Author/s:
Zheng, Y;Chen, L;Ji, NN;Wang, YL;Gao, C;Jin, SS;Hu, HW;Huang, Z;He, JZ;Guo, LD;Powell, JR

Title:
Assembly processes lead to divergent soil fungal communities within and among 12 forest ecosystems along a latitudinal gradient

Date:
2021-08-01

Citation:
Zheng, Y., Chen, L., Ji, N. N., Wang, Y. L., Gao, C., Jin, S. S., Hu, H. W., Huang, Z., He, J. Z., Guo, L. D. & Powell, J. R. (2021). Assembly processes lead to divergent soil fungal communities within and among 12 forest ecosystems along a latitudinal gradient. New Phytologist, 231 (3), pp.1183-1194. https://doi.org/10.1111/nph.17457.

Persistent Link:
https://hdl.handle.net/11343/298628

MR YONG ZHENG (Orcid ID : 0000-0001-5239-9824)
MR CHENG GAO (Orcid ID : 0000-0003-2522-7909)
DR HANG-WEI HU (Orcid ID : 0000-0002-3294-102X)
DR JEFF R POWELL (Orcid ID : 0000-0003-1091-2452)

Article type : Original Article

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

Yong Zheng[1,2,#], Liang Chen[3,#], Niu-Niu Ji[3], Yong-Long Wang[3], Cheng Gao[3], Sheng-Sheng Jin[1,2], Hang-Wei Hu[1,2], Zhiqun Huang[1,2], Ji-Zheng He[1,2], Liang-Dong Guo[3,4]*, Jeff R. Powell[5]*

[1] State Key Laboratory for Subtropical Mountain Ecology of the Ministry of Science and Technology and Fujian Province, Fujian Normal University, Fuzhou 350007, China.
[2] School of Geographical Sciences, Fujian Normal University, Fuzhou 350007, China.
[3] State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China.
[4] University of Chinese Academy of Sciences, Beijing 100049, China.
[5] Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia.

#These authors contributed equally to this work.
***Corresponding authors:**
Dr./Prof. Liang-Dong Guo
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of

Sciences, Beijing 100101, China.
Tel.: +86 10 64807510; *E-mail*: guold@im.ac.cn

Dr./Prof. Jeff R. Powell
Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia.
Tel.: +61 (0)2 4570 1093; *E-mail*: jeff.powell@westernsydney.edu.au

Received: 14 January 2021
Accepted: 14 April 2021

ORCID details for all authors:

Name	**ORCID**	**Link**
Yong Zheng	0000-0001-5239-9824	http://orcid.org/0000-0001-5239-9824
Liang Chen	0000-0002-5706-6559	https://orcid.org/0000-0002-5706-6559
Niu-Niu Ji	0000-0002-0526-705X	http://orcid.org/0000-0002-0526-705X
Cheng Gao	0000-0003-2522-7909	https://orcid.org/0000-0003-2522-7909
Hang-Wei Hu	0000-0002-3294-102X	http://orcid.org/0000-0002-3294-102X
Zhiqun Huang	0000-0002-8929-4863	https://orcid.org/0000-0002-8929-4863
Ji-Zheng He	0000-0002-9169-8058	http://orcid.org/0000-0002-9169-8058
Liang-Dong Guo	0000-0002-5203-3192	https://orcid.org/0000-0002-5203-3192
Jeff R. Powell	0000-0003-1091-2452	http://orcid.org/0000-0003-1091-2452

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 21.6° N to 50.8° 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 $Ed = 1 - \mathrm{Euc}_d / \mathrm{Euc}_{max}$, 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.

Acknowledgments

We are grateful to staff of the CForBio for soil sample collection. We appreciate Professors Ke-Ping Ma, Xiao-Jun Du, Wei-Guo Sang, Zhan-Qing Hao, Ming-Xi Jiang, Wan-Hui Ye, Min Cao, Xian-Kun Li, Guang-Ze Jin, Xiao-Yong Chen, and Bu-Hang Li for providing the plant information. We thank Dr. Jianjun Wang from Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences for the helpful comments on the early version of the manuscript. We also acknowledge the valuable comments and feedback provided by the editor and three anonymous referees. This study was financially supported by the Strategic Priority Research Program (B) of the Chinese Academy of Sciences (XDB31000000), National Natural Science Foundation of China (31971447 and 31210103910) and the Australian Research Council (DP130102501 and FT190100590).

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. Y.Z. and L.C. contributed equally to this work.

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.

References

Alzarhani AK, Clark DR, Underwood GJC, Ford H, Cotton TEA, Dumbrell AJ. 2019. Are drivers of root-associated fungal community structure context specific? *The ISME Journal* **13**: 1330–1344.

Beck S, Powell JR, Drigo B, Cairney JWG, Anderson IC. 2015. The role of stochasticity differs in the assembly of soil- and root-associated fungal communities. *Soil Biology & Biochemistry* **80**: 18–25.

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, De Wit P, Sánchez-García M, Ebersberger I, de Sousa F *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.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI *et al.* 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**: 335–336.

Caruso T, Hempel S, Powell JR, Barto EK, Rillig MC. 2012. Compositional divergence and convergence in arbuscular mycorrhizal fungal communities. *Ecology* **93**: 1115–1124.

Chase JM. 2003. Community assembly: when should history matter? *Oecologia* **136**: 489–498.

Chase JM. 2010. Stochastic community assembly causes higher biodiversity in more productive environments. *Science* **328**: 1388–1391.

Davison J, Moora M, Jairus T, Vasar M, Öpik M, Zobel M. 2016. Hierarchical assembly rules in arbuscular mycorrhizal (AM) fungal communities. *Soil Biology & Biochemistry* **97**: 63–70.

Davison J, Moora M, Öpik M, Adholeya A, Ainsaar L, Bâ A, Burla S, Diedhiou AG, Hiiesalu I, Jairus T *et al.* 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**: 970–973.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. *The ISME Journal* **4**: 337–345.

Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* **10**: 996–998.

Ellwood MDF, Manica A, Foster WA. 2009. Stochastic and deterministic processes jointly structure tropical arthropod communities. *Ecology Letters* **12**: 277–284.

Etienne RS. 2007. A neutral sampling formula for multiple samples and an 'exact' test of neutrality. *Ecology Letters* **10**: 608–618.

Ettema CH, Wardle DA. 2002. Spatial soil ecology. *Trends in Ecology & Evolution* **17**: 177–183.

Fargione J, Brown CS, Tilman D. 2003. Community assembly and invasion: an experimental test of neutral versus niche processes. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 8916–8920.

Feng G, Mi XC, Yan H, Li F, Svenning JC, Ma KP. 2016. CForBio: a network monitoring Chinese forest biodiversity. *Science Bulletin* **61**: 1163–1170.

Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 626–631.

Gadkar V, Rillig MC. 2006. The arbuscular mycorrhizal fungal protein glomalin is a putative homolog of heat shock protein 60. *FEMS Microbiology Letters* 263: 93–101.

Gao C, Zhang Y, Shi NN, Zheng Y, Chen L, Wubet T, Bruelheide H, Both S, Buscot F, Ding Q *et al.* 2015. Community assembly of ectomycorrhizal fungi along a subtropical secondary forest succession. *New Phytologist* **205**: 771–785.

Goslee SC, Urban DL. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* **22**: 1–19.

Guo X, Feng JJ, Shi Z, Zhou XS, Yuan MT, Tao XY, Hale L, Yuan T, Wang JJ, Qin YJ *et al.* 2018. Climate warming leads to divergent succession of grassland microbial communities. *Nature Climate Change* **8**: 813–818.

Holátko J, Brtnický M, Kučerík J, Kotianová M, Elbl J, Kintl A, Kynický J, Benada O, Datta R, Jansa J. 2021. Glomalin – Truths, myths, and the future of this elusive soil glycoprotein. *Soil Biology & Biochemistry* **153**: 108116.

Hurtt GC, Pacala SW. 1995. The consequences of recruitment limitation: reconciling chance, history and competitive differences between plants. *Journal of Theoretical Biology* **176**: 1–12.

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.

Kivlin SN, Winston GC, Goulden ML, Treseder KK. 2014. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales.

Fungal Ecology **12**: 14–25.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM *et al.* 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

Kraft NJB, Valencia R, Ackerly DD. 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. *Science* **322**: 580–582.

Legendre P. 2011. *lmodel2: Model II Regression. R package version 1.7-2.* URL https://CRAN.R-project.org/package=lmodel2.

Lozupone CA, Knight R. 2007. Global patterns in bacterial diversity. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 11436–11440.

Maaß S, Migliorini M, Rillig MC, Caruso T. 2014. Disturbance, neutral theory, and patterns of beta diversity in soil communities. *Ecology and Evolution* **4**: 4766–4774.

Maherali H, Oberle B, Stevens PF, Cornwell WK, McGlinn DJ. 2016. Mutualism persistence and abandonment during the evolution of the mycorrhizal symbiosis. *American Naturalist* **188**: E113–E125.

Mayor J, Bahram M, Henkel T, Buegger F, Pritsch K, Tedersoo L. 2015. Ectomycorrhizal impacts on plant nitrogen nutrition: emerging isotopic patterns, latitudinal variation and hidden mechanisms. *Ecology Letters* **18**: 96–107.

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P *et al.* 2013. Patterns and processes of microbial community assembly. *Microbiology and Molecular Biology Reviews* **77**: 342–356.

Nguyen NH, Song ZW, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. Vegan: community ecology package. R package version 2.0-10. Available at: http://CRAN.R-project.org/package=vegan.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M. 2010. The online database Maarj*AM* reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* **188**: 223–241.

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology* **11**: 789–799.

Powell JR, Bennett AE. 2016. Unpredictable assembly of arbuscular mycorrhizal fungal communities. *Pedobiologia* **59**, 11–15.

Powell JR, Karunaratne S, Campbell CD, Yao HY, Robinson L, Singh BK. 2015. Deterministic processes vary during community assembly for ecologically dissimilar taxa. *Nature Communications* **6**: 8444.

R Core Team. 2015. *R: a language and environment for statistical computing*. Version 3.0.2. URL http://www.R-project.org/.

Ranjard L, Dequiedt S, Chemidlin Prévost-Bouré N, Thioulouse J, Saby NPA, Lelievre M, Maron PA, Morin FER, Bispo A, Jolivet C, Arrouays D, Lemanceau P 2013. Turnover of soil bacterial diversity driven by wide-scale environmental heterogeneity. *Nature Communications* **4**: 1434.

Rillig MC, Ramsey PW, Morris S, Paul EA. 2003. Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. *Plant and Soil* **253**: 293–299.

Roberts DW. 2016. *labdsv: Ordination and Multivariate Analysis for Ecology. R package version 1.8-0*. URL https://CRAN.R-project.org/package=labdsv.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ *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.

Schroter K, Wemheuer B, Pena R, Schoning I, Ehbrecht M, Schall P, Ammer C, Daniel R, Polle A. 2019. Assembly processes of trophic guilds in the root mycobiome of temperate forests. *Molecular Ecology* **28**: 348–364.

Shi LL, Mortimer PE, Slik JWF, Zou XM, Xu J, Feng WT, Qiao L. 2014. Variation in forest soil fungal diversity along a latitudinal gradient. *Fungal Diversity* **64**: 305–315.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*. Cambridge, UK: Academic Press.

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A *et al.* 2014. Global diversity and geography of soil fungi. *Science* **346**: 1256688.

Tedersoo L, Jairus T, Horton BM, Abarenkov K, Suvi T, Saar I, Kõljalg U. 2008. Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. *New Phytologist* **180**: 479–490.

Toussaint A, Bueno G, Davison J, Moora M, Tedersoo L, Zobel M, Öpik M, Pärtel M. 2020. Asymmetric patterns of global diversity among plants and mycorrhizal fungi. *Journal of Vegetation Science* **31**: 355–366.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.

van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**: 1406–1423.

Veresoglou SD, Liu L, Xu T, Rillig MC, Wang M, Wang J, Chen Y, Hu Y, Hao Z, Chen B. 2019. Biogeographical constraints in Glomeromycotinan distribution across forest habitats in China. *Journal of Ecology* **107**: 684–695.

Wang B, Qiu Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**: 299–363.

Wang JJ, Pan FY, Soininen J, Heino J, Shen J. 2016. Nutrient enrichment modifies temperature-biodiversity relationships in large-scale field experiments. *Nature Communications* **7**: 13960.

Wang JJ, Shen J, Wu YC, Tu C, Soininen J, Stegen JC, He JZ, Liu XQ, Zhang L, Zhang EL. 2013. Phylogenetic beta diversity in bacterial assemblages across ecosystems: deterministic versus stochastic processes. *The ISME Journal* **7**: 1310–1321.

Wang YL, Gao C, Chen L, Ji NN, Wu BW, Li XC, Lü PP, Zheng Y, Guo LD. 2019. Host plant phylogeny and geographic distance strongly structure Betulaceae-associated ectomycorrhizal fungal communities in Chinese secondary forest ecosystems. *FEMS Microbiology Ecology* **95**: fiz037.

Zhang KP, Shi Y, Cui XQ, Yue P, Li KH, Liu XJ, Tripathi BM, Chu HY. 2019. Salinity is a key determinant for soil microbial communities in a desert ecosystem. *mSystems* **4**: e00225-18.

Zheng Y, Ji NN, Wu BW, Wang JT, Hu HW, Guo LD, He JZ. 2020. Climatic factors have unexpectedly strong impacts on soil bacterial β-diversity in 12 forest ecosystems. *Soil Biology & Biochemistry* **142**: 107699.

Zhou JZ, Deng Y, Shen LN, Wen CQ, Yan QY, Ning DL, Qin YJ, Xue K, Wu LY, He ZL *et al.* 2016. Temperature mediates continental-scale diversity of microbes in forest soils. *Nature Communications* **7**: 12083.

Zhou JZ, Liu WZ, Deng Y, Jiang YH, Xue K, He ZL, Van Nostrand JD, Wu LY, Yang YF, Wang AJ. 2013. Stochastic assembly leads to alternative communities with distinct functions in a bioreactor microbial community. *mBio* **4**: e00584-12.

Zhou JZ, Ning DL. 2017. Stochastic community assembly: does it matter in microbial ecology? *Microbiology and Molecular Biology Reviews* **81**: e00002-17.

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.

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.

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

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 $\alpha = 0.05$).

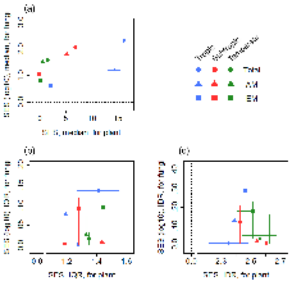
Table 2

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.

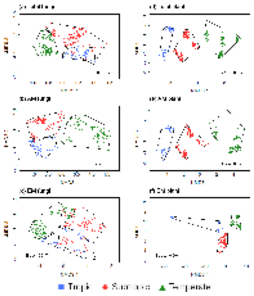
Factors	**Total fungi**		**AM fungi**		**EM fungi**	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value
Geographic distance	-0.006	0.692	0.014	0.101	0.0001	0.497
pH	**0.073*****[a]	0.001	**0.076*****	0.001	**0.044*****	0.001
TC[b]	**0.184*****	0.001	**0.321*****	0.001	**0.149*****	0.001
TN	-0.034	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, **, $P \leq 0.01$; ***, $P \leq 0.001$.

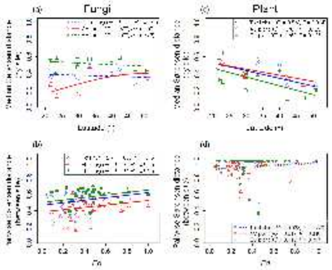
[b] TC, soil total carbon; TN, total nitrogen; TP, total phosphorus; C:N, ratio of TC to TN; N:P, ratio of TN to TP; BSA, plant basal area; MAT, mean annual temperature; MAP, mean annual precipitation. AM, arbuscular mycorrhizal; EM, ectomycorrhizal.



nph_17457_f1.tif



nph_17457_f2.tif



nph_17457_f3.tif