Assembly processes lead to divergent soil fungal communities within and among twelve forest ecosystems along a latitudinal gradient

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Summary

- Latitudinal gradients provide opportunities to better understand soil fungal community assembly and its relationship with vegetation, climate, soil and ecosystem function. Understanding the mechanisms underlying community assembly is essential for predicting compositional responses to changing environments.

- We quantified the relative importance of stochastic and deterministic processes in structuring soil fungal communities using patterns of community dissimilarity observed within and between twelve natural forests and related these to environmental variation within and among sites.

- The results revealed that whole fungal communities and communities of arbuscular and ectomycorrhizal fungi consistently exhibited divergent patterns but with less divergence for ectomycorrhizal fungi at most sites. Within those forests, no clear relationships were observed between the degree of divergence within fungal and plant communities. When comparing communities at larger spatial scales, among the twelve forests, we observed distinct separation in all three fungal groups among tropical, subtropical and temperate climatic zones. Soil fungal β-diversity patterns between forests were also greater when comparing forests exhibiting high environmental heterogeneity.

- Taken together, although large-scale community turnover could be attributed to specific environmental drivers, the differences among fungal communities in soils within forests was high even at local scales.

Key words: β-diversity; community assembly; forest; fungi; latitudinal gradient; neutral model

Introduction

Understanding the community assembly processes underlying observed patterns in community composition is critical for gaining a more mechanistic understanding of biodiversity maintenance, community stability and ecosystem functioning (Chase, 2010; Nemergut *et al.*, 2013). Stochastic (neutral or non-neutral with unstable outcomes) and deterministic (niche-based) processes are both important forces in determining the outcomes of community assembly for macro-organisms (Fargione *et al.*, 2003; Kraft *et al.*, 2008; Ellwood *et al.*, 2009). Neutral theory assumes that many natural community assembly patterns can arise due to ecological drift, resulting in communities that are dependent on stochastic aspects of colonisation, recruitment and mortality rather than adaptation of individuals to their habitats (Hurtt & Pacala, 1995; Hubbell, 2001; Alonso *et al.*, 2006; Zhou & Ning, 2017). Niche-based theory, however, states that deterministic factors, such as biotic interactions, species traits, and environmental variables, modulate the local community. When deterministic factors are of greater importance, local communities assembling under similar environmental conditions should converge upon a common composition, while divergence among local communities should be observed when environmental conditions differ (Chase, 2003; Zhou *et al.*, 2013).

Soil microorganisms, especially fungi, have remarkable biodiversity on Earth and play crucial roles in biogeochemical cycling and ecosystem functioning (Philippot *et al.*, 2013; Tedersoo *et al.*, 2014). Mycorrhizal fungi make up important functional guilds within soil fungal communities, forming mutualistic associations with 80% of land plants and obtaining carbon from hosts in exchange for nutrients (Smith & Read, 2008). Therefore, these fungi can greatly affect plant productivity, diversity, and ecosystem processes (van der Heijden *et al.*, 1998, 2015; Mayor *et al.*, 2015). Progress in molecular techniques to efficiently characterise fungal communities had resulted in researchers paying increasing attention to understanding the relative contributions of determinism and neutrality for fungal community assembly (*i.e.*, Dumbrell *et al.*, 2010; Gao *et al.*, 2015; Powell *et al.*, 2015; Alzarhani *et al.*, 2019). Previous studies have demonstrated that the relative role of deterministic and stochastic processes in structuring soil fungal community assembly is highly dependent on spatial scale (Caruso *et al.*, 2012; Kivlin *et al.*, 2014; Schroter *et al.*, 2019), although the factors underlying this spatial dependence are poorly understood.

Exploring fungal community turnover (β-diversity) can lead to insight into the mechanisms generating and maintaining fungal biodiversity in ecosystems (Ettema &

Wardle, 2002; Beck *et al.*, 2015). These studies are typically performed at large geographic scales, but with different environmental foci and differing results. Soil fungal communities across Scotland were more strongly affected by deterministic processes in systems with less disturbance (bogs, moors, and semi-natural grasslands) than in more intensive land use systems such as managed grasslands and arable systems (Powell *et al.*, 2015). Climatic factors, such as mean annual temperature (MAT) and precipitation (MAP) are often observed to be important predictors of soil fungal β-diversity (Tedersoo *et al.*, 2014). Temperature was identified as a key factor influencing soil fungal β-diversity across a large-scale latitudinal gradient in China and USA (*e.g.*, Shi *et al.*, 2014; Zhou *et al.*, 2016). Plants contribute organic carbon to fungi, which benefit the plants via decomposition or direct nutrients supply in symbiotic relationships, and a study of arbuscular mycorrhizal (AM) fungal communities found β-diversity to vary among individual host species (Powell & Bennett, 2016). However, the relationships between soil fungal β-diversity and that of vegetation communities are rarely considered and, therefore, whether and to what extent variation within these interacting communities is governed by similar assembly processes is an open question.

Using Illumina Miseq high-throughput sequencing, we examined soil fungal communities within twelve Chinese forests situated throughout eastern China, with the largest distance among forests being more than 4000 km (Fig. S1). To assess the relative importance of stochastic and deterministic factors for soil fungal community assembly in these forests, we employed a null model approach to generate neutral predictions, which were subsequently compared to the observed β-diversity. This analysis reveals three possible outcomes: the observed β-diversity not differing from the neutral prediction (neutral assembly overwhelms determinism) or the observed β-diversity being larger (divergent assembly) or smaller (convergent assembly) than the neutral prediction. Our first aim was to identify these patterns in soil fungal community assembly and their relationships with that of corresponding plant communities. Second, we estimated the relative importance of habitat turnover (*i.e.*, variation in environmental characteristics) based on soil, plant, and climatic parameters as drivers of fungal β-diversity. We hypothesized that the balance between deterministic and stochastic community assembly processes within individual forests and their relationship with assembly processes occurring within plant communities would differ depending on whether entire fungal communities or specific symbiotic (*i.e.*, AM and ectomycorrhizal [EM] fungal) guilds were assessed.

Materials and methods

Sites and sampling

This study was conducted in 12 permanent forest sites established by the Chinese Forest Biodiversity Monitoring Network (CForBio, Feng *et al.*, 2016), ranging in latitude from 21.6º N to 50.8º N in China (Fig. S1). The experimental forests were described previously by Ji *et al.* (2019). Briefly, a total of six forest types include tropical rain forest (TRF), subtropical evergreen broad-leaf forest (SEF), mixed evergreen broad-leaved and deciduous broad-leaved forest (MEDF), warm-temperate deciduous broad-leaved forest (WTDF), temperate broad-leaved Korean pine forest (TPF), and cold temperate monsoon coniferous forest (CTCF) were included in this study. During June to October in 2014, 20 preexisting plots (20 m × 20 m), each spaced more than 45 m (mean = 247 m) apart, were randomly selected from all plots at each site and soil samples were collected (Ji *et al.*, 2019). In each plot, ten soil cores (3.5 cm diameter, 10 cm depth, including organic and mineral horizons but excluding the litter layer) were randomly collected, bulked and subsampled to form a composite sample, which resulted in a total of 240 composite samples in the entire study. Samples were transported on ice to the laboratory and sieved through a 2-mm sieve to remove roots and debris. One portion of the soil samples was stored at −80°C until DNA extraction; another portion was air-dried for analyses of physicochemical soil properties. In the same plots that the soil samples were collected from, plant community data were obtained from CForBio in 2015 and species were classified as arbuscular mycorrhizal, ectomycorrhizal, ericoid mycorrhizal and non-mycorrhizal based on the published literature (Wang & Qiu, 2006; Maherali *et al.*, 2016). Soil, plant, and climatic variables in each study site are shown in Table S1, Table S2, and Table S3.

Soil and climatic factors

Soil pH, total carbon (TC), total nitrogen (TN), total phosphorus (TP), C:N, and N:P ratios were described in detail by Ji *et al.* (2019). Latitude, longitude, and plant data (basal area, richness and community composition) of the study sites were provided by the CForBio organization (obtained from colleagues as mentioned in the Acknowledgments section). Mean annual temperature (MAT) and mean annual precipitation (MAP) were obtained from the WorldClim database (www.worldclim.org) with a resolution of 2.5 min (Ji *et al.*, 2019).

Molecular analysis

Molecular analyses were performed independently on the 20 soil samples collected from each of the 12 sites. Genomic DNA was extracted from 0.25 g of frozen soil using a PowerSoil DNA isolation kit (MoBio Laboratories, Inc. USA) according to the manufacturer's instructions. The DNA was diluted 10-fold in water used in all downstream PCR experiments. The primers and PCR conditions for amplifying total fungal (including EM fungi – only 1% of sequence reads belonged to AM fungal; ITS) and AM fungi (18S) DNA are shown in Table S4. We acknowledge here that the two targeted regions differ in resolution; the potential for this limitation to introduce bias is addressed in the discussion section, as are arguments for the results still being robust. A barcode was added to the 5' end of the reverse primers (*i.e.*, ITS2 and AMDGR) to identify samples when demultiplexing sequence reads. The PCR products were purified using a PCR product gel purification kit (Axygen, Union City, CA, USA). The yields of purified PCR products were measured using a TBS 380 Fluorescence Spectrophotometer (Promega, USA), and 50 ng of DNA from each of the 240 purified PCR products were pooled and adjusted to 10 ng μL^{-1}. The pooled DNA was subjected to sequencing in two separate runs (one for ITS, one for 18S) on the Illumina MiSeq platform, using a 2 × 250 bp paired-read sequencing approach, at the Environmental Genome Platform in the Chengdu Institute of Biology, Chinese Academy of Sciences (Chengdu, China).

Bioinformatics analysis

Clean sequences were obtained from raw sequences after quality control using 'Quantitative Insights into Microbial Ecology' (QIIME v.1.7.0, Caporaso *et al.*, 2010). We used FLASH2 software to merge paired-end reads (mismatchRatio = 0.25, maxOverlap = 125 and minOverlap = 25). Quality control removed low quality reads with no valid primer sequence or barcode sequence, containing ambiguous bases, or with an average quality score < 20; in total, 280 429 ITS reads and 554 630 18S reads were removed. For ITS reads, the ITS1 region of each high-quality sequence was extracted using the fungal ITSx software package (Bengtsson-Palme *et al.*, 2013); a total of 8 427 416 sequences were retained after ITSx. Chimeric sequences were detected using the 'chimera.uchime' command in Mothur 1.31.2 (Schloss, 2009) and removed from further analysis. The non-chimeric sequences were grouped into different operational taxonomic units (OTUs) at a 97% similarity level

based on the UPARSE pipeline using the USEARCH v8.0 after dereplication and singleton exclusion (Edgar, 2013).

Representative sequences from OTUs were selected through the command of 'get.oturep' and were identified by a basic local alignment search tool (BLAST) search against the international nucleotide sequence databases collaboration (INSDc) and UNITE database (Kõljalg *et al.*, 2013) for ITS1 sequences and the Maarj*AM* 18S rRNA gene database (Öpik *et al.*, 2010) for 18S sequences (accessed on Apr. 9th, 2016 and on Sept. 28th, 2016, respectively). Briefly, for ITS1 OTUs, BLASTn search results were considered as sufficiently reliable assignments for fungi when e-values < e^{-50}, e-values between e^{-20} and e^{-50} were manually checked against the 10 best matches to ensure assignment accuracy for fungi, whereas those > e^{-20} were excluded from further analysis. For 18S rRNA gene sequences, we retained OTUs for further analyses if they were assigned to AM fungal taxa and if they had e-values < e^{-50}. Furthermore, the ITS1 OTUs were assigned to functional guilds at the 'highly probable' level following the methods of Tedersoo *et al.* (2014) and using the information in FunGuild (Nguyen *et al.*, 2016; accessed on Nov. 24th, 2016). The results of ITS sequences were described in detail by Ji *et al.* (2019). Briefly, 18 171 fungal OTUs (8 078 161 reads, read numbers ranged from 5290 to 88 715 in the 240 samples) were obtained. The read number per sample was normalized to that sample with the fewest reads (*i.e.*, 5290) using the 'sub.sample' command in Mothur. After normalizing, of 14 911 ITS OTUs (1 267 912 reads), a subset of 9883 ITS OTUs (1 260 949 reads, all OTUs ≥ 3 reads) was extracted to form the total fungal community, and all identified 2709 EM fungal OTUs (431 138 reads, 34.0% of all fungal sequences) were used for subsequent analyses.

In this study, after excluding 1796 OTUs (3 240 604 reads, *c.* 41.8% of all 18S reads) assigned to non-Glomeromycotina, we obtained a total of 4 517 918 high-quality AM fungal sequences, which were distributed across 638 18S OTUs (read numbers ranged from 1509 to 86 502 in the 237 samples). The read number per sample was normalized to the fewest reads (*i.e.*, 1509) using the 'sub.sample' command in Mothur. After normalization, we obtained 592 18S OTUs (357 633 reads) which all matched with known AM fungal sequences from the Maarj*AM* database. Three (*i.e.*, total, AM and EM) fungal community matrices (Table S5) were used in all analyses, including generation of null models based on the neutral model: total fungi (all ITS1 OTUs), EM fungi (ITS1 OTUs assigned to EM taxa) and AM fungi (18S OTUs assigned to Glomeromycota). The DNA sequences of the ITS and 18S rRNA gene amplicons were deposited in the National Center for Biotechnology

Information under accession nos. LT986405–LT998319 and MK352490–MK353081, respectively. The raw sequences (files as .fastq format) are available at the Microbiome Database (http://egcloud.cib.cn) with Nos: PRJ-AMPLI-cda51de0ddacdbaba567b0157b2f3696 (ITS data) and PRJ-AMPLI-d7487575b3f2e06c694c86014e409da8 (18S data).

Statistical analyses

Neutral model simulation and comparison analysis

We estimated distributions of β-diversity that would be expected if community assembly was driven primarily by neutral processes, assuming that the twenty samples (*i.e.*, each local community) within each forest site were linked to a common metacommunity, and compared these to observed β-diversity estimates within the relevant forest site. This approach is a type of null-model analysis in that expectations under the null hypothesis are derived from the collected data, here using observed species abundance distributions associated with each sample. As stated above, these comparisons have three possible outcomes: the observed β-diversity not differing from the neutral prediction (neutral assembly overwhelms determinism) or the observed β-diversity being larger (divergent assembly) or smaller (convergent assembly) than the neutral prediction.

To generate null-model predictions, we used the neutral sampling formula for multiple samples (Etienne, 2007). This approach was used independently for each of the twelve forest sites to estimate parameters (immigration and diversity) from a neutral model, and these estimates were used to simulate communities that could have assembled under only neutral processes. This analysis constrains the analysis to a metacommunity of realistic size and diversity for each site independently. First, the fungal (total, AM, and EM) sample-OTU data obtained from each site was used to estimate the theta (Θ, diversity index) and immigration rate (I) parameters of the neutral model. The formula was used for multiple samples to estimate neutral parameters using the PARI/GP code included in Etienne (2007). Parameters were estimated for the total, AM, and EM fungal communities separately from each of the 12 forest sites. Using the estimated parameters, the PARI/GP function 'urn2.gp' was performed to create 100 communities of matching size. The detailed processes conducted for simulating the neutral community were described by Maaß *et al.* (2014).

The PARI/GP output files were imported into R (R Core Team, 2015) to calculate

pairwise Sørensen community dissimilarities (β-diversity) among all communities within each simulation (Powell *et al.*, 2015). We compared estimates of observed β-diversity (median, interquantile range [IQR] and interdecile range [IDR] of pairwise β-diversity distributions) within each site to the null distribution of these estimates from simulations for the relevant site after calculating standardized effect size (SES) values. Each SES was calculated as the difference in the observed statistic associated with the β-diversity distribution and corresponding expectations produced by the neutral models using the following formula: ($estimate_{obs.}$ – mean of $estimate_{exp.}$) / standard deviation of the $estimate_{exp}$, where $estimate_{obs.}$ denotes the estimate (median/IQR/IDR) associated with the observed distribution of Sørensen dissimilarities and $estimate_{exp.}$ denotes the estimate (median/IQR/IDR) associated with the distribution of Sørensen dissimilarities for each of the individual simulated communities. Variability in the estimates was assessed as bootstrapped 95% confidence intervals. If the SES value was not significantly ($P < 0.05$) different from zero, the community assembly was interpreted as being driven primarily by neutral process; otherwise, the community assembly was regarded as deterministic. In the case of a significant difference in the SES from zero, a positive effect size indicated that the estimate was higher than predicted (divergence), while a negative effect size indicated that the estimate was lower than predicted (convergence) under the neutral hypothesis. The SES values were compared amongst the three fungal groups within each site and the mean values of all 12 sites. The same analyses were conducted for the total plant community, as well as the AM and EM plant communities. In addition to presenting SES values, the distributions of β-diversities for the observed fungal/plant communities and relevant subsets are presented as probability density curves in Figure S2.

Estimation of community and habitat turnover

By applying the approach described in Ranjard *et al.* (2013), we estimated relationships between fungal β-diversity along the latitudinal gradient and in relation to habitat turnover among forests. Briefly, fungal β-diversities (as Sørensen index) were calculated based on the OTU-sample matrix using the 'labdsv' package (Roberts, 2016) in R. To compare with latitude, β-diversity was estimated as the median Sørensen dissimilarity ($n = 12$) among local communities within each of the 12 forests. To compare with habitat turnover among forests, we aggregated the twenty fungal communities in each forest site into a single row (sample) of the OTU-sample matrix by calculating the sum of reads in each column (OTU).

This resulted in 12 composite fungal communities representing 12 forests, and β-diversity was estimated as the Sørensen dissimilarity between each pair of forests (n = 66 pairwise comparisons). Habitat turnover (or environmental dissimilarity, *Ed*) was estimated from a distance matrix based on the site-level soil, plant, and climatic factors. According to the approach described in previous studies (Ranjard *et al.*, 2013; Powell *et al.*, 2015), we calculated the Euclidean distance between sites ('dist' function in R) and then standardised this value using the formula $Ed = 1 - Euc_d / Euc_{max}$, in which Euc_d is the Euclidean distance and Euc_{max} is the maximum distance between sites.

Statistical hypothesis testing

To detect the effect of site on environmental factors, the observed β-diversity, and SES values, one-way analysis of variance (ANOVA) or nonparametric Kruskal–Wallis tests (where data did not satisfy homogeneity of variance) were performed. Subsequently, multiple comparisons of group means among the 12 forest sites were carried out where appropriate (at $P < 0.05$ level) with Tukey's HSD test after significant ANOVA using pairwise *t*-tests ("bonferroni" method of *P*-value correction) of rank-transformed responses after significant Kruskal–Wallis tests. Similar analyses of one-way ANOVA followed by multiple comparisons were also conducted for the observed β-diversity amongst different fungal/plant groups and different climate zones; analyses were performed separately on data subsets consisting of each combination of plant/fungal group and climate zone.

Fungal community compositions were ordinated using nonmetric multidimensional scaling (NMDS) with Sørensen dissimilarity matrices using the 'metaMDS' function in the 'vegan' package (Oksanen *et al.*, 2013). We used the 'lmodel2' package (Legendre, 2011) to perform type II linear regression (ordinary least squares) on the relationships between the observed fungal β-diversity and latitude and habitat turnover (*Ed*) after estimating these at the site level. We also checked for heterogeneity of variation in observed β-diversity (beta dispersion) for fungal and plant communities within each climate zone (tropical, subtropical, and temperate) using the 'betadisper' function (Simpson dissimilarity; free variation in richness) in the 'vegan' package. To estimate the effects of climate zone and site (across all sites and within subsets of tropical, subtropical, and temperate sites) on compositional turnover within each group of fungi (total, AM, and EM fungi), permutational multivariate analysis of variance (PerMANOVA) was conducted based on distance matrices (Sørensen dissimilarity) using the 'adonis' function in the 'vegan' package with 999 permutations.

To explore the independent influence of dispersal limitation on the fungal community assembly, partial Mantel tests were further performed to analyze the correlation between fungal communities and geographic distance after excluding the effects of abiotic (soil and climate related) and biotic (plant related) factors using the 'ecodist' package (Goslee & Urban, 2007). In turn, partial Mantel tests were carried out to explore the relationships between the fungal community and abiotic/biotic factors after excluding the influence of geographic distance. All analyses were conducted in R 3.0.2 (R Core Team, 2015).

Results

Divergence dominated fungal and plant community assembly outcomes within forests

Distributions of observed community dissimilarities (β-diversity) among the total, AM, and EM fungal communities and among total, AM and EM plant communities are presented as probability densities (Fig. S2). Their distributions indicate that substantial variation was observed among communities for all groups across many sites, with some of the distributions being bimodal (indicating convergence among some samples within a site resulting in two different community states). SES values for all these fungal groups were consistently greater than zero (Table S6), indicating significant divergence. The highest fungal SES values within a site were generally associated with total or AM fungal communities, and the lowest SES values were always observed for the EM fungal communities except for site Baotianman (BTM, Table S6). Estimates of *Theta* and *I* are provided in Table S7.

The results of null model analyses indicated that there was strong evidence for divergent assembly among total, AM, and EM fungal communities in each of the three climatic zones (tropical, subtropical, and temperate zones) based on deviations in observed dispersion (Fig. 1b, c) and/or average dissimilarity (Fig. 1a) from the 100 simulations under the null model. For the plant communities, not all SES estimates associated with average dissimilarity differed from zero (Fig. 1a). However, IQR and IDR estimates were substantial in all cases (Fig. 1b, c), reflecting the large range of dissimilarities observed at most sites (Fig. S2) and indicating greater dispersion among local communities than predicted using the null model, providing evidence for divergent assembly.

We did not observe any similar patterns between SESs for fungal and plant communities when comparing responses at individual sites (Table S6) or among climatic

zones (Fig. 1). The most important landscape-level environmental variables for explaining variation in SES values for total fungi were temperature ($P = 0.009$), latitude ($P = 0.012$) and total plant species richness ($P = 0.034$), with plant community composition ($P = 0.053$) and precipitation ($P = 0.051$) being marginally nonsignificant (Table 1). For AM fungi, SES values were significantly correlated with soil C:N ratio ($P = 0.020$) and marginally nonsignificantly correlated with pH ($P = 0.065$) and plant community composition ($P = 0.079$) (Table 1). No environmental factors were correlated with EM fungal SES values (Table 1). SES values for plant communities tended to be correlated more closely with plant community variables, including plant species richness (all and AM plants), basal area (AM plants), and community composition (EM plants).

Environmental drivers of fungal β-diversity across forests

The mean observed β-diversity of EM fungi was consistently and significantly greater than that of total and AM fungi ($P < 0.0001$, Table S8). However, there was no significant difference in plant β-diversity among the three plant groups ($P = 0.179$, Table S8). Fungal β-diversity was significantly greater within temperate forests, followed by tropical forests and then subtropical forests ($P < 0.0001$, Table S9). There was no significant difference in plant β-diversity across tropical, subtropical, and temperate forests ($P = 0.764$, Table S9). Climate zone and site had significant effects on β-diversity of both fungal and plant compositions (Fig. 2, Table S10), regardless of whether whole communities or AM/EM subsets were assessed. We observed larger effects of climate zone and site on AM fungi ($R^2 = 0.280$) than on total ($R^2 = 0.095$) and EM ($R^2 = 0.069$) fungi, based on larger R^2 values in PerMANOVA analyses (Table S10). Among all groups of total, AM, and EM fungi, the effects of site on β-diversities were larger (*i.e.*, greater R^2 values) in the subtropical (R^2, 0.222, 0.403, 0.172, respectively) zone as compared with in temperate (R^2, 0.183, 0.247, 0.156, respectively) and tropical (R^2, 0.086, 0.266, 0.064, respectively) zones (Table S10). Variation explained by climate zone and site was similar when comparing the total, AM, and EM plant groups (Table S10).

At the sampling site level, we found that the observed AM fungal β-diversity was positively correlated with latitude ($P < 0.0001$, Fig. 3a), which is the opposite pattern as was observed for AM plant β-diversity ($P = 0.025$, Fig. 3c). The increase in AM fungal β-diversity with latitude was mirrored by a decline in AM fungal richness with latitude ($P <$

0.001, Fig. S3). No significant correlation with latitude was observed for β-diversity within the other fungal groups (Fig. 3a) despite total (P = 0.004) and EM (P = 0.023) plant β-diversity exhibiting negative relationships with latitude (Fig. 3c). We observed the highest β-diversity in soil EM fungal communities followed by total fungal communities and then AM fungal communities (Fig. 3b). β-diversity in all fungal groups increased with greater habitat turnover (*Ed*; P values < 0.05 for total and EM fungi, P = 0.063 for AM fungi; Fig. 3b), although the explanatory power of these relationships was low (R^2 < 0.07). The β-diversity for all plant groups were not significantly associated with habitat turnover, which may be due to the limited overlap in plant composition among forest sites (Fig. 3d).

Finally, we performed partial Mantel tests to reveal relationships among geographic distance and individual environmental factors on fungal community composition. Pure geographic distance was not significantly correlated with any fungal community groupings (Table 2). Soil pH and total C were significant variables explaining variation in all fungal groups after accounting for geographic distance, plants and climatic factors, while temperature and plant community composition also explained variation in all fungal groups after accounting for other variables (Table 2). Variation in AM fungal community composition was further explained by variation in AM plant basal area (Table 2).

Discussion

Divergence-dominated fungal community assembly

The relative importance of stochastic and deterministic processes in structuring a microbial community is currently being debated (Zhou & Ning, 2017). Previous studies documented that the assembly of microbial communities depends largely on deterministic processes (niche-based) driven by contemporary environmental changes, such as pH, temperature, precipitation, salinity and nutrients (Fierer & Jackson, 2006; Lozupone & Knight, 2007; Dumbrell *et al.*, 2010; Wang *et al.*, 2016; Zhou *et al.*, 2016; Zhang *et al.*, 2019; Zheng *et al.*, 2020). Microbial communities can also be driven by stochastic processes facilitated by geographical separation and dispersal limitation (Wang *et al.*, 2013). Compared with most cases focusing on bacteria from the available microbial community studies, the importance of stochastic processes in generating and maintaining fungal biodiversity is rarely appreciated, and a few studies have addressed the importance of neutrality for these communities in soil and roots (Caruso *et al.*, 2012; Davison *et al.*, 2016; Schroter *et al.*,

2019).

Here, we observed evidence for assembly processes leading to strong divergence among soil fungal communities within forests each distributed along a large environmental gradient. This was the case for whole fungal communities and for subsets consisting of AM and EM fungi, although divergence was less among communities of the latter group. The degree of divergence at whole fungal communities was greater in more diverse forests in warmer climates at lower latitudes, which is consistent with how each of the forest sites was ranked in terms of degree of divergence (tropical > subtropical > temperate). Interpreting these differences is challenging due to the possibility of fungal community shifts leading to differences in abundance of functional guilds among these communities, so we mainly discuss the results of the AM and EM fungal community subsets.

These results indicating a greater tendency toward divergence are consistent with expectations under two scenarios: (1) environmental heterogeneity (*e.g.*, in soil properties, vegetation and/or microclimate) within forests leads to strong niche-based assembly within soil fungal communities or (2) strong biotic interactions lead to communities diverging more than expected under similar environmental conditions. Powell and Bennett (2016) observed similar results for AM fungal communities sampled in a global survey (Davison *et al.*, 2015). In that analysis, divergence and patterns consistent with neutrality were much more frequent outcomes than convergence when trying to compare local communities sampled from similar environments (roots of a single host species collected from multiple plants within a single plot). Here, we observed greater dispersion among AM fungal communities within sites in the higher latitude forests (greater NMDS area of temperate and subtropical than that tropical forest sites and Betadisper $P < 0.0001$; Fig. 2b), but this pattern was confounded by the reduced AM fungal richness at higher latitudes. Similarly, the AM fungal value associated with the null model approach revealed stronger divergence, on average, in temperate and subtropical forests than in tropical forests (Fig. 1a). Veresoglou *et al.* (2019) also reported that latitudinal differences in AM fungal β-diversity were driven to a greater extent by forest types than by latitude.

Less divergence was observed among EM fungal communities in the current study than what was observed for AM fungi and whole fungal communities, which might be attributed to relatively high host specificity for EM fungi compared with AM fungi (Tedersoo *et al.*, 2008; Davison *et al.*, 2015; Wang *et al.*, 2019). If this was the case we might expect a positive relationship between the degree of divergence and variation in EM fungal host

communities. However, we did not observe such a relationship. We did not observe as much difference in the degree of divergence among the three plant subsets (total, AM and EM) either. These results might suggest that neutral processes may play a greater role in structuring EM fungal communities than for communities of AM and other fungi, with niche-based assembly playing a lesser role. That said, distributions of pairwise β-diversity estimates were clearly bimodal or multimodal at most sites (Fig. S2c) and dispersion estimates were consistently high (Fig. 1b, c), indicating the existence of multiple community states which may be linked to the existence of a few discrete niches within individual forest sites. Our results may also reflect the limitations associated with trying to interpret assembly processes in EM fungal communities from small amounts of soil (0.25 g of a composite of ten cores per plot) instead of root samples, where in the latter there might be stronger evidence of niche-based assembly and we may more completely sample rarer taxa that are active in the environment. Regardless, there is some validity to interpreting these data as these samples do reflect the composition of EM fungal communities available to colonise root samples in these environments.

Drivers of soil fungal β-diversity in forests at large scales

In addition to a greater prevalence of divergent community assembly for soil fungi within each forest, we observed high levels of divergence among soil fungal communities across the forests. This divergence could be partially explained by climate, vegetation and soil properties. For the most part, the environmental drivers associated with fungal community turnover were consistent among the three fungal groups, with soil C, plant community composition, MAT and soil pH being the most important variables. These results are consistent with other large scale studies of soil fungal diversity (*e.g.*, Tedersoo *et al.*, 2014; Zhou *et al.*, 2016; Guo *et al.*, 2018).

However, we did observe that AM fungal β-diversity under these forests was lower than the other groups and its relationship with habitat turnover overall was not as strong. It is difficult to draw conclusions from these particular results given the possibility that this is determined, in part or as a whole, by greater variation in the ITS region (used to sample total fungi and EM fungi) than that of the 18S rRNA gene (used to sample AM fungi). That said, when comparing compositional shifts in soil fungal communities among the 12 forests, strengths of relationships with some individual variables tended to be greater for AM fungi

than for the other two groups. This difference was particularly large for soil C, which is intriguing given hypotheses regarding the role that AM fungal-derived proteins (*i.e.*, glomalin) might play in determining the recalcitrance of soil C stocks (Rillig *et al.*, 2003; Gadkar & Rillig, 2006; Holátko *et al.*, 2021). The AM fungal subset was also the only fungal group to exhibit a significant relationship between composition and the basal area of probable host trees. This may reflect the more generalist nature of AM fungal and plant associations, where frequency of host plants may be a greater driver of composition than host identity, than in more specialist EM fungal and plant associations. We note, however, that Toussaint *et al.* (2020) observed that diversities of both AM and EM fungal communities were correlated with frequencies of probable host trees of each fungal group in a global synthesis.

Conclusions

Here we were able to demonstrate substantial divergence among soil fungal communities, including mycorrhizal fungal communities both within and among these twelve, relatively intensively sampled forests. Within forests, fungal communities were consistently more divergent than what we would expect if neutral processes dominated during community assembly. Our results also indicated that the relative strength of deterministic assembly processes differed among fungi and plants in these forests. We did observe that the degree of divergence was related, in part, to environmental variation when looking at whole fungal communities or those of AM fungi. However, we were unable to detect patterns to explain the degree of divergence among EM fungal communities despite the inclusion of variables that were related to compositional shifts at large scales. Taken together, although soil fungal community turnover at large scales could be attributed to specific environmental drivers, strong divergence during fungal community assembly in forest soils at local scales limits the predictability of fungal community assembly outcomes. We encourage future studies to employ similar high-intensity sampling designs across more forest sites and other vegetation types to better assess the generality of these conclusions, derived from a limited number of sites, and to better understand nuanced relationships with environmental variation.

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Author Contributions

Y.Z., L.C. and L.-D.G. designed the experiments and analysed the data in consultation with C.G., S.-S.J., H.-W.H., Z.H., J.-Z.H., and J.R.P. The field surveys, soil samplings, and molecular work were conducted by Y.Z., N.-N.J., L.C., Y.-L.W., and C.G. The manuscript was written by Y.Z. and J.R.P. with contributions from all co-authors.

Data Availability

The data that support the findings of this study are openly available on figshare at https://www.doi.org/10.6084/m9.figshare.13543046.

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Supporting information figure and table captions:

Figure S1. Geographic distribution of sampling sites.

Figure S2. Distributions of Sørensen dissimilarities (β-diversity) of the observed fungal communities.

Figure S3. A significant relationship was observed between the arbuscular mycorrhizal fungal diversity and latitude.

Table S1. The basic soil, plant, and climatic information of 12 forest sites

Table S2. The plant community composition data.

Table S3. The raw data of environmental factors including soil, plant basal area and richness, climatic parameters, and sampling time.

Table S4. Primer sets and PCR conditions used to amplify fungal DNA.

Table S5. The fungal community composition data.

Table S6. Standardized effect sizes (SES) associated with median fungal community dissimilarities.

Table S7. Estimated parameters associated with the neutral model of biodiversity for fungal and plant communities at each site.

Table S8. The observed β-diversity (Sørensen index) of fungal and plant communities at each site.

Table S9. The observed β-diversity (Sørensen index) of fungal and plant communities across tropic, subtropic, and temperate climate zones.

Table S10. Detailed PerMANOVA outcomes for all data subsets.

Figure legends

Figure 1. Estimates of fungal and plant community compositional divergence or convergence by community type and climate zone. The X (plants) and Y (fungi) axes represent standardized effect sizes (SESs) for observed community dissimilarities relative to 100 simulations based on a null model of neutral community assembly. Estimates were calculated in relation to central tendencies, from the median **(a)**, and dispersion from the interquartile range (IQR, middle 50% of observations; **b**) and interdecile range (IDR, middle 80% of observations; **c**). Analyses were performed separately for total, arbuscular (AM) and ecto- (EM) mycorrhizal fungi and plants. The median (points) and 95% confidence interval (lines) of the estimates are presented for communities sampled from tropical, subtropical and temperate forest ecosystems. Central tendency estimates **(a)** focus on shifts in community composition that tend to occur across all communities while dispersion estimates **(b, c)** focus on extreme shifts between pairs of communities. A reduction in the central tendency **(a)** or dispersion **(b, c)** is evidence of community convergence from a common composition, whereas an increase in these two parameters reflects evidence of communities diverging to a greater extent than expected if neutral processes dominate assembly.

Figure 2. Non-metric multidimensional scaling (NMDS) plots showing shifts in community composition for total **(a)**, arbuscular mycorrhizal (AM, **b**), and ectomycorrhizal (EM, **c**) fungi, as well as total **(d)**, AM **(e)**, and EM **(f)** plants, across tropical, subtropical, and temperate climate zones. Solid lines indicate the two-dimensional space that contains all observations within a climate zone. Tests for homogeneity of beta dispersion indicated differences in β-diversity among climate zones for total ($P = 0.0002$), AM ($P < 0.0001$), and EM ($P < 0.001$) fungal groups and for AM ($P = 0.009$), EM ($P = 0.004$), but not total ($P = 0.163$) plant groups.

Figure 3. Relationships of observed fungal **(a, b)** and plant **(c, d)** community dissimilarity (β-diversity) with latitude **(a, c)** and habitat turnover (or environmental dissimilarity, *Ed*; **b, d)**. To compare with latitude, β-diversity was estimated as the median Sørensen dissimilarity among local communities within each of the 12 forests. To compare with habitat turnover among forests, β-diversity was estimated as the Sørensen dissimilarity between each pair of forests after aggregating each OTU/species-sample matrix within each forest. Pairwise environmental dissimilarities were calculated based on Euclidean distances among site-level environmental variables. Solid lines indicate that the predicted relationship is significant ($P < 0.05$) or marginally nonsignificant ($0.05 \leq P < 0.10$) and dashed lines indicate that the relationship is not significant ($P \geq 0.10$). Relationships within each group are based on type II linear regression estimated using ordinary least squares and *P*-values are as follows: between total ($P = 0.573$), AM ($P < 0.0001$) and EM ($P = 0.372$) fungal β-diversity and latitude **(a)**; between total ($P = 0.034$), AM ($P = 0.063$) and EM ($P = 0.043$) fungal β-diversity and *Ed* **(b)**; between total ($P = 0.004$), AM ($P = 0.025$) and EM ($P = 0.023$) plant β-diversity and latitude **(c)**; between total ($P = 0.220$), AM ($P = 0.346$)

and EM (P = 0.387) plant β-diversity and *Ed* **(d)**. AM: arbuscular mycorrhizal; EM: ectomycorrhizal.

Fig. 1

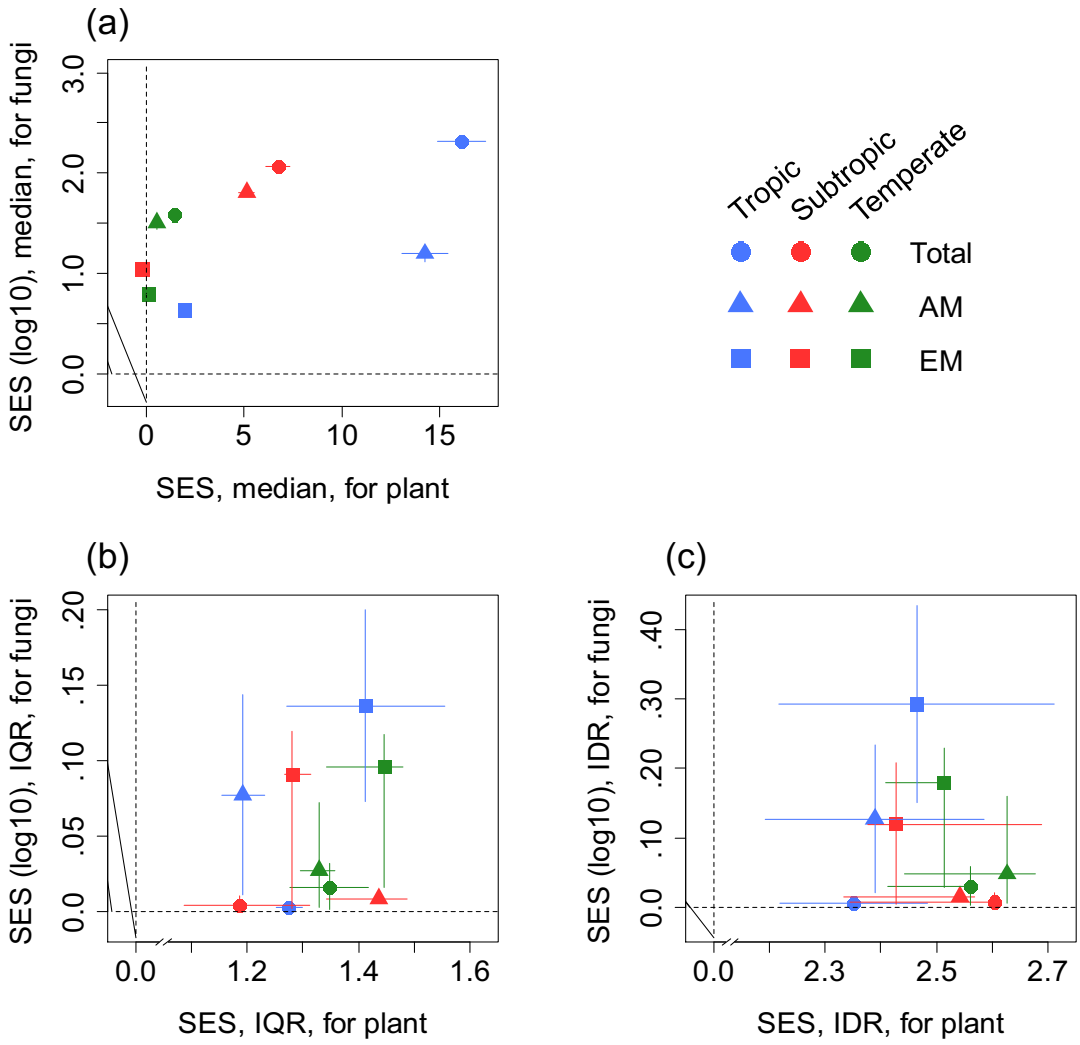

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Fig. 2

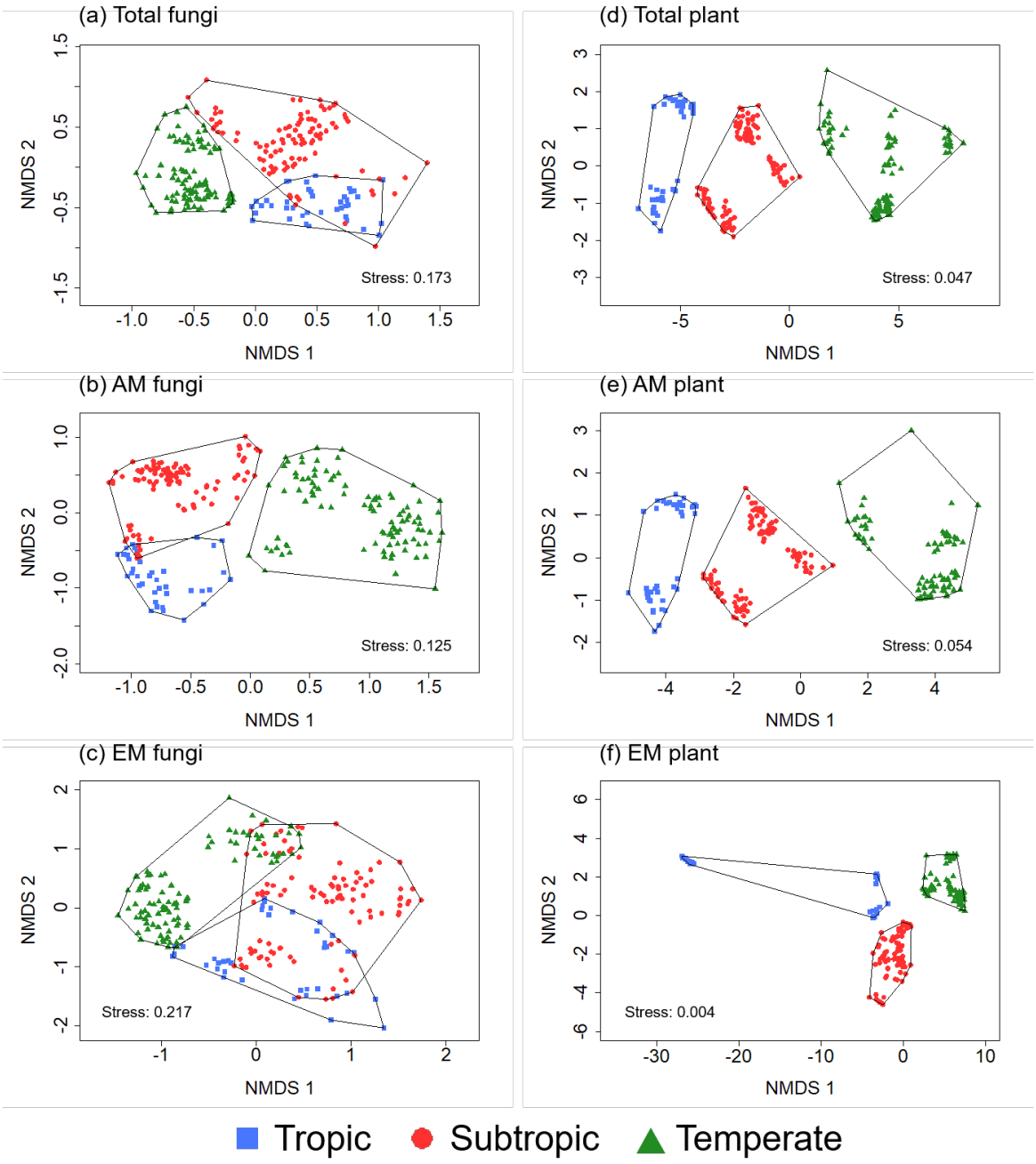

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Fig. 3

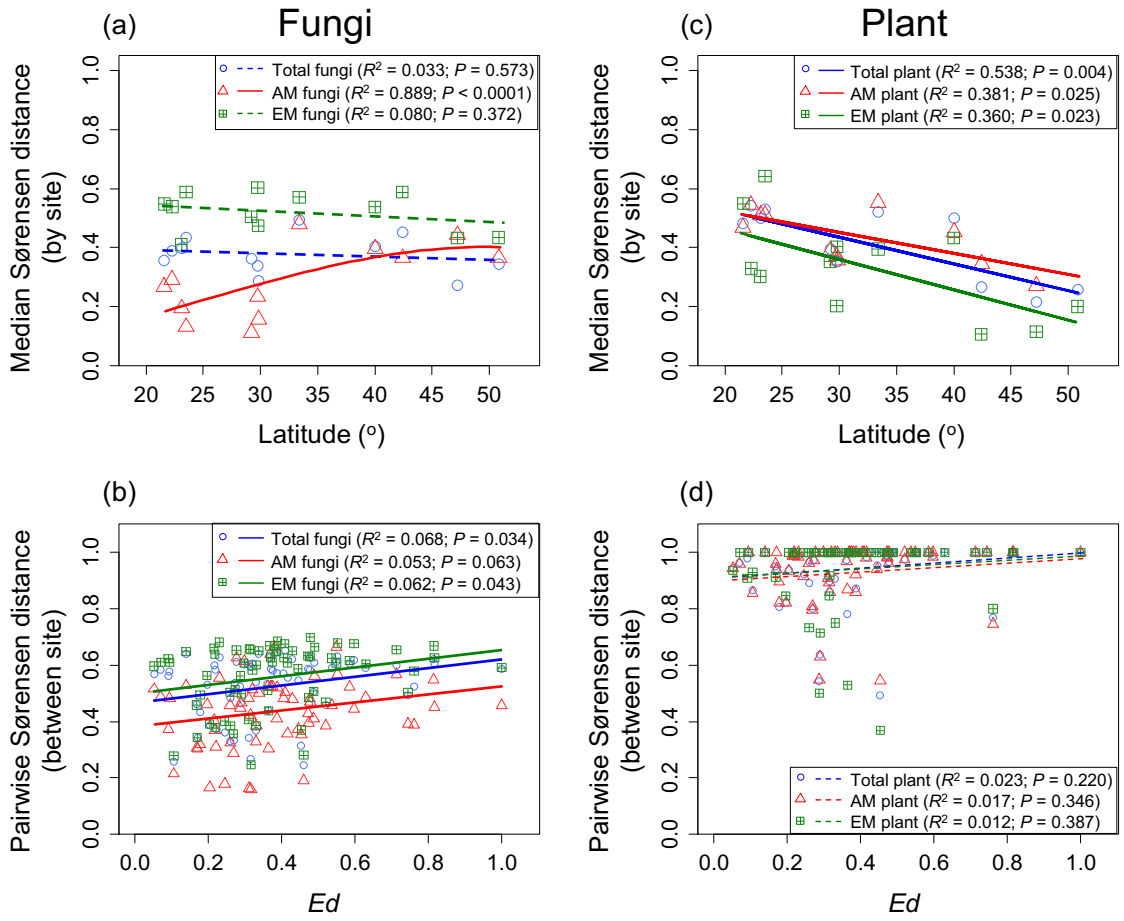

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Table 1

Results of Pearson's correlation analyses between site-level environmental variables and the standardized effect sizes (SESs) associated with shifts in median community composition relative to predictions assuming dominance of neutral processes during community assembly. Separate analyses were performed for total, arbuscular mycorrhizal (AM), and ectomycorrhizal (EM) fungal and plant community groups and environmental parameters. *r*-values are presented in bold in cases where $P < 0.1$ (indicating statistical significance or marginal nonsignificance at $\alpha = 0.05$).

Environmental parameters	**Total fungi**		**Total plants**		**AM fungi**		**AM plants**		**EM fungi**		**EM plants**	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value
pH	-0.327	0.300	-0.324	0.305	**-0.548**	0.065	-0.330	0.321	-0.432	0.161	0.302	0.429
TC	-0.269	0.398	-0.120	0.711	0.227	0.478	-0.235	0.488	-0.149	0.645	-0.574	0.106
TN	-0.141	0.662	-0.054	0.867	-0.165	0.609	-0.232	0.493	-0.300	0.343	-0.587	0.096
TP	-0.357	0.255	-0.239	0.454	-0.336	0.286	-0.293	0.383	-0.486	0.109	-0.427	0.252
C:N	-0.358	0.254	-0.284	0.371	**0.659**	0.020	-0.246	0.465	0.165	0.608	-0.254	0.510
N:P	0.147	0.649	-0.070	0.828	0.336	0.286	-0.071	0.835	0.173	0.591	0.111	0.776
Plant basal area	-0.199	0.536	0.016	0.960	-0.028	0.931	**0.665**	0.025	0.021	0.949	-0.272	0.478
Plant richness	**0.612**	0.034	**0.563**	0.057	-0.050	0.877	**0.670**	0.024	-0.129	0.688	-0.325	0.393
Plant comm. comp.	**0.570**	0.053	0.106	0.743	**-0.551**	0.079	0.082	0.810	0.130	0.688	**0.696**	0.037
Plant SES	0.483	0.112	/	/	0.117	0.732	/	/	0.183	0.638	/	/
Latitude	**-0.693**	0.012	-0.464	0.129	0.238	0.457	-0.533	0.091	-0.216	0.500	-0.293	0.444
MAT	**0.716**	0.009	0.463	0.129	-0.280	0.378	0.525	0.098	0.279	0.381	0.330	0.385
MAP	**0.574**	0.051	0.287	0.365	0.090	0.781	0.270	0.422	0.191	0.553	-0.125	0.749

Table 2

Results of partial Mantel tests (*r* and *P* values) between each fungal community matrix and matrices of environmental parameters associated with geographic distance, soil properties, corresponding plant parameters, and climatic factors.

Factors	**Total fungi**		**AM fungi**		**EM fungi**	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value
Geographic distance	-0.006	0.692	0.014	0.101	0.0001	0.497
pH	**0.073*****[a]	0.001	**0.076*****	0.001	**0.044*****	0.001
TC[b]	**0.184*****	0.001	**0.321*****	0.001	**0.149*****	0.001
TN	-0.034	0.982	0.009	0.253	-0.017	0.923
TP	0.009	0.272	-0.028	0.994	0.001	0.454
C:N	-0.093	1.000	0.025	0.053	-0.076	1.000
N:P	-0.083	1.000	-0.081	1.000	-0.084	1.000
Plant basal area (Tot., AM, EM, respectively)	-0.029	0.961	**0.083*****	0.001	-0.005	0.598
Plant richness (Tot., AM, EM, respectively)	-0.039	0.998	0.016	0.121	0.019	0.063
Plant community composition	**0.270*****	0.001	**0.213*****	0.001	**0.199*****	0.001
MAT	**0.072*****	0.001	**0.108*****	0.001	**0.032****	0.008
MAP	-0.092	1.000	-0.176	1.000	-0.075	1.000

[a]The relationships which were found to be significant are indicated in bold, **, $P \leq 0.01$; ***, $P \leq 0.001$.

[b]TC, soil total carbon; TN, total nitrogen; TP, total phosphorus; C:N, ratio of TC to TN; N:P, ratio of TN to TP; BSA, plant basal area; MAT, mean annual temperature; MAP, mean annual precipitation. AM, arbuscular mycorrhizal; EM, ectomycorrhizal.

Supplementary Information

Title:

Assembly processes lead to divergent soil fungal communities within and among twelve forest ecosystems along a latitudinal gradient

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Running title: Fungal community assembly in forest soils.

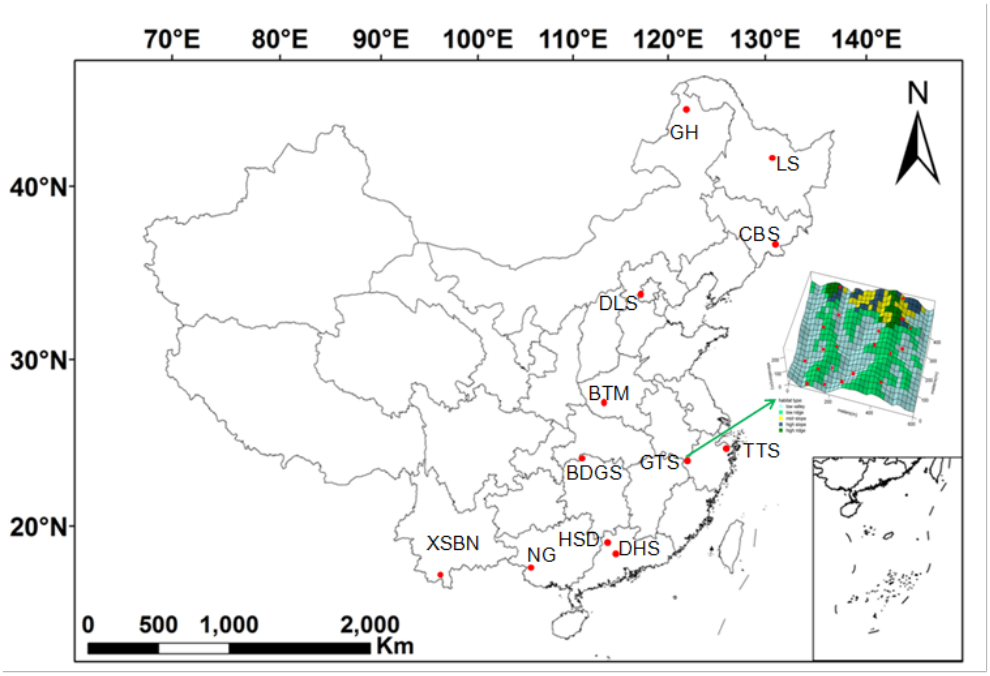
Figure legends

Figure S1. Geographic distribution of sampling sites in a map of China, which was slightly modified with an inset map based on Ji *et al.* (2019). Temperate forests include Genhe (GH), Liangshui (LS), Changbaishan (CBS), Donglingshan (DLS), and Baotianman (BTM); subtropical forests include Gutianshan (GTS), Badagongshan (BDGS), Tiantongshan (TTS), Heishiding (HSD), and Dinghushan (DHS); tropical forests include Nonggang (NG) and Xishuangbanna (XSBN). The inset color map shows the distribution of 20 plots in GTS site.

Figure S2. Distributions of Sørensen dissimilarities (β-diversity) of the observed communities of total fungi **(a)**, AM (arbuscular mycorrhizal) fungi **(b)**, EM (ectomycorrhizal) fungi **(c)**, total plant **(d)**, AM plant **(e)**, and EM plant **(f)** across twelve forests are presented as probability densities.

Figure S3. A significant relationship was observed between the arbuscular mycorrhizal (AM) fungal diversity (OTU richness) and latitude. The parameters of this strong relationship were: AIC = 118.7; $R^2_{adj} = 0.763$; $F = 18.72$; $P < 0.001$.

Fig. S1



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Ji NN, Gao C, Sandel B, Zheng Y, Chen L, Wu BW, Li XC, Wang YL, Lü PP, Sun X *et al.* 2019. Late Quaternary climate change explains soil fungal community composition rather than fungal richness in forest ecosystems. *Ecology and Evolution* **9**: 6678–6692.

Fig. S2

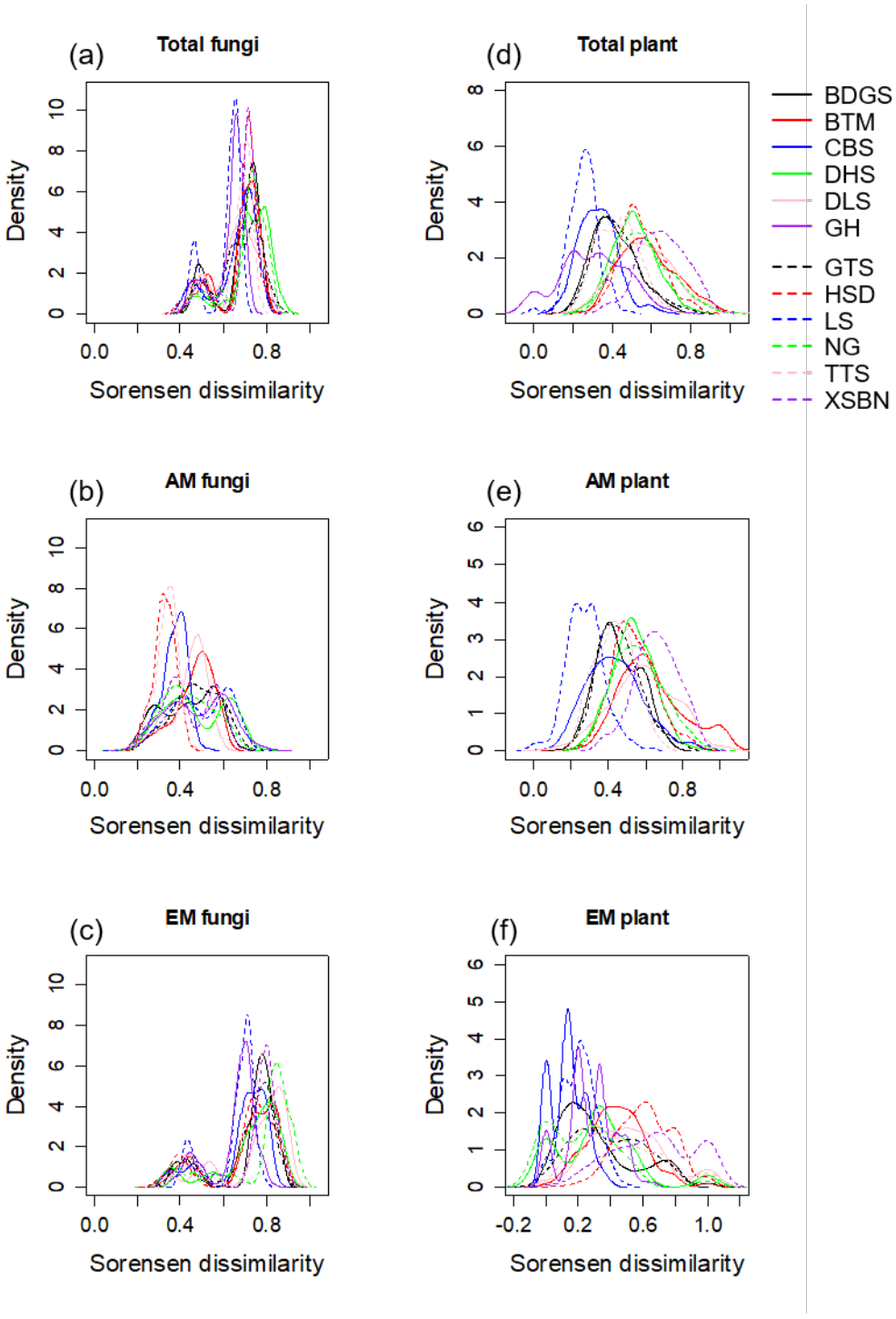

Figure S2. Distributions of Sørensen dissimilarities (β-diversity) of the observed communities of total fungi **(a)**, AM (arbuscular mycorrhizal) fungi **(b)**, EM (ectomycorrhizal) fungi **(c)**, total plant **(d)**, AM plant **(e)**, and EM plant **(f)** across twelve forests are presented as probability densities.

Fig. S3

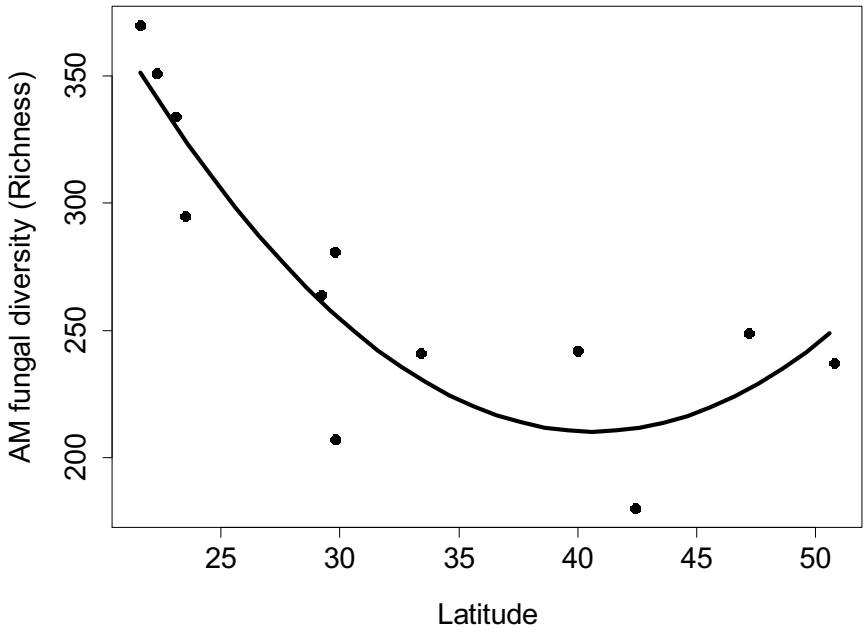

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Table S1.

The basic soil, plant, and climatic information of 12 forest sites (content supplement based on Table 1 in Zheng *et al.* 2020).

Site	Soil factors						Plant factors						Climatic factors		
	pH	TC[b] (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	C:N	N:P	Forest type[c]	Richness	Total basal area (×1000 cm^2)	AMP basal area (×1000 cm^2)[d]	EMP basal area (×1000 cm^2)[d]	Relative abund. (%) of EMP[e]	Climate zone	MAT (°C)	MAP (mm)
BDGS	4.21±0.04f [a]	11.39±0.422a	0.796±0.019a	0.778±0.046cd	14.28±0.329def	1.080±0.058c	SEF	45.35±1.390a	20.48±1.243ab	9.09±0.71ab	7.83±0.98bcde	15.28±1.442def	Subtropic	11.5	2105
BTM	4.88±0.07cd	6.233±0.508cd	0.444±0.041d	0.536±0.050de	14.44±0.384de	0.900±0.117cd	WTDF	24.10±2.531de	15.52±0.876bcd	2.00±0.58ef	13.5±1.08bc	36.54±5.625c	Temperate	15.1	886
CBS	5.48±0.05b	7.950±0.529bc	0.652±0.044abc	1.162±0.053ab	12.26±0.188fg	0.553±0.022d	TPF	11.40±0.600f	17.71±1.169ab	5.70±0.94cd	12.0±1.48bcd	38.30±2.494bc	Temperate	3.6	700
DHS	3.78±0.01g	4.078±0.248def	0.290±0.015e	0.156±0.007f	13.98±0.272defg	1.894±0.102b	SEF	26.65±2.045cd	10.58±0.645cde	4.89±0.52cde	4.79±0.61ef	7.272±1.176f	Subtropic	20.9	1929
DLS	6.71±0.05a	7.104±0.581c	0.536±0.042bcd	0.838±0.052c	13.26±0.213defg	0.633±0.023cd	WTDF	10.10±0.680fg	9.770±0.521de	2.49±0.40def	7.23±0.76cdef	27.30±4.158cd	Temperate	4.8	550
GH	4.93±0.07c	11.93±1.364a	0.507±0.051cd	0.921±0.047bc	23.50±0.830a	0.572±0.070d	CTCF	3.150±0.274g	14.43±1.692bcde	0	14.4±1.70b	100±0.000a	Temperate	-5.3	450
GTS	4.68±0.04de	4.034±0.254def	0.208±0.016e	0.136±0.018f	19.79±0.450b	1.948±0.224b	SEF	36.55±2.222b	17.10±1.326bc	6.42±0.51bc	9.98±1.17bcde	23.50±2.616de	Subtropic	15.3	1964
HSD	4.12±0.03f	3.056±0.188ef	0.231±0.010e	0.078±0.003f	13.08±0.353efg	3.014±0.114a	SEF	45.80±2.022a	16.30±1.328bcd	10.0±1.00a	6.18±0.89def	11.09±1.274ef	Subtropic	19.6	1744
LS	5.64±0.08b	11.80±0.561a	0.703±0.038a	1.026±0.060abc	17.24±0.799c	0.702±0.031cd	TPF	17.10±0.695ef	24.07±3.426a	2.24±0.30ef	21.8±3.51a	49.49±4.033b	Temperate	-0.3	676
NG	6.54±0.05a	5.327±0.174cde	0.549±0.014bcd	1.210±0.166a	9.696±0.136h	0.575±0.059d	TRF	32.60±1.645bc	8.552±0.429e	7.88±0.42abc	0.67±0.25f	4.952±1.194f	Tropic	22	1500
TTS	3.78±0.05g	10.37±0.964ab	0.667±0.047ab	0.446±0.022e	15.25±0.367cd	1.545±0.121b	MEDF	30.90±1.174bcd	14.58±0.833bcde	10.5±0.74a	3.95±0.51ef	9.906±1.553f	Subtropic	16.2	1375
XSBN	4.51±0.04e	2.285±0.099f	0.187±0.005e	0.252±0.013ef	12.19±0.278g	0.782±0.049cd	TRF	47.65±2.833a	15.13±1.343bcde	9.58±1.21ab	5.54±1.23def	13.23±1.072ef	Tropic	21.8	1493

[a] Values (mean ± SE) in the same column without shared letters denote significant difference at $P < 0.05$, as indicated by Tukey's HSD test. [b] TC, total carbon content; TN, total nitrogen content; TP, total phosphorus content; C:N, ratio of TC to TN; N:P, ratio of TN to TP; MAT, mean annual temperature; MAP, mean annual precipitation. [c] Forest types: TRF, tropical rain forests (XSBN and NG); SEF, subtropical evergreen broad-leaf forests (DHS, HSD, GTS, and BDGS); MEDF, mixed evergreen broad-leaved and deciduous broad-leaved forest (TTS); WTDF, warm-temperate deciduous broad-leaved forests (BTM and DLS); TPF, temperate broad-leaved Korean pine forests (CBS and LS); CTCF, cold temperate monsoon coniferous forest (GH). [d] AMP, arbuscular mycorrhizal plants; EMP, ectomycorrhizal plants. [e] The relative abundance (%) of the EM plants to the total plant individuals.

Table S2.

The plant community composition data (available at the link below).

https://www.doi.org/10.6084/m9.figshare.13543046

Table S3.

The raw data of environmental factors including soil, plant basal area and richness, climatic parameters, and sampling time information (available at the link below).

https://www.doi.org/10.6084/m9.figshare.13543046

Table S4.

Primer sets and PCR conditions used to amplify fungal DNA prior to Illumina sequencing.

Target	Nested PCR	Primer	Sequence (5'–3')	Thermal condition	Reference
Fungi (incl. EM fungi)[a]	First PCR	ITS1F	CTTGGTCATTTAGAGGAAGTAA	95°C for 5 min, 30 cycles of at 94°C for 50 s, 52°C for 1 min, 68°C for 1 min.	White *et al.*, 1990
		ITS4	TCCTCCGCTTATTGATATGC		Gardes & Bruns, 1993
	Second PCR	ITS5	GGAAGTAAAAGTCGTAACAAGG	95°C for 3 min, 30 cycles of at 94°C for 50 s, 52°C for 1 min, 68°C for 1 min.	White *et al.*, 1990
		ITS2	GCTGCGTTCTTCATCGATGC		White *et al.*, 1990
AM fungi[b]	First PCR	GeoA2	CCAGTAGTCATATGCTTGTCTC	95°C for 5 min, 30 cycles of at 94°C for 1 min, 58°C for 50 s, 68°C for 1 min.	Schwarzott & Schüßler, 2001
		AML2	GAACCCAAACACTTTGGTTTCC		Lee *et al.*, 2008
	Second PCR	NS31	TTGGAGGGCAAGTCTGGTGCC	95°C for 3 min, 30 cycles of at 94°C for 1 min, 58°C for 50 s, 68°C for 1 min.	Simon *et al.*, 1992
		AMDGR	CCCAACTATCCCTATTAATCAT		Sato *et al.*, 2005

Note: AM, arbuscular mycorrhizal; EM, ectomycorrhizal.

[a] The PCR recipes of ITS primers: (1) The first amplification of the entire ITS region with primers ITS1F (White *et al.*, 1990) and ITS4 (Gardes & Bruns, 1993) was carried out in a final 25 μL reaction solution including 2.5 μL 10× buffer, 1.5 mM $MgSO_4$, 200 μM of each dNTP, 0.75 μM of each primer, 0.5 U KOD-plus-Neo polymerase (Toyobo), and 10 ng of template DNA. (2) The conditions for the nested (second) PCR were the same as the first PCR, except for the template (40 times diluted solution of the products of the first PCR) and primer sets of ITS5 and ITS2 (White *et al.*, 1990) linked to a barcode sequence (6 bases). All amplifications were conducted in triplicate.

[b] The PCR recipes of 18S primers: (1) The first amplification with primers with primers GeoA2 (Schwarzott & Schüßler, 2001) and AML2 (Lee *et al.*, 2008) was carried out in a final 25 μL reaction solution including 2.5 μL 10× buffer, 1.5 mM $MgSO_4$, 200 μM of each dNTP, 0.75 μM of each primer, 0.5 U KOD-plus-Neo polymerase, and 1 μL (*c.* 10 ng) template DNA. (2) The nested PCR products were amplified from the template (20 times diluted solution of the products of the first PCR) with three replicates, using the primers NS31 (Simon *et al.*, 1992) and AMDGR (Sato *et al.*, 2005) linked to a 6-bases barcode.

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Table S5.

The fungal community composition data (available at the link below).

https://www.doi.org/10.6084/m9.figshare.13543046

Table S6.

The values of standardized effect size (SES) associated with median community dissimilarities for the observed total, arbuscular mycorrhizal (AM), and ectomycorrhizal (EM) fungal and plant (in parentheses). β-diversities are presented as the mean of the estimate relative to those of 100 simulated communities for each of the 12 sites.

Zone	Site	SES value[a]					
		Total fungi	Total plants	AM fungi	AM plants	EM fungi	EM plants
Subtropic	BDGS	227.2[b]	8.513	27.87	5.660	5.373	-1.621
Temperate	BTM	295.9	2.787	5.592	1.816	36.18	-0.052
Temperate	CBS	7.881	0.023	23.35	0.152	1.090	-1.210
Subtropic	DHS	260.2	1.557	95.31	1.139	61.30	n.a.[b]
Temperate	DLS	34.38	0.345	7.183	0.364	4.680	1.812
Temperate	GH	19.27	-0.245	197.4	n.a.	6.519	-0.506
Subtropic	GTS	49.28	2.063	87.38	2.320	5.739	0.048
Subtropic	HSD	53.85	1.959	67.43	3.141	3.232	0.846
Temperate	LS	56.00	4.164	185.8	-0.337	6.412	0.516
Tropic	NG	217.0	7.319	4.617	5.976	2.859	n.a.
Subtropic	TTS	131.8	19.56	68.66	13.32	7.148	n.a.
Tropic	XSBN	197.7	24.93	55.34	22.55	7.174	1.954
Fungi	ANOVA[c]	Df = 2; $F = 7.922$; $P = 0.002$					
	Mean[d]	129.2±30.17A		68.83±18.89AB		12.31±5.177B	
Plants	KW test[c]	$\chi^2 = 8.530$, $P = 0.014$					
	Mean[d]		129.2±30.17A		5.099±2.105AB		0.199±0.410B

[a] The SES values were calculated according to the formula as '(observed estimate−mean of the simulated estimates) / standard deviation of the simulated estimates' using the Sørensen dissimilarities of total, AM, and EM fungal and plant community data.

[b] Consistently positive effect size (SES value > 0) indicates that the estimate is higher than predicted (divergence) under the neutral hypothesis; n.a., Result was not available because of the limited plant community data in these sites.

[c] The outcomes of one-way ANOVA or nonparametric Kruskal-Wallis (KW) test analyses on the effect of site on the SES values amongst different fungal and plant groups.

[d] Values (mean ± SE) in that row without shared capital letters denote significant difference amongst fungal and plant groups at $P < 0.05$, as indicated by Tukey's HSD test.

Table S7.

The estimated parameters associated with the neutral model of biodiversity fit at the level of Chinese forest soil for total, AM, and EM fungal and plant communities.

Site	**Theta (Θ)**						***I* (median)**					
	Total fungi	Total plants	AM fungi[a]	AM plants	EM fungi[a]	EM plants	Total fungi	Total plants	AM fungi	AM plants	EM fungi	EM plants
BDGS	16.72	22.83	13.76	18.35	12.57	3.39	4.1E+12	128	672	110	18	3.40
BTM	20.40	16.51	22.70	15.38	7.52	4.14	3.6E+12	29	26	20.9	21377	3.91
CBS	50.06	6.19	12.33	4.66	29.57	1.68	89	15.5	191	6.80	13	6.72
DHS	12.85	23.42	9.08	21.84	4.29	n.a.[b]	5.1E+12	30.5	4537	24.1	2.6E+12	n.a.
DLS	34.85	8.59	19.29	4.58	14.24	1.91	501	4.53	46	3.10	26.5	6.41
GH	35.56	1.80	6.46	n.a.	13.93	1.31	275	1.46	2.5E+12	n.a.	46.5	1.37
GTS	25.08	24.16	6.86	16.57	12.39	3.13	8189	40.25	3.0E+12	33.9	24	3.47
HSD	32.31	47.12	8.97	35.20	16.76	9.91	868	48.44	3346	51.6	14	4.46
LS	31.29	6.65	8.31	7.07	15.46	1.50	1016	56.23	10764	13.8	51	54.5
NG	16.72	21.13	20.46	20.79	16.98	n.a.	3.8E+12	40.59	108	37.5	23.5	n.a.
TTS	16.26	12.72	10.37	10.34	10.88	n.a.	4.7E+12	491	1727	313	48.5	n.a.
XSBN	20.40	19.14	11.78	16.98	12.82	3.03	3.1E+12	5.4E+11	2251	4.6E+11	26	4.02

[a] AM, arbuscular mycorrhizal; EM, ectomycorrhizal.

[b] n.a., Result was not available because of the limited plant community data in these sites.

Table S8.

The observed β-diversity (Sørensen index) of the total, arbuscular mycorrhizal fungi (AM), and ectomycorrhizal (EM) fungal and plant communities at each of 12 forest sites.

Sequence	Sites	Total fungi	Total plants	AM fungi	AM plants	EM fungi	EM plants
1	BDGS	0.338	0.352	0.233	0.371	0.603	0.203
2	BTM	0.493	0.520	0.480	0.552	0.571	0.396
3	CBS	0.453	0.266	0.365	0.346	0.588	0.107
4	DHS	0.399	0.499	0.194	0.510	0.410	0.303
5	DLS	0.405	0.499	0.396	0.454	0.538	0.434
6	GH	0.345	0.258	0.365	n.a.[c]	0.435	0.201
7	GTS	0.364	0.396	0.112	0.390	0.505	0.352
8	HSD	0.434	0.530	0.132	0.512	0.588	0.642
9	LS	0.272	0.215	0.443	0.272	0.434	0.115
10	NG	0.390	0.543	0.291	0.545	0.539	0.330
11	TTS	0.287	0.354	0.156	0.357	0.474	0.403
12	XSBN	0.357	0.482	0.269	0.468	0.548	0.549
	ANOVA[a]	**Fungi:** Df = 2; F = 20.62; P < 0.0001; **Plants:** Df = 2; F = 1.815; P = 0.179					
	Mean[b]	0.378±0.019B	0.410±0.034a	0.286±0.036C	0.434±0.028a	0.519±0.019A	0.336±0.047a

[a] The outcomes of one-way ANOVA analyses on the effect of site on the observed β-diversity amongst different fungal and plant groups.

[b] Values (mean ± SE) in that row without shared letters denote significant difference in observed β-diversity amongst fungal (uppercase) and plant (lowercase) groups at P < 0.05, as indicated by Tukey's HSD test.

[c] n.a., Result was not available because of the limited plant community data in that site.

Table S9.

The observed β-diversity (Sørensen index) of the total, arbuscular mycorrhizal (AM), and ectomycorrhizal (EM) fungal and plant communities across tropic, subtropic, and temperate climate zones.

Type		Tropic		Subtropic		Temperate	
		Mean[a]	Median[a]	Mean	Median	Mean	Median
Fungi	Total	0.712	0.734	0.463	0.437	0.770	0.818
	AM	0.754	0.774	0.526	0.574	0.792	0.826
	EM	0.716	0.744	0.514	0.533	0.765	0.807
	ANOVA[b]	Df = 2; F = 115; P < 0.0001					
	Mean	0.739 ± 0.010B[c]		0.508 ± 0.020C		0.796 ± 0.010A	
Plants	Total	0.800	0.930	0.828	0.921	0.824	0.961
	AM	0.801	0.927	0.830	0.922	0.799	0.875
	EM	0.757	0.990	0.854	1.000	0.783	1.000
	ANOVA[b]	Df = 2; F = 0.281; P = 0.764					
	Mean	0.786 ± 0.015A[c]		0.837 ± 0.008A		0.802 ± 0.012A	

[a] The values were shown as the mean (median) of the Sørensen index within each climate zone for each fungal/plant group.

[b] The outcomes of one-way ANOVA analyses on the fungal and plant observed β-diversity amongst three different climate zones.

[c] Values (mean ± SE) in that row with shared capital letters denote non-significant difference amongst three climate zones at P < 0.05, as indicated by Tukey's HSD test.

Table S10.

Detailed PerMANOVA outcomes using all data focusing on the effects (*i.e.*, predictors) of climate zone (n = 240) and site (n = 240) and re-analyzed to reveal the site effect for each three climate zones of tropic (n = 40), subtropic (n = 100), and temperate regions (n = 100). The analyses were conducted for total, arbuscular mycorrhizal (AM), and ectomycorrhizal (EM) fungi and plant communities individually.

Effect	**Df**	**Total fungi**		**Total plants**		**AM fungi**		**AM plants**		**EM fungi**		**EM plants**	
		R^2	*P* value	R^2	*P* value	R^2	*P* value	R^2	*P* value	R^2	*P* value	R^2	*P* value
Climate zone	2	0.095	0.001	0.143	0.001	0.280	0.001	0.157	0.001	0.069	0.001	0.130	0.001
Site (All data)	11	0.262	0.001	0.572	0.001	0.490	0.001	0.540	0.001	0.207	0.001	0.598	0.001
Site (Tropic)	1	0.086	0.001	0.359	0.001	0.266	0.001	0.361	0.001	0.064	0.003	0.327	0.001
Site (Subtropic)	4	0.222	0.001	0.492	0.001	0.403	0.001	0.480	0.001	0.172	0.001	0.528	0.001
Site (Temperate)	4	0.183	0.001	0.562	0.001	0.247	0.001	0.465	0.001	0.156	0.001	0.620	0.001