Mycosphere

ARTICLE

Open Access

https://doi.org/10.48130/mycosphere-0026-0011

Mycosphere **2026**, 17: e010

Latitudinal pattern in diversity, community assembly, and network structure of phyllosphere fungi in Chinese forests

Xingchun Li[1,2#], Jie Li[1,3#], Adam Frew[4], Yong Zheng[5], Cong Wang[1,2], Lin Jiang[6], Liangdong Guo[1,2*], Claire Elizabeth Willing[7,8] and Cheng Gao[1,2*]

[1] *State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*
[2] *College of Life Sciences, University of Chinese Academy of Sciences, Beijing 101408, China*
[3] *China General Microbiological Culture Collection Center (CGMCC), Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*
[4] *Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales 2747, Australia*
[5] *Key Laboratory for Humid Subtropical Eco-geographical Processes of the Ministry of Education, Fujian Normal University, Fuzhou 350117, China*
[6] *School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA*
[7] *Department Biology, Stanford University, Stanford, CA 94305, USA*
[8] *School of Environmental and Forest Science, University of Washington, Seattle, WA 98195, USA*
[#] Authors contributed equally: Xingchun Li, Jie Li
[*] Correspondence: guold@im.ac.cn (Guo L); gaoc@im.ac.cn (Gao C)

Abstract

Deciphering latitudinal diversity patterns is critical for understanding biodiversity generation and its ecosystem consequences. However, the latitudinal distribution of community diversity, assembly, and network structure of phyllosphere fungi in forest ecosystems remains poorly understood. In this study, we examined phyllosphere epiphytic and endophytic fungal communities from 172 plant species spanning 21 Chinese forests (18° to 53° N) using amplicon sequencing techniques. The alpha and beta diversity of phyllosphere epiphytic and endophytic fungi displayed a U-shaped latitudinal pattern, which was mainly driven by plant identity, species pool, space, and climatic factors. The community assembly of phyllosphere epiphytic and endophytic fungi was predominantly governed by stochastic processes, and the relative importance of stochastic processes on community assembly of epiphytic fungi rather than endophytic fungi showed a unimodal latitudinal pattern. The plant-epiphytic fungus networks were more stable but less complex than plant-endophytic fungus networks in most forests. The complexity of plant-epiphytic fungus networks, rather than plant-endophytic fungus networks, decreased with increasing latitude. These findings enhance our understanding of species co-occurrence and community stability in forest ecosystems.

Keywords: Alpha diversity, Beta diversity, Community assembly, Latitudinal pattern, Network structure, Phyllosphere fungi

Citation: Li X, Li J, Frew A, Zheng Y, Wang C, et al. 2026. Latitudinal pattern in diversity, community assembly, and network structure of phyllosphere fungi in Chinese forests. *Mycosphere* 17: e010 https://doi.org/10.48130/mycosphere-0026-0011

Introduction

As biodiversity supports ecosystem function and resilience, extensive research has focused on identifying the ecological forces shaping diversity across spatial scales[1,2]. While ecologists have long recognized predictable shifts in species diversity along environmental gradients, much of this work has centered on plants, animals, and soil microbes[1,3–6]. As an important component of aboveground microorganisms, phyllosphere fungi, including epiphytes living on leaf surfaces and endophytes living inside leaves, are of high species diversity and have important ecological functions[7–9]. These fungal symbionts shape patterns of plant community assembly, productivity, and ecosystem stability[10–14]. In return, plants may affect the phyllosphere fungal community via specificity and supply of carbon resources from hosts[9,15,16]. Although latitudinal gradients in plant diversity are well-established, the mechanisms driving the latitudinal diversity pattern of phyllosphere fungal communities remain unclear. Therefore, understanding these microbial biogeographic patterns is essential for uncovering the forces that govern biodiversity and for enhancing our ability to predict how ecological communities will respond to climate change-driven disturbances and their ecological consequences.

The fungal diversity distribution in different ecosystems may be driven by plant and abiotic factors, such as temperature and precipitation[9,17,18]. There are many studies concerning the alpha and beta diversity distribution of soil fungi in ecosystems, and some have found various latitudinal patterns, such as a decreased[1,5,19–21], a hump-shaped[22,23], a concave[24], and no[25] latitudinal patterns of soil fungal alpha and beta diversity. However, a few studies have been conducted on the distribution pattern of phyllosphere fungal diversity[18,26–29]. For example, some studies reported a decreased latitudinal pattern of phyllosphere endophytic fungal alpha diversity in land plants[26,27] and in the savanna species *Themeda triandra*[18]. By contrast, a hump-shaped latitudinal pattern and an increased latitudinal pattern of phyllosphere fungal alpha diversity were found in *Quercus robur* (42.0–61.0° N)[29] and in *Pinus sylvestris* (55.0–66.5° N)[28], respectively. For beta diversity distribution, a previous study found a higher beta diversity of endophytic fungi in boreal forest (three plant species) than in temperate (three plant species) and tropical (six plant species) forests[26]. In addition, a previous study found that the alpha and beta diversity of endophytic and epiphytic fungi increased with plant species diversity in a subtropical forest[9]. These various distribution patterns may be ascribed to different energy availability caused by temperature and rainfall, distinct host specificity, or edge effect of host distribution. Generally, the high plant species diversity and favorable hydrothermal conditions in low-latitude regions may support high fungal diversity, compared with high-latitude regions. However, a comprehensive assessment of the latitudinal pattern of both alpha and beta diversity in phyllosphere epiphytic and endophytic fungal communities in forest ecosystems is still lacking.

A general framework of assembly rules reflects that species co-occurrence results from phylogeographic (speciation and migration) and ecological assembly processes[30]. Historical speciation, extinction, and migration form a regional species pool[31], which may drive local diversity patterns[4,32]. For example, previous studies have found that regional species pools affected the alpha and beta diversity patterns of plants[4,32–34] and animals[35]. By contrast, a few studies elucidated the effect of species pool on fungal diversity patterns[36,37]. For example, Chen et al.[36] found a positive correlation between regional species pool and fungal beta diversity in forest, grass, and wetland soils on a national scale, suggesting a relative contribution of species pool to fungal community assembly. However, the effect of species pool on community assembly of phyllosphere epiphytic and endophytic fungi in forest ecosystems has not been studied.

In addition to species pools, ecological processes, including deterministic and stochastic processes, play important roles in community assembly[38–40]. Most previous studies reported that deterministic processes mainly shaped community assembly of phyllosphere epiphytic or/and endophytic fungi[41–44], while some showed that stochastic processes were predominant[9,45,46]. Furthermore, the relative importance of ecological assembly processes for regulating species diversity patterns differed across various biogeographical regions and was mediated by local environmental factors, such as temperature and precipitation[47–52]. For plant communities, some studies found that environmental filtering played a more important role in temperate compared to tropical forests[47,48]. This latitudinal gradient is often attributed to increasing environmental harshness at higher latitudes strengthening deterministic filtering[53,54], whereas higher productivity at lower latitudes could increase the importance of stochastic processes, such as ecological drift and priority effect[55,56]. In contrast to the well-documented patterns in plant communities, the latitudinal patterns in fungal community assembly remain poorly understood[49,50]. For example, the relative importance of stochastic processes in soil fungal community assembly was higher in low compared to high latitudinal paddy soils, due to less environmental filtering in tropical regions[49,50]. These findings imply that deterministic processes may play a more important role in shaping phyllosphere fungal communities in high-latitude than in low-latitude regions. However, the latitudinal pattern in phyllosphere epiphytic and endophytic fungal community assembly in forest ecosystems has not been studied.

Unveiling plant-fungus interactions using ecological network analysis can enhance our understanding of the mechanisms underlying biodiversity maintenance and community stability[57,58]. In the main topological properties of a network, high modularity enhances overall network stability by confining perturbations to individual modules and preventing them from spreading to others, thereby protecting communities from secondary extinctions[59]. Nestedness and connectance, shaped by variations in climate and plant community composition[9,60], can reflect network complexity[9,61]. Many studies have been conducted on network analysis of phyllosphere epiphytic and endophytic fungi in forest[9,16,27], grassland[62], coastal[63], and mangrove[15,64] ecosystems, and some found that they were mainly characterized by high specialization and modularity, but less connectance and nestedness. Furthermore, a previous study revealed a higher specialization of plant-endophytic fungus networks in subboreal than in subtropical forests[27]. In addition, a positive relationship between the complexity of plant-epiphytic and plant-endophytic fungus networks and tree species diversity was reported in a subtropical forest[9], which may be due to more potential species interactions caused by a greater host range overlap in higher tree diversity forests. This finding suggests that the complexity of the phyllosphere fungus network is higher in low-latitude than in high-latitude regions, as plant species diversity is higher in low latitude compared with high latitude regions. However, the latitudinal pattern of the network structure of phyllosphere epiphytic and endophytic fungi in forest ecosystems has rarely been investigated.

In order to better understand the latitudinal pattern of diversity, community assembly and network structure of phyllosphere epiphytic and endophytic fungi, we examined phyllosphere fungal communities from 172 plant species spanning 21 Chinese forests (18° to 53° N) using Illumina Novaseq high-throughput sequencing techniques. We hypothesized that: (1) the alpha and beta diversity of epiphytic and endophytic fungi decrease with increasing latitude; (2) the community assembly of epiphytic and endophytic fungi is mainly shaped by deterministic processes, and the strength of deterministic processes increases with increasing latitude; and (3) the complexity of plant-epiphytic and plant-endophytic fungus networks decreases with increasing latitude in forest ecosystems.

Materials and methods

Study site and sampling

This study was conducted at 21 forest sites along a latitudinal gradient in China (21.36°–53.33° N, 100.36°–130.01° E; Supplementary Fig. S1a). Leaf samples were collected from July to October 2020. At each site, we selected nine to 12 common woody plant species, and 30 healthy leaves (*ca*. 20 g) were collected from each of five individuals (replicates, 50 m away from each other) of each plant species. The collected leaf samples were immediately placed in sterile plastic bags, labeled, transported to the laboratory in an ice box, and stored at −80 °C until DNA extraction. In total, 1,197 leaf samples were collected from 172 plant species across 103 genera and 54 families (Supplementary Fig. S1b). Additionally, estimates of the mean annual temperature (MAT), mean annual precipitation (MAP), temperature seasonality (TS), and precipitation seasonality (PS) were obtained from the WorldClim global climate data (www.worldclim.org) set with a resolution of 2.5 min. Geographic coordinates (latitude and longitude) and altitude were recorded using an M-241 GPS instrument (Holux Technology Inc., Taiwan, China). Information on the plant species, geography, and climate of each site is provided in Supplementary Table S1.

DNA extraction, amplicon sequencing, and bioinformatics

We extracted DNA of epiphytic and endophytic fungi following Yao et al.[15]. Briefly, epiphytic fungi from each leaf sample (5 g frozen leaves) were washed from leaf surfaces using sonication (45 s) and vortexing (30 s). For endophytic fungi, the surface sterilization of the above leaf samples was carried out in 75% ethanol (1 min), 3.25% sodium hypochlorite (3 min), and 75% ethanol (30 s), followed by three rinses in sterile distilled water. The DNA of the epiphytic and endophytic fungi was extracted using the modified CTAB method[15], and the DNA concentration was measured using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, USA).

We amplified the fungal internal transcribed spacer 1 (ITS1) region of rDNA using the primer pairs ITS1F and ITS2[65] linked with 12-base barcode sequences to enable sample identification following Yao et al.[15]. The PCR products from three amplifications of each sample were pooled, purified with EZNA Gel Extraction Kit (Omega, Norcross, GA, USA), and quantified with Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). We combined equal

amounts (100 ng) of purified DNA from each sample and adjusted the mixture to 10 ng μL^{-1}. Following the manufacturer's instructions of the VAHTS Universal DNA Library Prep Kit for Illumina V3 (Illumina, CA, USA), we added an Illumina sequencing adaptor (5′-GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG-3′) to the products to construct the sequencing library. Finally, the library was sequenced on the Illlumina NovaSeq PE250 platform with NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

The raw sequence data were processed using Quantitative Insights into Microbial Ecology 2 (QIIME 2)[66]. After quality filtering (requiring a quality score > 25, no ambiguous bases, with valid primer sequence or barcode sequence and length > 250 bp), the DADA2 pipeline was applied to perform denoising, dereplication, and chimeras removal[67]. The ITS1 sequences were extracted using the ITSx software package[68] and clustered into operational taxonomic units (OTUs) at a 97% sequence similarity level with VSEARCH v2.9.1[69]. For taxonomic assignment, the most abundant ITS1 sequence from each OTU (≥ 10 reads) was searched against the 'unified system for the DNA-based fungal species linked to the classification' (UNITE) database[70] using the sintax function[71] in USEARCH with a confidence cut-off (p) value of 0.65. Finally, the sequence number per sample was rarefied to the smallest sample size using the sub.sample command in MOTHUR[72]. In addition, the potential pathogenic fungi were predicted based on the FungalTraits database[73]. Information about fungi detected in the study is summarized in Supplementary Table S2.

Statistical analysis

All the statistical analyses were implemented in R v4.0.3, except for the network modularity analysis in the program MODULAR[74]. The alpha diversity of fungi was measured using two indices: OTU richness (the number of fungal OTUs in a sample) and Shannon's diversity index. The beta diversity of fungi, as the fungal community dissimilarities of paired samples, was measured using Bray–Curtis and Simpson dissimilarity metrics. Bray–Curtis metric, which incorporates species abundance data[75], can be confounded by variations in fungal richness[76]. To account for variation in plant species and fungal richness among sites, fungal beta diversity was calculated using the Simpson dissimilarity metric based on presence/absence data among the five replicates of the same plant species in each site using the beta.pair command in the Betapart package[77]. The gamma diversity is defined as the total number of fungal OTUs in a site, which represents the species pool. The relationships between the alpha and beta diversity of fungi and latitude were evaluated using first- and second-order polynomial regression models, with plant identity as a random factor, and model selection was performed using the Akaike Information Criterion (AIC).

We constructed plant phylogenetic trees based on the phylogeny[78] as the backbone using phylomatic-awk[79]. Pairwise patristic distances of plant phylogeny (pairwise sums of the branch lengths connecting terminal taxa) were calculated using the cophenetic.phylo command in the Ape package[80]. Principal coordinate analysis (PCoA) was used to convert the pairwise patristic distances to phylogenetic eigenvectors using the cmdscale command in the Vegan package[81]. We generated the spatial variables using Moran's eigenvector maps (MEM)[82] from the site coordinates (latitude and longitude) using the dbmem command in the Adespatial package[83]. All the environmental variables were standardized prior to subsequent statistical analyses. In order to avoid multicollinearity among the variables, we deleted the variables with the variance inflation factor (VIF) > 10.

The variation in alpha (OTU richness) and beta (Simpson dissimilarity) diversity of epiphytic and endophytic fungi was partitioned by plant identity, species pool, space, and climatic variables using the varpart command in the Vegan package. Additionally, the distance matrices of community composition (Hellinger transformation of the OTU read data) of epiphytic and endophytic fungi were constructed by calculating dissimilarities using the Bray–Curtis method. Subsequently, non-metric multidimensional scaling (NMDS) was used to visualize the community dissimilarities of epiphytic and endophytic fungi using the metaMDS command in the Vegan package. Furthermore, permutational multivariate analysis of variance (PerMANOVA) was applied to explore the effect of site on the epiphytic and endophytic fungal community compositions. The relative abundance of abundant epiphytic and endophytic fungal OTUs (> 0.2% of total fungal reads) among different sites was depicted using the pheatmap function in the Pheatmap package v1.0.8[84].

We built a structural equation model (SEM) to test for the direct and indirect effects of host plant species, climate, space, and species pool on fungal alpha and beta diversity using the PiecewiseSEM package[85]. Prior to modeling, bivariate correlations were performed among all variables to assess the suitability of a linear model, and all predictors and response variables were standardized before analyses using the z-score to interpret parameter estimates on a comparable scale. Additionally, we used Fisher's C-test (when $0.05 < p < 1.00$) to confirm the goodness of the modelling results. We then modified our models according to the significance ($p < 0.05$) and the goodness of the model. We used plant phylogenetic eigenvectors and spatial eigenvectors to represent host plant species and space variables, respectively. For graphical simplicity, we constructed three composite variables for climate, plant phylogenetic eigenvectors, and spatial eigenvectors.

To assess the effect of species pool on beta diversity of epiphytic and endophytic fungi, we first simulated the expected relationship between beta diversity among communities that are randomly sampled from a species pool without any community assembly processes using a lognormal species abundance distribution and gamma diversity[4]. Then, we examined the correlation between the observed beta diversity and gamma diversity using Spearman's correlation. If the observed correlation is significant and consistent with the expected correlation, that indicates the species pool has an influence on beta diversity.

Furthermore, we used beta deviation based on the null model method to compare the relative importance of species pool and ecological assembly processes on fungal beta diversity. Briefly, we calculated observed beta diversity and expected beta diversity from the null model as the distance from a sample to the centroid of the group of all samples within a site (distance-to-centroid) using the betadisper function in the Vegan package. Then, beta deviation was calculated using the formula: Beta deviation = [Beta-diversity$_{observed}$ – Mean (beta-diversity$_{expected}$)]/SD [beta-diversity$_{expected}$]. In addition, a one-sample t-test was used to compare mean beta deviation against zero in each site. If a mean beta deviation is statistically indistinguishable from zero, this indicates that the species pool dominates beta diversity within a site. By contrast, if a mean beta deviation is significantly different from zero, this indicates that ecological assembly processes dominate beta diversity within a site[86].

Additionally, we further assessed the relative importance of different ecological assembly processes on epiphytic and endophytic fungal community assembly using inferred community assembly mechanisms by phylogenetic bin-based null model analysis (iCAMP)[87]. We calculated the beta Net Relatedness Index (βNRI) of fungal OTUs in each bin based on a fungal phylogenetic tree

(constructed from 18S + 28S rDNA sequences using the taxonomy_to_tree.pl script of Tedersoo et al.[88]) and the Raup–Crick index (RC_{bray}) using the scripts of Chase et al.[89] and Ning et al.[87]. For each bin, heterogeneous selection was recognized as βNRI > 1.96, homogenizing selection as βNRI < −1.96, homogenizing dispersal as |βNRI| < 1.96 and RC_{bray} < −0.95, dispersal limitation as |βNRI| < 1.96 and RC_{bray} > 0.95, and drift or others (mostly consists of weak selection, weak dispersal, diversification, and/or drift) as |βNRI| < 1.96 and $|RC_{bray}|$ < 0.95. Subsequently, the relative importance of individual ecological assembly processes at the whole community level was assessed by weighting with the relative abundance of each bin. Then, the relationships between the relative importance of different ecological processes and latitude were evaluated using the lm function in the Stats package[90].

According to Toju et al.[91], we examined the architecture properties of plant-fungus networks, including the H_2' metric of the network-level interaction specialization[92], the weighted connectance[93], the weighted nestedness metric based on overlap and decreasing fill (WNODF)[94], and Barber's metric of modularity[95]. Briefly, original species-level matrices were obtained from the OTU-level matrix. Randomized matrices were obtained using a shuffle-sample null model with 1,000 permutations for performing a randomization test. Based on the original and randomized species-level matrices, we calculated the weighted connectance, H_2', and WNODF using the network-level command in the Bipartite package[96] and modularity using the MODULAR program[74]. The significance of the difference between observed and random network properties was examined using a two-tailed t-test at $p < 0.05$. To compare network properties in different forest sites and correct variances in network properties caused by species richness and number of interactions, we performed Z-score normalization for the network structure indices. The Z-score is defined as follows: $Z = (E_{observed} - E_{randomized})/SD_{randomized}$, where $E_{observed}$ is the observed value, $E_{randomized}$ and $SD_{randomized}$ are the mean value and the standard deviation of randomized matrices[97]. Then, the relationships between the structures of plant-fungus networks and latitude were evaluated using the lm function in the Stats package.

Results

General characterization of high-throughput sequencing data

After quality control, 42,366,526 high-quality ITS1 sequences were obtained from 45,779,701 raw sequences and divided into 56,804 OTUs at a 97% sequence similarity level. Of these OTUs, 20,391 (36,053,961 sequences) were identified as fungi. As the fungal sequence numbers ranged from 1 to 128,780 across 2,394 samples, the sequence number was normalized to 510 (3 epiphytic samples and 103 endophytic samples with < 510 sequences excluded). The normalized dataset contained 13,187 epiphytic fungal OTUs, which mainly consisted of 9,368 Ascomycota (71.9% of total epiphytic fungal reads) and 2,920 Basidiomycota (13.0%) (Supplementary Fig. S2a). It also contained 6,146 endophytic fungal OTUs, which mostly consisted of 4,337 Ascomycota (77.9% of total endophytic fungal reads) and 1,054 Basidiomycota (13.1%) (Supplementary Fig. S2b).

Additionally, we identified 399 potential epiphytic pathogenic fungal OTUs (8.8% of total epiphytic fungal reads) and 344 potential endophytic pathogenic fungal OTUs (19.4% of total endophytic fungal reads). The potential plant pathogens were dominated by a few genera, such as *Ramularia*, *Strelitziana,* and *Pseudocercospora* in the epiphytic community and *Colletotrichum*, *Ramularia,* and *Pseudocercospora* in the endophytic community (Supplementary Fig. S3).

Latitudinal pattern of epiphytic and endophytic fungal diversity

The OTU richness and Shannon's diversity index of epiphytic fungi ranged from 78.5 ± 24.9 to 192.7 ± 33.6 and from 2.9 ± 0.5 to 4.5 ± 0.6 (means ± SD) in different sites, and exhibited strong quadratic relationships with latitude (Fig. 1a, b; Supplementary Table S3). Similarly, the OTU richness and Shannon's diversity index of endophytic fungi ranged from 23.2 ± 11.0 to 83.3 ± 48.9 and from 1.4 ± 0.9 to 3.2 ± 1.2 in different sites, and displayed a U-shaped pattern with latitude (Fig. 1c, d; Supplementary Table S3). The beta diversity (Bray–Curtis dissimilarity) of epiphytic and endophytic fungi showed a U-shaped latitudinal pattern (Fig. 1e, f). Similarly, the beta diversity (Simpson dissimilarity) of epiphytic and endophytic fungi fitted well with quadratic regression models after controlling for host plant species and fungal richness (Fig. 1g, h; Supplementary Table S3).

Furthermore, the OTU richness of both epiphytic and endophytic pathogenic fungi showed a U-shaped latitudinal pattern (Supplementary Fig. S4a, b). Similarly, the beta diversity (Simpson dissimilarity) of epiphytic pathogenic fungi decreased with increasing latitude after controlling for host plant species and pathogenic fungal richness (Supplementary Fig. S4c), while that of endophytic pathogenic fungi fitted well with quadratic regression models (Supplementary Fig. S4d). Additionally, the relative abundance of potential epiphytic and endophytic pathogenic fungi decreased with increasing latitude (Supplementary Fig. S4e, f).

Variation partitioning analysis showed that 66.8% and 50.9% of the variation in epiphytic and endophytic fungal OTU richness was explained by plant identity (64.3% and 45.4%), species pool (44.0% and 27.1%), spatial distance (41.0% and 22.1%), and climatic factors (27.1% and 17.9%) (Fig. 2a, b).

The epiphytic fungal community was dominated by Dothideomycetes and Eurotiomycetes, and the endophytic fungal community was dominated by Dothideomycetes and Sordariomycetes at the class level, with various relative abundances among sites (Fig. 3a, b). For example, Dothideomycetes were relatively abundant in mid-latitudinal regions, but Sordariomycetes were relatively abundant in low-latitudinal regions (Fig. 3a, b). The heatmap revealed that the occurrence of some relatively abundant epiphytic and endophytic fungal OTUs was different among forest sites (Supplementary Fig. S5). For example, epiphytic fungal OTU8803 (*Paraconiothyrium*) occurred mainly in DLS and TYS, while OTU9678 (*Ramichloridium*) occurred mainly in DHS (Supplementary Fig. S5a). Endophytic fungal OTU 9698 (*Pseudocercospora*) occurred mainly in DHS, NG, BB, and NBH, while OTU 8803 (*Paraconiothyrium*) occurred mainly in DLS, TYS, and BTM (Supplementary Fig. S5b). The NMDS ordination and PERMANOVA showed that the community composition was significantly different between epiphytic and endophytic fungi ($R^2 = 0.042$, $p = 0.001$; Fig. 3c). Furthermore, the site had a significant effect on the community composition of epiphytic and endophytic fungi ($R^2 = 0.457$, $p = 0.001$; $R^2 = 0.200$, $p = 0.001$; Fig. 3d, e). Variation partitioning analysis showed that 47.9% and 44.0% of the variation in epiphytic and endophytic fungal beta diversity was explained by plant identity (41.4% and 32.4%), species pool (32.0% and 21.4%), spatial distance (29.1% and 18.1%), and climatic factors (18.5% and 4.4%) (Fig. 2c, d).

The SEM analysis showed that the alpha diversity of epiphytic fungi was influenced by plant phylogeny, species pool, and climate directly and by plant phylogeny, space, and climate through species pool indirectly (Fig. 4a). The beta diversity of epiphytic fungi was influenced by plant phylogeny, species pool, space, and epiphytic fungal richness directly and by plant phylogeny, space, and climate through species pool and epiphytic fungal richness indirectly

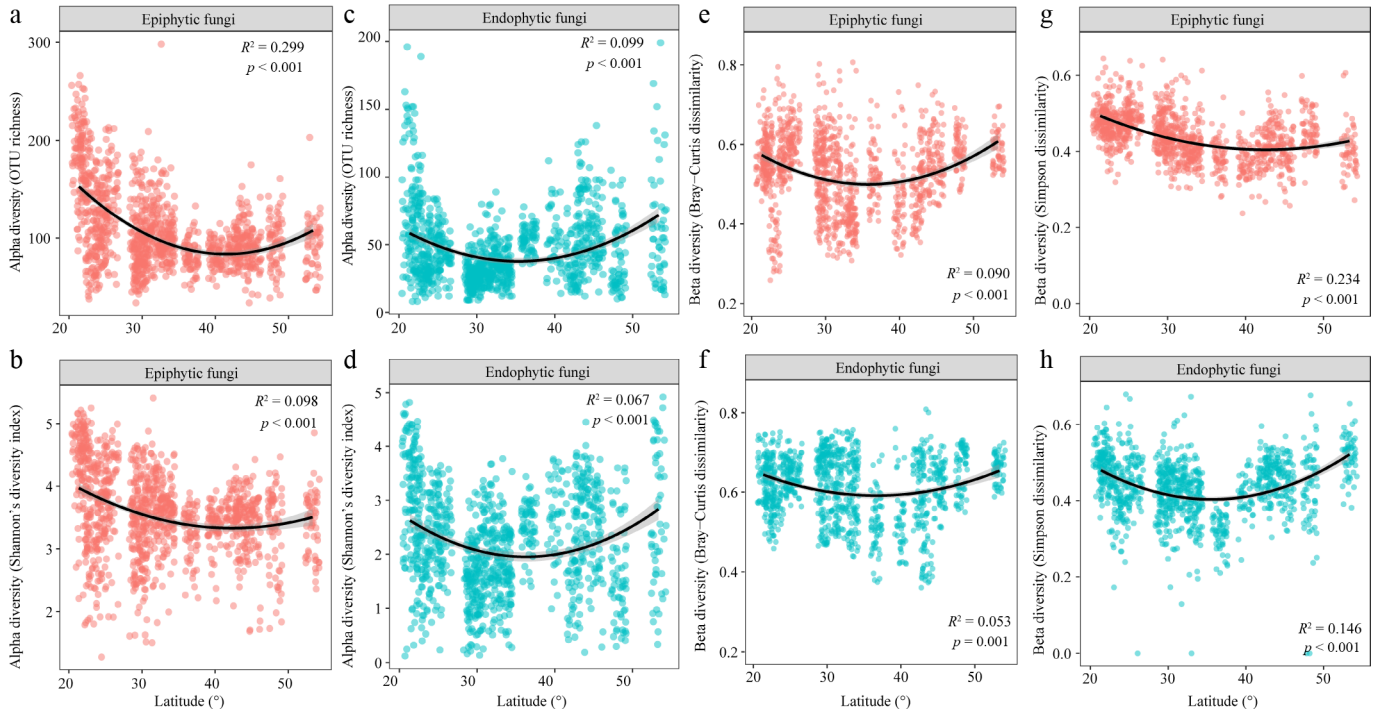

Fig. 1 Latitudinal patterns of the alpha and beta diversity of phyllosphere epiphytic and endophytic fungi.

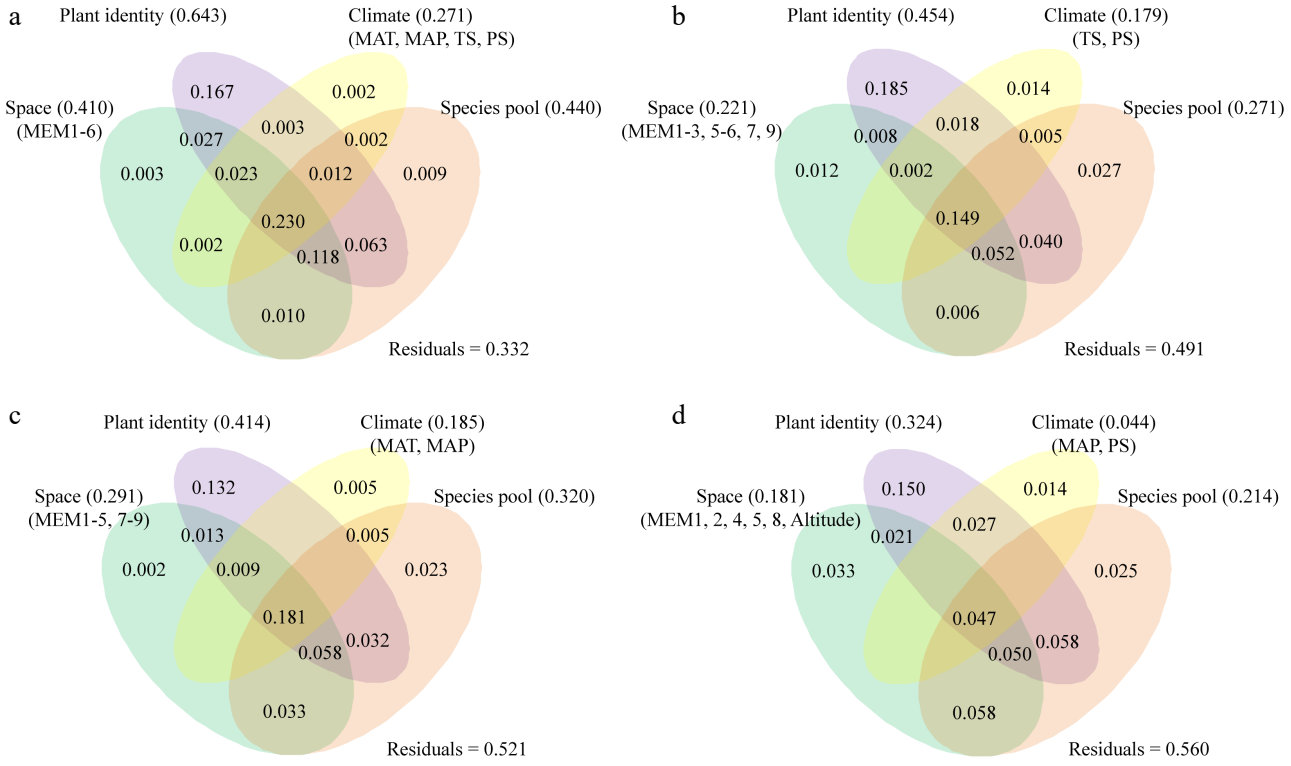

Fig. 2 Result of variation partitioning analysis showing the pure and shared effects of plant identity, species pool, space, and climatic variables on phyllosphere fungal diversity. (a) Richness of epiphytic fungal operational taxonomic units (OTUs). (b) Richness of endophytic fungal OTUs. (c) Beta diversity (Simpson dissimilarity) of epiphytic fungi. (d) Beta diversity (Simpson dissimilarity) of endophytic fungi. Numbers indicate proportions of explained variation (adjusted R^2 values). MAT, mean annual temperature; MAP, mean annual precipitation; TS, Temperature seasonality; PS, precipitation seasonality; MEM, Moran's eigenvector maps.

(Fig. 4a). The alpha diversity of endophytic fungi was influenced by plant phylogeny, species pool, and space directly and by plant phylogeny and space through species pool indirectly (Fig. 4b). The beta diversity of endophytic fungi was influenced by plant phylogeny, species pool, space, and climate directly and by plant phylogeny and space through species pool indirectly (Fig. 4b).

https://doi.org/10.48130/mycosphere-0026-0011

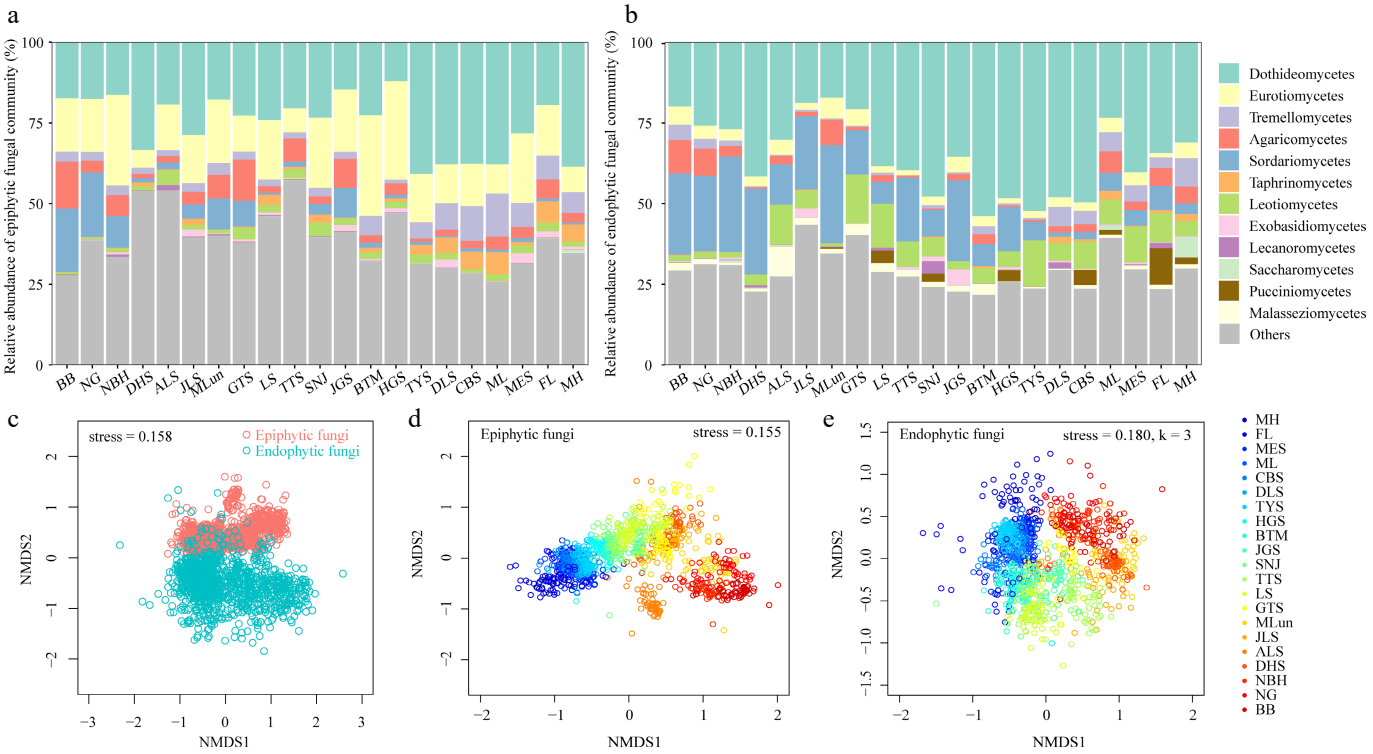

Fig. 3 Community composition of the phyllosphere epiphytic and endophytic fungi in different forest sites. Relative abundance of (a) epiphytic, and (b) endophytic fungi at the class level. (c)–(e) Non-metric multidimensional scaling (NMDS) ordination of the community composition (Bray–Curtis dissimilarity) of epiphytic and endophytic fungi in different sites. 'Others' includes the fungal classes with < 0.5% of the total epiphytic and endophytic fungal reads and operational taxonomic units unclassified to fungal class level. The sites are ranked from lowest to highest latitude. BB, Bubeng; NG, Nonggang; NBH, Nabanhe; DHS, Dinghushan; ALS, Ailaoshan; JLS, Jiulianshan; MLun, Mulun; GTS, Gutianshan; LS, Lushan; TTS, Tiantongshan; SNJ, Shennongjia; JGS, Jigongshan; BTM, Baotianman; HGS, Huangguanshan; TYS, Taiyueshan; DLS, Donglingshan; CBS, Changbaishan; ML, Muling; MES, Maoershan; FL, Fenglin; MH, Mohe.

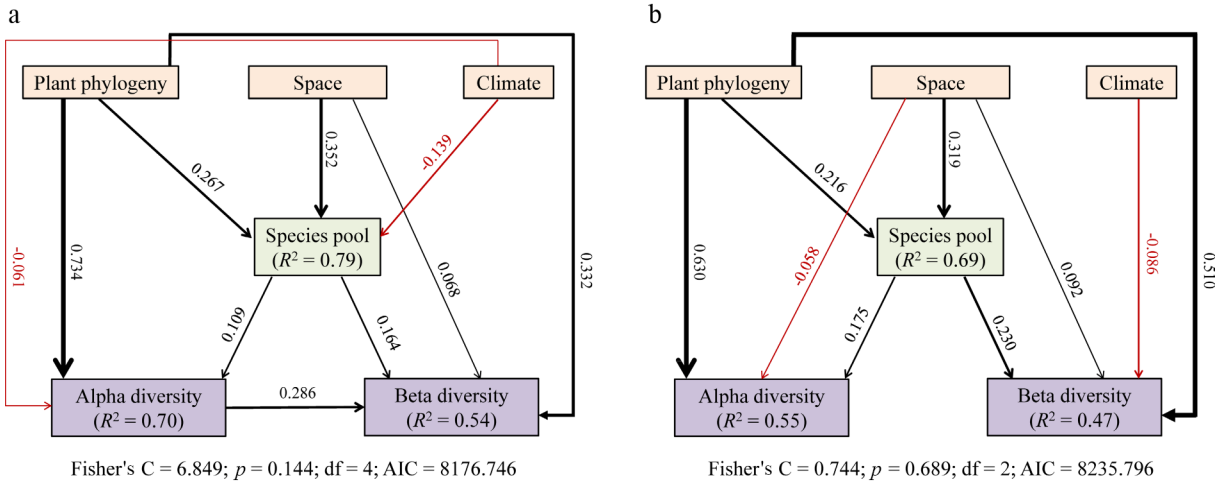

Fig. 4 Structural equation models (SEMs) showing the hypothesized relationships between plant phylogeny, species pool, space, climate, and fungal diversity. (a) Epiphytic fungi. (b) Endophytic fungi. The width of the lines represents the strength of the relationship (p < 0.05). Black and red solid lines indicate significant positive or negative correlations, respectively. Numbers above the lines indicate path coefficients. R^2 is the proportion of variance explained by the model. df, degree of freedom; AIC, akaike information criterion. The detailed information of plant phylogeny, space, and climate is listed in Supplementary Table S5.

Latitudinal pattern of epiphytic and endophytic fungal community assembly

The results of sampling simulation using a lognormal species abundance distribution revealed that the expected beta diversity was increased with increasing gamma diversity, regardless of the number of individuals (Fig. 5a). A significant correlation between the observed beta and gamma diversity of epiphytic and endophytic fungi was found and was consistent with the expected pattern (Fig. 5b, c). These results indicated that the species pool had an effect on the beta diversity of epiphytic and endophytic fungi.

The null model analyses showed that the mean beta deviation of epiphytic and endophytic fungal communities in each site was

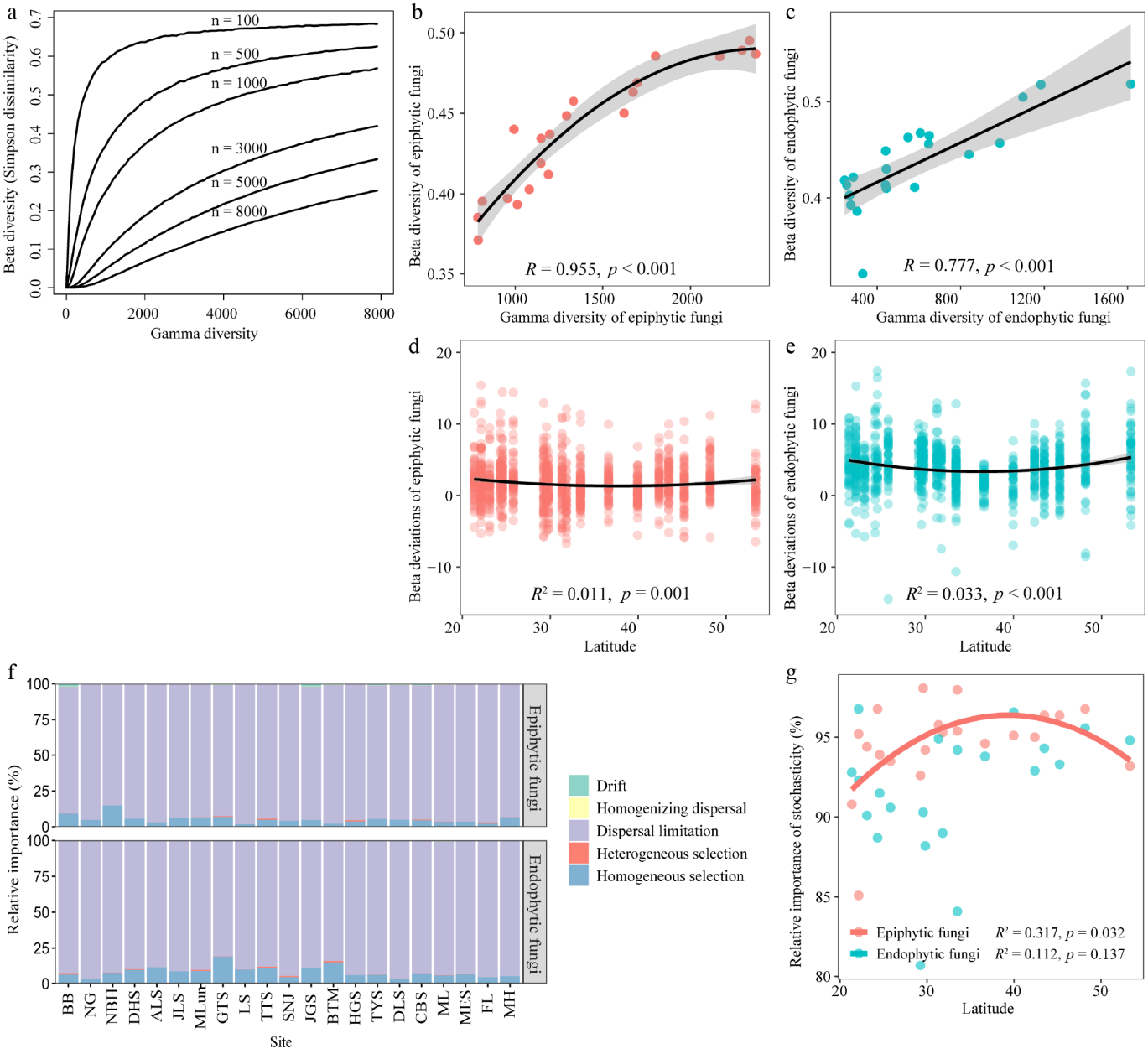

Fig. 5 Community assembly of phyllosphere epiphytic and endophytic fungi. (a) The expected beta diversity increased with increasing gamma diversity produced by a simple sampling simulation, using a lognormal species abundance distribution, and 'n' represented the number of individuals in each sample. Relationship between the observed beta diversity (Simpson dissimilarity) and gamma diversity in (b) epiphytic, and (c) endophytic fungi. Latitudinal pattern of beta deviation of (d) epiphytic, and (e) endophytic fungi. (f) Phylogenetic-bin-based null model analysis (iCAMP) showing the relative importance of each process on community assembly of epiphytic and endophytic fungi in each site, and the sites are ranked from lowest to highest latitude. (g) Latitudinal pattern of the relative importance of stochastic processes on epiphytic and endophytic fungal community assembly. BB, Bubeng; NG, Nonggang; NBH, Nabanhe; DHS, Dinghushan; ALS, Ailaoshan; JLS, Jiulianshan; MLun, Mulun; GTS, Gutianshan; LS, Lushan; TTS, Tiantongshan; SNJ, Shennongjia; JGS, Jigongshan; BTM, Baotianman; HGS, Huangguanshan; TYS, Taiyueshan; DLS, Donglingshan; CBS, Changbaishan; ML, Muling; MES, Maoershan; FL, Fenglin; MH, Mohe.

significantly greater than zero after controlling for species pool (Fig. 5d, e; Supplementary Table S4). This indicated that the ecological assembly processes, but not the species pool, predominantly governed the beta diversity pattern of epiphytic and endophytic fungi. Furthermore, the iCAMP analysis showed that epiphytic and endophytic fungal communities were mainly shaped by stochastic processes, including dispersal limitation (80.7%–98.0%) and drift (0%–1.9%), followed by deterministic processes, including homogeneous selection (1.9%–19.0%) and heterogeneous selection (0%–1.4%) in each site (Fig. 5f). In addition, the relative importance of stochastic processes on epiphytic but not endophytic fungal community assembly showed a hump-shaped latitudinal pattern (Fig. 5g).

Latitudinal pattern of plant-epiphytic and plant-endophytic fungus network structures

The network structure of plant-epiphytic and plant-endophytic fungi was generally characterized by high specialization and modularity but low connectance and nestedness (Supplementary Fig. S6). Namely, compared with the null models, the observed value of

weighted connectance was significantly lower (except for endophytic fungal networks in BB, ALS, JLS, GTS, CBS, and MH sites), whereas that of H_2' and modularity was significantly higher (except for epiphytic and endophytic fungal networks at the JGS site) across the forest sites (Supplementary Fig. S6a–S6c, S6e–S6g). Additionally, compared with the null models, the observed value of WNODF in epiphytic and endophytic fungus networks was not significantly different at most sites (57.1%, 81.0%) (Supplementary Fig. S6d, S6h).

Furthermore, Z-score normalization analysis revealed that plant-epiphytic fungus networks were more highly specialized and modular, but less connected and nested than plant-endophytic fungus networks in all forest sites (except for modularity in BB, DHS, and BTM, and nestedness in NG, MLun, and HGS; Fig. 6a–d), suggesting that plant-epiphytic fungus networks were more stable but less complex than plant-endophytic fungus networks in most sites. Additionally, the connectance and nestedness of plant-epiphytic fungus networks decreased, while the specialization increased with increasing latitude (Fig. 6e–h). By contrast, the network structure of plant-endophytic fungi had no significant latitudinal pattern (Fig. 6e–h). These results suggest that the complexity of plant-epiphytic but not plant-endophytic fungus networks decreased with increasing latitude.

Discussion

The first hypothesis was not supported by our findings that the alpha and beta diversity of epiphytic and endophytic fungi showed a U-shaped latitudinal pattern, as reported in soil fungi in Chinese forests[24]. This pattern may be partly attributed to the influence of the species pool, as both epiphytic and endophytic fungal species pools exhibited a significant U-shaped pattern in our study (Supplementary Fig. S7). Compared to mid-latitude forests, a larger species pool at low and high latitudes provided great and heterogeneous fungal sources, which contributed to higher alpha and beta diversity in these regions. Indeed, a significantly positive relationship between species pool and the alpha and beta diversity of fungi was observed in our study (Fig. 5b, c; Supplementary Fig. S8) and previous[36,37] studies. It is highly possible that larger species pools contain greater species with diverse adaptive traits that allow them to thrive in different environments[98], or due to greater random differences in community composition[4,99,100].

In addition to the species pool, the alpha and beta diversity of epiphytic and endophytic fungi was also affected by plant identity, as well as spatial and climatic variables, as reported in previous studies[9,15,18,101]. The effect of plant identity on fungal diversity is exerted through direct host preferences, which are mediated by leaf nutrients, volatile organic compounds, and surface topography. Indeed, we found that phyllosphere fungal diversity in most plant species was lower in mid-latitudinal than in low- and high-latitudinal forests (Supplementary Figs S9, S10). In addition, soil heterogeneity is lower in mid-latitudinal than in low- and high-latitudinal regions[24]. The homogeneous soil environment possibly contributes to a more homogeneous leaf environment[102], which may enhance homogeneous filtering and lead to low diversity. Another reason is that greater human disturbance (e.g., land use change, fragmentation, or deposition) in central China may have damaged diversity, as reported in soil fungi and other macro-organisms[103,104]. However, some previous studies found that endophytic fungal alpha diversity decreased toward boreal forests using culturable methods[26,27]. The differences may be attributed to the different detection methods, as culture-based approaches can detect more Ascomycota than Basidiomycota[105,106], which are dominant at high latitudes[20].

Inconsistent with the second hypothesis, we found that community assembly of epiphytic and endophytic fungi was mainly structured by stochastic processes (e.g., dispersal limitation). Similarly, some previous studies reported that fungal community assembly in leaves and soil was governed primarily by stochastic processes in shrub[45,46], agriculture[107], and forest[1,108] ecosystems. The reason could be that geographic isolation among sampling sites generates dispersal barriers and reduces migration rates of microbial propagules, especially at a large spatial scale[109,110], as we found an important influence of space on community of epiphytic and endophytic fungi by variation partitioning analysis (Fig. 2). Furthermore, our results showed that a considerable proportion of epiphytic and endophytic fungal community variance was not explained (52.1%–56.0%; Fig. 2c, d), which may be due to stochastic processes such as diversification or speciation, which was not directly detected in the natural environment[111]. Moreover, fungal species have different dispersal and colonization abilities[112], and the arrival order of fungal species may affect the fungal community via priority effects[113].

Furthermore, we found that the relative importance of stochastic processes on the community assembly of epiphytic fungi had a hump-shaped latitudinal pattern. Compared to low and high latitudes, the epiphytic fungal community was more homogeneous in mid-latitudinal forests in this study, which possibly led to stronger interspecific competition[24]. In this case, the priority effect may have a greater impact on community assembly because the species that arrives first gains a competitive advantage by preemptively occupying resources[114], leading to greater stochasticity in mid-latitudinal forests. Additionally, the complex topography and large elevational variation in mid-latitude regions may cause greater dispersal limitation for epiphytic fungi, further contributing to the high stochasticity. However, we found no latitudinal pattern in the relative importance of stochastic processes on community assembly of endophytic fungi. This result may be attributed to the strong selective effect of the host on endophytic fungi during their colonization[15,115].

Our third hypothesis was partially supported by our findings that the complexity of plant-epiphytic fungus networks, rather than plant-endophytic fungus networks, decreased with increasing latitude, as the connectance and nestedness of plant-epiphytic fungus networks but not plant-endophytic fungus networks decreased with increasing latitude. The higher connectance may be due to a great species pool and favorable hydrothermal conditions in low-latitudinal forests, promoting potential interactions between epiphytic fungi and plants, thereby enhancing network complexity. Additionally, the specialization of epiphytic fungus networks increased with increasing latitude, as found in root-associated fungi[60]. It reflects a stronger niche differentiation in temperate regions, leading to less competition for host plants among fungal species, which decreases the nestedness of plant-fungus networks, thus suggesting a lower network complexity at high latitudes. However, the network structure of plant-endophytic fungi had no significant latitudinal pattern. This result could be ascribed to stronger host selection and greater resistance and/or resilience to environmental perturbations compared to epiphytic fungi[15,116].

Additionally, we found that the plant-endophytic fungus network was more complex but less stable compared to the plant-epiphytic fungus network, as indicated by its higher connectance and nestedness but lower modularity compared to those of the epiphytic network. Similar results have also been found in a subtropical mangrove ecosystem[64]. The lower nestedness of plant-epiphytic fungus networks suggests that host plants may prefer to interact with specialists over generalists among epiphytic fungi rather than

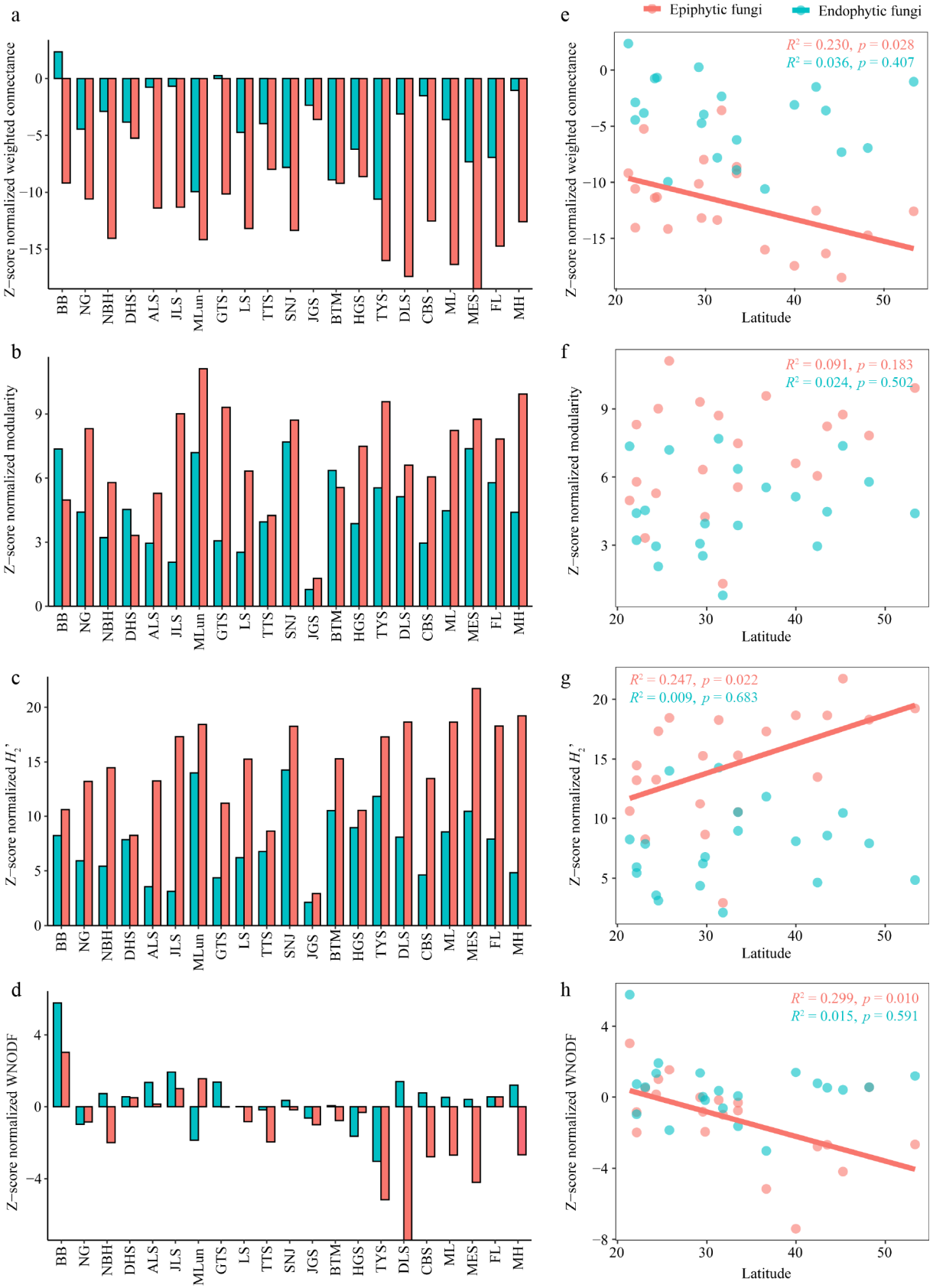

Fig. 6 Structure of plant-epiphytic and endophytic fungus networks. (a)–(d) Z-score-normalized properties of epiphytic and endophytic fungus networks. (e)–(h) Latitudinal pattern of plant-epiphytic and endophytic fungus networks. H_2', H_2' metric of the network-level interaction specialization; Modularity, Barber's metric of modularity; WNODF, weighted nestedness metric based on overlap and decreasing fill. BB, Bubeng; NG, Nonggang; NBH, Nabanhe; DHS, Dinghushan; ALS, Ailaoshan; JLS, Jiulianshan; MLun, Mulun; GTS, Gutianshan; LS, Lushan; TTS, Tiantongshan; SNJ, Shennongjia; JGS, Jigongshan; BTM, Baotianman; HGS, Huangguanshan; TYS, Taiyueshan; DLS, Donglingshan; CBS, Changbaishan; ML, Muling; MES, Maoershan; FL, Fenglin; MH, Mohe.

among endophytic fungi[117], thereby further promoting the specialization of these networks. This higher specialization indicates strong niche differentiation[118], which could reduce interspecific interactions and contribute to the low complexity of epiphytic fungal networks. Such strong niche differentiation may be driven by leaf surface microenvironments, including sunlight intensity, moisture, and nutrient content. Additionally, the high specialization was followed by high modularity, which enhances the stability of epiphytic fungal networks by limiting the impact of perturbations within a single module[59].

Finally, we should note that the leaf samples were stored at –80 °C, which may damage the tissue and cellular structure of broadleaves, introducing bias into the isolation of epiphytic and endophytic fungi that could potentially influence the latitudinal patterns of phyllosphere fungi.

Conclusions

This study provides the first example of research revealing the latitudinal pattern of diversity, community assembly, and network structure of phyllosphere epiphytic and endophytic fungi in Chinese forests. The alpha and beta diversity of epiphytic and endophytic fungi exhibited a U-shaped latitudinal pattern, which was mainly driven by plant identity, species pool, space, and climatic factors. The community assembly of phyllosphere epiphytic and endophytic fungi was predominantly governed by stochastic processes, and the relative importance of stochastic processes on epiphytic fungi rather than endophytic fungi showed a unimodal latitudinal pattern. The plant-epiphytic fungus networks were more stable but less complex than plant-endophytic fungus networks in most forests. The complexity of plant-epiphytic fungus networks, rather than plant-endophytic fungus networks, decreased with increasing latitude. These results enhance our understanding of species co-occurrence and community stability in forest ecosystems.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Guo L, Gao C; data collection: Li X, Guo L, Gao C, Zheng Y, Wang C; analysis and interpretation of results: Li X, Li J, Guo L, Gao C; draft manuscript preparation: Li X, Li J, Guo L, Gao C, Willing CE, Frew A, Jiang L. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The raw reads have been deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA1221103, PRJNA1221115, and PRJNA1221120), and the other relevant data can be found within the manuscript and its supporting materials.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (32322053, 32170120, 32471693, 32170129, and 31700543). We thank support from the Chinese Forest Biodiversity Monitoring Network. We are grateful to Yong-Wong Wang, Xiang Sun, Liang Chen, Zi-Xuan Guo, Yue Zhang, Yu-Xuan Liu, Yong-Qing Lin, Qing-Yi Yu, Jing Cai, Hui Yao, Hui-Yun Gan, Min Cao, Ye Chen, Fu-Ping Zeng, Yang Wang, Song-Yan Tian, Ming-Xi Jiang, Han Xu, Xi-Hua Wang, Xiao-Yong Chen, Xiao-Bao Deng, Hong-Jin Dong, Xiao-Jun Du, Yong-Jun Fan, Sheng-Lei Fu, Nan-Nan Shi, Xiao-Wei Gao, Zhan-Qing Hao, Xiu-Hai Zhao, Lai Jiang, Ming-Xi Jiang, Guang-Ze Jin, Bu-Hang Li, Xian-Kun Li, Yu-Hong Liu, Ke-Ping Ma, Jin-Hua Qi, Wei-Guo Sang, Li-Yan Sun, Zong-Qiang Xie, Ji Ye, Wan-Hui Ye, Shui-Sheng You, Ming-Jian Yu, Shu-Sheng Zhang, Yong-Liang Zhang for help in field sampling. We are grateful to Yu-Xuan Liu, Yong-Qing Lin, and Yu-Jin Cui for help in performing experiments, and Yi-Fu Wang, Peng-Fa Li, Yong-Wong Wang, Zi-Xuan Guo, Yue Zhang, Yu-Xuan Liu, and Hui Yao for help in data analysis. We thank all members of the Gao and Guo Lab for their discussion and communication.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information accompanies this paper online at: https://doi.org/10.48130/mycosphere-0026-0011.

Dates

Received 1 April 2026; Revised 21 May 2026; Accepted 6 June 2026; Published online 17 July 2026

References

[1] Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, et al. 2018. Structure and function of the global topsoil microbiome. *Nature* 560:233–237

[2] Bernardo-Madrid R, González-Suárez M, Rosvall M, Rueda M, Revilla E, et al. 2025. A general rule on the organization of biodiversity in Earth's biogeographical regions. *Nature Ecology & Evolution* 9:1193–1204

[3] Dyer LA, Singer MS, Lill JT, Stireman JO, Gentry GL, et al. 2007. Host specificity of Lepidoptera in tropical and temperate forests. *Nature* 448:696–699

[4] Kraft NJ, Comita LS, Chase JM, Sanders NJ, Swenson NG, et al. 2011. Disentangling the drivers of beta diversity along latitudinal and elevational gradients. *Science* 333:1755–1758

[5] Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, et al. 2014. Global diversity and geography of soil fungi. *Science* 346:1256688

[6] Li P, Tedersoo L, Crowther TW, Wang B, Shi Y, et al. 2023. Global diversity and biogeography of potential phytopathogenic fungi in a changing world. *Nature Communications* 14:6482

[7] Vorholt JA. 2012. Microbial life in the phyllosphere. *Nature Reviews Microbiology* 10:828–840

[8] Koskella B. 2020. The phyllosphere. *Current Biology* 30:R1143–R1146

[9] Li J, Li XC, Gan HY, Zhang Y, Guo ZX, et al. 2025. Plant diversity increases diversity and network complexity rather than alters community assembly processes of leaf-associated fungi in a subtropical forest. *Science China Life Sciences* 68:846–858

[10] Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, et al. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences of the United States of America* 100:15649–15654

[11] Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, et al. 2011. Microbially mediated plant functional traits. *Annual Review of Ecology, Evolution, and Systematics* 42:23–46

[12] Purahong W, Hyde KD. 2011. Effects of fungal endophytes on grass and non-grass litter decomposition rates. *Fungal Diversity* 47:1–7

[13] Christian N, Herre EA, Clay K. 2019. Foliar endophytic fungi alter patterns of nitrogen uptake and distribution in *Theobroma cacao*. *New Phytologist* 222:1573–1583

[14] He Y, Han B, Geng Y, Shi L, Peng C, et al. 2025. Unveiling the role of foliar fungi in mediating leaf photosynthesis under global change. *Journal of Ecology* 113:3735–3747

[15] Yao H, Sun X, He C, Maitra P, Li XC, et al. 2019. Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem. *Microbiome* 7:57

[16] Yang T, Tedersoo L, Soltis PS, Soltis DE, Sun M, et al. 2023. Plant and fungal species interactions differ between aboveground and belowground habitats in mountain forests of Eastern China. *Science China Life Sciences* 66:1134−1150
[17] U'Ren JM, Lutzoni F, Miadlikowska J, Zimmerman NB, Carbone I, et al. 2019. Host availability drives distributions of fungal endophytes in the imperilled boreal realm. *Nature Ecology & Evolution* 3:1430−1437
[18] Harris MA, Kemler M, Slippers B, Hassel N, Tsamba J, et al. 2025. Productivity drives leaf mycobiome diversity patterns at global and continental scales. *Global Ecology and Biogeography* 34:e70094
[19] Zhou J, Deng Y, Shen L, Wen C, Yan Q, et al. 2016. Temperature mediates continental-scale diversity of microbes in forest soils. *Nature Communications* 7:12083
[20] Hu Y, Veresoglou SD, Tedersoo L, Xu T, Ge T, et al. 2019. Contrasting latitudinal diversity and co-occurrence patterns of soil fungi and plants in forest ecosystems. *Soil Biology and Biochemistry* 131:100−110
[21] Liu S, Wang H, Tian P, Yao X, Sun H, et al. 2020. Decoupled diversity patterns in bacteria and fungi across continental forest ecosystems. *Soil Biology and Biochemistry* 144:107763
[22] Shi LL, Mortimer PE, Ferry Slik JW, Zou XM, Xu JC, et al. 2014. Variation in forest soil fungal diversity along a latitudinal gradient. *Fungal Diversity* 64:305−315
[23] Wang P, Chen Y, Sun Y, Tan S, Zhang S, et al. 2019. Distinct biogeography of different fungal guilds and their associations with plant species richness in forest ecosystems. *Frontiers in Ecology and Evolution* 7:216
[24] Huang YT, Zhang X, Fu SL, Zhang WX. 2019. Environmental filtering drives local soil fungal beta diversity more than dispersal limitation in six forest types along a latitudinal gradient in Eastern China. *Forests* 10:863
[25] Zheng Y, Chen L, Ji NN, Wang YL, Gao C, et al. 2021. Assembly processes lead to divergent soil fungal communities within and among 12 forest ecosystems along a latitudinal gradient. *New Phytologist* 231:1183−1194
[26] Arnold AE, Lutzoni F. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* 88:541−549
[27] Ikeda A, Matsuoka S, Masuya H, Mori AS, Hirose D, et al. 2014. Comparison of the diversity, composition, and host recurrence of xylariaceous endophytes in subtropical, cool temperate, and subboreal regions in Japan. *Population Ecology* 56:289−300
[28] Millberg H, Boberg J, Stenlid J. 2015. Changes in fungal community of Scots pine (*Pinus sylvestris*) needles along a latitudinal gradient in Sweden. *Fungal Ecology* 17:126−139
[29] Gaytán Á, Abdelfattah A, Faticov M, Moreira X, Castagneyrol B, et al. 2022. Changes in the foliar fungal community between oak leaf flushes along a latitudinal gradient in Europe. *Journal of Biogeography* 49:2269−2280
[30] Götzenberger L, de Bello F, Bråthen KA, Davison J, Dubuis A, et al. 2012. Ecological assembly rules in plant communities-approaches, patterns and prospects. *Biological Reviews of the Cambridge Philosophical Society* 87:111−127
[31] Keddy PA. 1992. Assembly and response rules: two goals for predictive community ecology. *Journal of Vegetation Science* 3:157−164
[32] Catano CP, Dickson TL, Myers JA. 2017. Dispersal and neutral sampling mediate contingent effects of disturbance on plant beta-diversity: a meta-analysis. *Ecology Letters* 20:347−356
[33] Li L, Liu Y, Wang X, Fang J, Wang Q, et al. 2015. Different effects of regional species pool on plant diversity between forest and grassland biomes in arid Northwest China. *PLoS ONE* 10:e0131982
[34] Medina NG, Bowker MA, Hortal J, Mazimpaka V, Lara F. 2018. Shifts in the importance of the species pool and environmental controls of epiphytic bryophyte richness across multiple scales. *Oecologia* 186:805−816
[35] Lessard JP, Borregaard MK, Fordyce JA, Rahbek C, Weiser MD, et al. 2012. Strong influence of regional species pools on continent-wide structuring of local communities. *Proceedings: Biological Sciences, the Royal Society* 279:266−274
[36] Chen B, Pan H, Song X, Yao Y, Qi J, et al. 2024. Linking regional species pool size to dispersal-selection relationships in soil fungal communities across terrestrial ecosystems. *Global Ecology and Biogeography* 33:e13876
[37] Xing H, Chen W, Liu Y, Cahill Jr JF. 2024. Local community assembly mechanisms and the size of species pool jointly explain the beta diversity of soil fungi. *Microbial Ecology* 87:58
[38] MacArthur R, Levins R. 1967. The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist* 101:377−385
[39] Hubbell SP. 2011. *The Unified Neutral Theory of Biodiversity and Biogeography (MPB-32)*. Princeton: Princeton University Press. doi: 10.1515/9781400837526
[40] Chase JM, Myers JA. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences* 366:2351−2363
[41] Xiong C, He JZ, Singh BK, Zhu YG, Wang JT, et al. 2021. Rare taxa maintain the stability of crop mycobiomes and ecosystem functions. *Environmental Microbiology* 23:1907−1924
[42] Yan ZZ, Chen QL, Li CY, Thi Nguyen BA, He JZ, et al. 2022. Contrasting ecological processes shape the *Eucalyptus* phyllosphere bacterial and fungal community assemblies. *Journal of Sustainable Agriculture and Environment* 1:73−83
[43] Yang H, Yang ZJ, Wang QC, Wang YL, Hu HW, et al. 2022. Compartment and plant identity shape tree mycobiome in a subtropical forest. *Microbiology Spectrum* 10:e01347-22
[44] Schimann H, Vacher C, Coste S, Louisanna E, Fort T, et al. 2023. Determinants of the vertical distribution of the phyllosphere differ between microbial groups and the epi- and endosphere in a tropical forest. *Phytobiomes Journal* 7:312−323
[45] Lai Z, Sun Y, Yu Y, Liu Z, Bai Y, et al. 2023. Plant selection and ecological microhabitat drive the shrub-associated microbiome selection in revegetated shrub ecosystems. *Applied Soil Ecology* 190:105023
[46] Chen DQ, Yang JJ, Wang SF, Lan SR, Wang YL, et al. 2025. Comparative analysis of community composition and network structure between phyllosphere endophytic and epiphytic fungal communities of *Mussaenda pubescens*. *Microbiology Spectrum* 13:e0101924
[47] Tang Z, Fang J, Chi X, Feng J, Liu Y, et al. 2013. Patterns of plant beta-diversity along elevational and latitudinal gradients in mountain forests of China. *Ecography* 35:1083−1091
[48] Qiao X, Jabot F, Tang Z, Jiang M, Fang J. 2015. A latitudinal gradient in tree community assembly processes evidenced in Chinese forests. *Global Ecology and Biogeography* 24:314−323
[49] Jiao S, Yang Y, Xu Y, Zhang J, Lu Y. 2020. Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. *ISME Journal* 14:202−216
[50] Huang X, Wang J, Dumack K, Liu W, Zhang Q, et al. 2021. Protists modulate fungal community assembly in paddy soils across climatic zones at the continental scale. *Soil Biology and Biochemistry* 160:108358
[51] Nishizawa K, Shinohara N, Cadotte MW, Mori AS. 2022. The latitudinal gradient in plant community assembly processes: A meta-analysis. *Ecology Letters* 25:1711−1724
[52] Wang Z, Jiang Y, Zhang M, Chu C, Chen Y, et al. 2023. Diversity and biogeography of plant phyllosphere bacteria are governed by latitude-dependent mechanisms. *New Phytologist* 240:1534−1547
[53] Chase JM. 2007. Drought mediates the importance of stochastic community assembly. *Proceedings of the National Academy of Sciences of the United States of America* 104:17430−17434
[54] Guo H, Więski K, Lan Z, Pennings SC. 2014. Relative influence of deterministic processes on structuring marsh plant communities varies across an abiotic gradient. *Oikos* 123:173−178
[55] Chase JM. 2010. Stochastic community assembly causes higher biodiversity in more productive environments. *Science* 328:1388−1391
[56] Mori AS, Shiono T, Koide D, Kitagawa R, Ota AT, et al. 2013. Community assembly processes shape an altitudinal gradient of forest biodiversity. *Global Ecology and Biogeography* 22:878−888

[57] Rohr RP, Saavedra S, Bascompte J. 2014. On the structural stability of mutualistic systems. *Science* 345:1253497
[58] van der Heijden MG, Martin FM, Selosse MA, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* 205:1406–1423
[59] Stouffer DB, Bascompte J. 2011. Compartmentalization increases food-web persistence. *Proceedings of the National Academy of Sciences of the United States of America* 108:3648–3652
[60] Toju H, Sato H, Yamamoto S, Tanabe AS. 2018. Structural diversity across arbuscular mycorrhizal, ectomycorrhizal, and endophytic plant-fungus networks. *BMC Plant Biology* 18:292
[61] Jordano P, Stouffer D. 2016. Sampling networks of ecological interactions. *Functional Ecology* 30:1883–1893
[62] Guo CY, Yang A, Zhang WH. 2024. Host identity determines the bacterial and fungal community and network structures in the phyllosphere of plant species in a temperate steppe. *Phytobiomes Journal* 8:143–154
[63] Sun X, Li JL, He C, Li XC, Guo LD. 2021. Specific network and phylosymbiosis pattern in endophyte community of coastal halophytes. *Fungal Ecology* 53:101088
[64] Zhu C, Lin Y, Wang Z, Luo W, Zhang Y, et al. 2023. Community assembly and network structure of epiphytic and endophytic phyllosphere fungi in a subtropical mangrove ecosystem. *Frontiers in Microbiology* 14:1147285
[65] White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, eds. Innis MA, Gelfand DH, Sninsky JJ, White TJ. Amsterdam: Elsevier, Academic Press. pp. 315–322 doi: 10.1016/B978-0-12-372180-8.50042-1
[66] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* 37:852–857
[67] Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, et al. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* 13:581–583
[68] Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, et al. 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution* 4:914–919
[69] Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4:e2584
[70] Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, et al. 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* 22:5271–5277
[71] Edgar RC. 2016. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. *bioRxiv* 1-20. https://doi.org/10.1101/074161
[72] Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75:7537–7541
[73] Põlme S, Abarenkov K, Henrik Nilsson R, Lindahl BD, Clemmensen KE, et al. 2021. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Diversity* 105:1–16
[74] Marquitti FMD, Guimarães Jr PR, Pires MM, Bittencourt LF. 2014. MODULAR: software for the autonomous computation of modularity in large network sets. *Ecography* 37:221–224
[75] Clarke KR, Somerfield PJ, Chapman MG. 2006. On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray–Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology* 330:55–80
[76] Baselga A. 2010. Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography* 19:134–143
[77] Baselga A, Orme CDL. 2012. betapart: an R package for the study of beta diversity. *Methods in Ecology and Evolution* 3:808–812
[78] Zanne AE, Tank DC, Cornwell WK, Eastman JM, Smith SA, et al. 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* 506:89–92
[79] Webb CO, Donoghue MJ. 2005. Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Notes* 5:181–183
[80] Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290
[81] Oksanen J, Blanchet F, Friendly M, Kindt R, Legendre P, et al. 2017. *Vegan: Community ecology package. R package version 2.6-2.* https://CRAN.R-project.org/package=vegan
[82] Dray S, Legendre P, Peres-Neto PR. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological Modelling* 196:483–493
[83] Dray S, Bauman D, Blanchet G, Borcard D, Clappe S, et al. 2026. adespatial: multivariate multiscale spatial analysis. R package version 0.3-29. https://CRAN.R-project.org/package=adespatial
[84] Kolde R. 2025. pheatmap: pretty heatmaps. R package version 1.0.13. https://CRAN.R-project.org/package=pheatmap
[85] Lefcheck JS. 2016. piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution* 7:573–579
[86] Zhang X, Liu S, Wang J, Huang Y, Freedman Z, et al. 2020. Local community assembly mechanisms shape soil bacterial β diversity patterns along a latitudinal gradient. *Nature Communications* 11:5428
[87] Ning D, Yuan M, Wu L, Zhang Y, Guo X, et al. 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. *Nature Communications* 11:4717
[88] Tedersoo L, Sánchez-Ramírez S, Kõljalg U, Bahram M, Döring M, et al. 2018. High-level classification of the fungi and a tool for evolutionary ecological analyses. *Fungal Diversity* 90:135–159
[89] Chase JM, Kraft NJB, Smith KG, Vellend M, Inouye BD. 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. *Ecosphere* 2:1–11
[90] Grömping U. 2006. Relative importance for linear regression in R: the Package relaimpo. *Journal of Statistical Software* 17:1–27
[91] Toju H, Tanabe AS, Ishii HS. 2016. Ericaceous plant-fungus network in a harsh alpine-subalpine environment. *Molecular Ecology* 25:3242–3257
[92] Blüthgen N, Menzel F, Hovestadt T, Fiala B, Blüthgen N. 2007. Specialization, constraints, and conflicting interests in mutualistic networks. *Current Biology* 17:341–346
[93] Tylianakis JM, Tscharntke T, Lewis OT. 2007. Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature* 445:202–205
[94] Almeida-Neto M, Ulrich W. 2011. A straightforward computational approach for measuring nestedness using quantitative matrices. *Environmental Modelling & Software* 26:173–178
[95] Barber MJ. 2007. Modularity and community detection in bipartite networks. *Physical Review E: Statistical, Nonlinear, and Soft Matter Physics* 76:066102
[96] Dormann CF, Frund J, Bluthgen N, Gruber B. 2009. Indices, graphs and null models analyzing bipartite ecological networks. *The Open Ecology Journal* 2:7–24
[97] Ulrich W, Almeida-Neto M, Gotelli NJ. 2009. A consumer's guide to nestedness analysis. *Oikos* 118:3–17
[98] Spasojevic MJ, Catano CP, LaManna JA, Myers JA. 2018. Integrating species traits into species pools. *Ecology* 99:1265–1276
[99] Fukami T. 2004. Community assembly along a species pool gradient: implications for multiple-scale patterns of species diversity. *Population Ecology* 46:137–147
[100] Brownstein G, Steel JB, Porter S, Gray A, Wilson C, et al. 2012. Chance in plant communities: a new approach to its measurement using the nugget from spatial autocorrelation. *Journal of Ecology* 100:987–996
[101] Kembel SW, Mueller RC. 2014. Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. *Botany* 92:303–311
[102] Fan H, Wu J, Liu W, Yuan Y, Hu L, et al. 2015. Linkages of plant and soil C : N : P stoichiometry and their relationships to forest growth in subtropical plantations. *Plant and Soil* 392:127–138
[103] Shi L, Dossa GGO, Paudel E, Zang H, Xu J, et al. 2019. Changes in fungal communities across a forest disturbance gradient. *Applied and Environmental Microbiology* 85:e00080-19

[104] de Thoisy B, Richard-Hansen C, Goguillon B, Joubert P, Obstancias J, et al. 2010. Rapid evaluation of threats to biodiversity: human footprint score and large vertebrate species responses in French Guiana. *Biodiversity and Conservation* 19:1567−1584
[105] Arnold AE. 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biology Reviews* 21:51−66
[106] Guo LD, Hyde KD, Liew EC. 2001. Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequences. *Molecular Phylogenetics and Evolution* 20:1−13
[107] Gao C, Montoya L, Xu L, Madera M, Hollingsworth J, et al. 2020. Fungal community assembly in drought-stressed sorghum shows stochasticity, selection, and universal ecological dynamics. *Nature Communications* 11:34
[108] Bowman EA, Arnold AE. 2021. Drivers and implications of distance decay differ for ectomycorrhizal and foliar endophytic fungi across an anciently fragmented landscape. *ISME Journal* 15:3437−3454
[109] Peay KG, Garbelotto M, Bruns TD. 2010. Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands. *Ecology* 91:3631−3640
[110] Peay KG, Schubert MG, Nguyen NH, Bruns TD. 2012. Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Molecular Ecology* 21:4122−4136
[111] Zhou J, Ning D. 2017. Stochastic community assembly: does it matter in microbial ecology? *Microbiology and Molecular Biology Reviews* 81:e00002−00017
[112] Luan L, Jiang Y, Cheng M, Dini-Andreote F, Sui Y, et al. 2020. Organism body size structures the soil microbial and nematode community assembly at a continental and global scale. *Nature Communications* 11:6406
[113] Kennedy PG, Peay KG, Bruns TD. 2009. Root tip competition among ectomycorrhizal fungi: are priority effects a rule or an exception? *Ecology* 90:2098−2107
[114] Fukami T. 2015. Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Annual Review of Ecology, Evolution, and Systematics* 46:1−23
[115] Santamaría J, Bayman P. 2005. Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). *Microbial Ecology* 50:1−8
[116] Mahmoudi M, Almario J, Lutap K, Nieselt K, Kemen E. 2024. Microbial communities living inside plant leaves or on the leaf surface are differently shaped by environmental cues. *ISME Communications* 4:ycae103
[117] Chagnon P, U'Ren JM, Miadlikowska J, Lutzoni F, Arnold AE. 2016. Interaction type influences ecological network structure more than local abiotic conditions: evidence from endophytic and endolichenic fungi at a continental scale. *Oecologia* 180:181−191
[118] Blüthgen N. 2010. Why network analysis is often disconnected from community ecology: A critique and an ecologist's guide. *Basic and Applied Ecology* 11:185−195