant pathogens compared with that of saprotrophs (Tables S9 and S12), and the correlation coefficients between fungal community composition and plant phylogenetic distance were larger for EcM fungi and plant pathogens than that for saprotrophs (Fig. 3). These results support our second hypothesis and suggest that the influence of plant phylogeny on fungal communities may differ by guilds. Previously, Nguyen et al. [79] found that species richness of EcM fungi and saprotrophs was linked to different tree community attributes in a tree diversity field experiment, and the effect of plant species composition on fungal community composition was much greater for EcM fungi than for saprotrophs. Along an alpine treeline ecotone, Vasutova et al. [81] observed distinct environmental driving forces on the community composition of mycorrhizal and saprotrophic fungi, respectively.

For host-dependent fungal guilds, niche breadth and ecological specialization are expected to link more closely to plant phylogeny, considering their genetic compatibility and coevolutionary processes [82, 83]. For free-living fungal guilds (mainly saprotrophs), there is suspected to be a relatively high functional redundancy [42] and susceptibility of local species pools to abiotic environmental filtering [23, 36], which will substantially obscure the effects of plant

phylogeny. Of note, the biotrophic fungi assigned in this study were not strictly sampled from fine roots or diseased leaves as implemented in other studies [e.g.,18, 21, 22]. Thus, verification of functional guild assignments of the detected fungal taxa by simultaneously collecting diseased leaves, fine roots, rotten wood and litters would be valuable in future studies.

The nested PERMANOVA showed that the variation in fungal community composition was significantly explained by multiple plant taxonomic levels, from phyla to species, but the majority (24.1%) of the variation was explained by species identity (Table S15). When partitioning the plant species effect and phylogeny effect by VPA, we found that the effect of phylogenetic eigenvectors on soil fungal communities almost overlapped that of species identity (Fig. S17–S18). Of note, plant species identity still exclusively explained 0.4%–1.0% of the variation in fungal community composition. As previously stated by Tedersoo et al. [22], the eigenvector approach for the quantification of plant phylogeny accounts for both deep-diverging clades and terminal taxa across the overall phylogenetic tree and thus covers both the species identity and phylogenetic distance *per se*. In this study, plant species effect and phylogeny effect were very similar and hard to disentangle.

Biogeographic pattern of forest soil mycobiome and its possible causes

Although we observed the significant phylogenetic imprint and species identity effect of woody plants on the soil mycobiome in natural mountain forests of eastern China, abiotic environmental filtering was still the primary driving force on soil fungal biogeography in this study (Table S8, Fig. S11). Previously, Bonito et al. [84] proposed that soil geographic origin rather than soil properties had a stronger influence on root-associated fungal communities than did plant species identity, whereas more studies reported a larger influence of abiotic environmental variables on microbial community composition relative to that of plant factors [16, 36, 85, 86].

In this study, environmental variables explained 24.1% of the variation in soil fungal community composition (Table S8), which was larger than that explained by space (7.2%) or plant phylogeny (11.4%). Soil pH, MAT, and MAP were chosen by the function *bioenv* as the best subset of environmental drivers of community composition with the maximum correlation with fungal community dissimilarity matrix (Mantel $r = 0.68$, Fig. S11). In alpine grasslands of the Tibetan Plateau and temperate grasslands of northern China, soil pH, MAT, and MAP were included in the best subset of environmental drivers of soil fungal community composition [38, 87]. In the Arctic, Timling et al. [88] also reported that soil fungal community composition was strongly correlated with soil pH, temperature and precipitation. These results revealed that the environmental factors that generate and maintain biogeographic patterns in macroorganisms, such as available water and ambient heat [89], similarly operate in the soil fungal world; soil pH, which typically drives soil bacterial communities [90, 91], also plays a pivotal role in soil fungal distributions.

Soil fungal biogeography in forests consists of a mixed effect of biotic interaction, abiotic environmental filtering and geographic isolation, and there are complex interactions among the three factors. Additional studies similar to what we present here are strongly encouraged for comparison; comparison of the cross-continental biogeographic pattern between similar forests in eastern Asia and eastern North America is especially intriguing as a future investigation. In this study, 11.7% of the explanatory rate on the variation in fungal community composition was shared by abiotic environmental variables and plant factors, and 7.1% of that was shared by spatial vectors and plant factors (Fig. S17). In an investigation of a relict *Pseudotsuga japonica* forest, Murata et al. [92] found that most of the EcM fungi of *P. japonica* originated from host shifts in neighboring areas, which represented an integrated function of plant selection and spatial distance *per se*. Indeed, because of the overlapping nature of belowground networks (e.g., mycelium and fine roots) and aboveground canopies in natural forests, the soil fungal communities around tree trunks may also be influenced by the fine roots and fallen leaves from other adjacent plants. Likewise, several studies have reported that tree species identity can affect a series of soil properties in local forests, such as pH, element content and physical structure [93, 94]. In turn, plant-mediated variance in soil properties has been inferred to affect soil fungal communities directly [28], which reflected the interactive effects of biotic and abiotic variables on fungal communities [95].

Conclusion

Each individual tree is a superorganism, and soil fungal communities around trees should be regarded as an extension of their bodies [96] or considered as a key plant trait [20]. We identified a significant relationship between soil fungal community composition and woody plant phylogeny in this survey encompassing 58 plant species from temperate to subtropical forests in eastern China. In the multivariate analyses, both plant phylogeny and species identity strongly influenced soil fungal community composition, suggesting either partner choice or shared habitat preferences of trees and their adjacent soil fungal communities. Abiotic environmental filtering was the primary driving force in soil fungal biogeography, and intertwined with the

plant effects and geographic isolation. Edaphic variables, as representative of abiotic environmental filtering, explained the largest variation of soil fungal community composition. The communities of EcM fungi and plant pathogens were significantly more strongly affected by plant phylogeny than that of saprotrophs, suggesting the role of trophic status. This study provides novel insights into the association between soil fungi and woody plants with respect to phylogeny and indicates that the organization of plant-fungal biodiversity is at least partially genetically based and non-neutral. The findings are also integral to a better understanding of species coexistence and diversity maintained in the fragmented mountain forests of China as well as other areas of the world.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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