Soil fungal assemblage complexity is dependent on soil fertility and dominated by deterministic processes

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Summary

- In the processes controlling ecosystem fertility, fungi are increasingly acknowledged as key drivers. However, the understanding of the rules of fungal community assembly regarding the effect of soil fertility level is still limited.
- Using soil samples from typical tea plantations spanning approximately a 2,167 km north–east to south–west across China, this study investigated the assemblage complexity and assembly processes of 140 fungal communities along a soil fertility gradient.
- The community dissimilarities of total fungi and fungal functional guilds increased with increasing soil fertility index dissimilarity. The symbiotrophs were more sensitive to the variation of soil fertility than those of pathotrophs and saprotrophs. Fungal networks exhibited larger size and higher connectivity as well as greater potential for inter-module connection in more fertile soils. Environmental factors had a slightly greater influence on fungal community composition than spatial factors. Species abundance fitted the Zipf-Mandelbrot distribution (niche-based mechanisms), which provided evidence for deterministic-based processes.
- Overall, the soil fungal communities in tea plantations responded in a deterministic manner to soil fertility, with high fertility correlated with complex fungal community assemblages. This study provides new insights that might contribute to predictions of fungal community complexity.

Keywords： Community assembly; Ecological processes; Fungal ecology; Fungal functional guilds; Soil fertility

Introduction

A central challenge in ecology is the identification of the community assemblage patterns and related fundamental assembly processes (Weiher *et al.*, 2011). The assembly of microbial communities has become a major research focus in ecology (Hanson *et al.*, 2012; Nemergut *et al.*, 2013), as soil microbes are major drivers on biogeochemical cycles at a global scale and play vital roles in the regulation of natural ecosystems (Falkowski *et al.*, 2008; van der Heijden *et al.*, 2008; Bardgett & van der Putten, 2014). Some of these soil microorganisms display intimate relationships with plants that are critical for plant productivity, resistance, and resilience (Vandenkoornhuyse *et al.*, 2015). In the soil microbial community, fungi are ecologically important because they mediate soil carbon and nutrient cycling, and are involved in nutrient and water uptake to support plant nutrition and protect the plant from biotic and abiotic stresses such as drought and phytopathogen attacks (Read & Perez-Moreno, 2003; Sikes *et al.*, 2009; Schneider *et al.*, 2012; Tedersoo *et al.*, 2014). Soil fungi are often regarded as important drivers of soil fertility (Duhamel & Vandenkoornhuyse, 2013) and help to feed the world (Marx, 2004). Thus, knowledge of the characterization, ecological function, and assembly process of soil fungal community are current hot topics in microbial ecology.

The current ecological theories implied that a positive feedback relationship was existed between soil fertility and soil biodiversity (Delgado-Baquerizo *et al.*, 2017). Soil fertility is under the control of the ecological efficiency of soil fungi as decomposers and other functional guilds involved in symbiotic relationships with plants (Kyaschenko *et al.*, 2017). In turn, fertility-related soil properties can control soil fungal community structure and function by allowing the more appropriately adapted species to grow (Abbott & Johnson, 2017). However, soil fertility is multifactorial, whereas the majority of studies addressing soil fertility have focused mainly on one particular component, most often the mineral nutrient concentration, which may not accurately represent the soil fertility-related ecosystem service. Despite this fact, most studies aiming to address patterns of soil fungal assemblages were often developed under the focus of isolated soil characteristics, including soil pH (Rousk *et al.*, 2010; Tedersoo *et al.*, 2014), salinity (Mohamed & Martiny, 2011), organic matter/carbon content (Liu *et al.*, 2015) and soil nutrient content

(Egerton-Warburton & Allen, 2000). Distinct soil fertility components that potentially exhibit synergistic or antagonistic biotic interactions may result in differences between soil fungal assemblages (Siciliano *et al.*, 2014; Grau *et al.*, 2017). In this study, we used a soil fertility index (hereafter called the soil fertility proxy) as a quantitative measurement of soil fertility (Andrews *et al.*, 2004; Shang *et al.*, 2014) to explicitly link the multiple soil fertility components and the complexity of soil fungal assemblages.

The assembly of microbial communities has traditionally been considered predominantly driven through deterministic (*e.g.,* selection) processes (Fierer & Jackson, 2006; Lozupone & Knight, 2007). It can be suggested that a given combination of microbial species will be selected deterministically according to environmental filtering through the organisms' fitness (De Wit & Bouvier, 2006). Emerging evidence has demonstrated that deterministic processes alone are not sufficient to explain the inherent variance in microbial community composition observed in natural ecosystems (Ramette & Tiedje, 2007). A large portion of the remaining unexplained variation reflects the existence of ecological stochasticity (Zhou & Ning, 2017), which implies that the community patterns with respect to species dynamics may be a result of stochastic processes through birth, death, and dispersal events (Chave, 2004). As opposed to a dichotomous debate, a more comprehensive perspective suggests that both deterministic and stochastic processes are jointly responsible for microbial community assembly (Dumbrell *et al.*, 2010; Wang *et al.*, 2013; Morrison-Whittle & Goddard, 2015; Tripathi *et al.*, 2018). Despite the well-recognized role of these ecological processes, the debate regarding the relative importance of deterministic and stochastic processes to soil fungal community assembly remains largely unresolved.

Tea plants (*Camellia sinensis* L.) are perennial shrubs that grow preferentially in acidic soils. Given that fungi generally exhibit a higher tolerance to acidic conditions than bacteria (Rousk *et al.*, 2010), soil fungi have been assumed to be more important than bacteria in tea plantation ecosystems (Huang *et al.*, 2017). Extensive tea cultivation in South China provides a system for studying patterns and processes of soil fungal community assembly at a broad scale. In this study, a total of 140 soil fungal communities inhabiting fourteen representative tea plantations across eleven provinces of South China spanning 2,167 kilometers were analyzed and evaluated. Using

this design, variations in fungal community assemblages along a soil fertility gradient were explored, and the relative importance of deterministic and stochastic processes in defining fungal community assembly was examined. Specifically, considering the mechanistic links between soil fertility and soil mycobiota, we hypothesized that the complexity of fungal assemblages could be closely dependent on the soil fertility condition, that is, the more soil fertility proxy is, the more complex of fungal community. We also addressed the possible mechanisms that explain the fungal community assembling along soil fertility gradient.

This study aimed to provide a better understanding of fungal community diversity along a soil fertility gradient by analyzing key fungal functional guilds, namely, plant symbionts, decomposers (saprotrophs), and plant pathogens. A second objective of this study was to characterize the relative importance of deterministic and stochastic processes to fungal community assembly. In a broader view, this work aimed to highlight the importance of considering fungi and fungal community assemblages as important compartments that drive ecosystem fertility.

Materials and Methods

Soil sampling, physicochemical analyses, and soil fertility assessment

Soils were sampled from 14 tea plantation sites across eleven provinces of South China (spanning approximately 21.99°–32.10°N and 100.43°–119.89°E, an approximately 2,167 km north–east to south–west gradient; Figure 1a) in between mid-November to mid-December 2016. Within each sampling site, ten plots were randomly selected, and a composited soil sample (consisting of five discrete soils, 0–20 cm depth) was collected from each plot. A total of 140 soil samples (fourteen sites × ten plots) were included in the present study. Each fresh soil was sieved through a 2-mm mesh to remove visible roots and rocks. Then soil sample was further homogenized before subdividing each for analyses. Aliquots of samples were stored at −80 °C until the molecular analyses. The remaining samples were air-dried for the assessment of soil pH, electrical conductivity (EC), soil organic matter (SOM), total nitrogen (TN), available phosphorus (AP), and available potassium (AK).

Soil pH and EC were determined in a soil-water (1:2.5 mass/volume) suspension using a

combination electrode (PE-10, Sartorious, Germany) and conductivity meter, respectively. Soil organic carbon (SOC) and TN content were measured by a vario MACRO cube element analyzer (Elementar Analysen systeme GmbH, Hanau, Germany). A conversion factor of 1.724 from SOC to SOM was used to calculate the SOM content (Maillard & Angers, 2014). Soil AP content was analyzed following the molybdenum-blue colorimetric method after soil samples were extracted by $NaHCO_3$ (0.5 M). Soil AK was extracted with a 1 M ammonium acetate solution and then determined by flame photometry (AP1200, Shanghai Aopu Analytical Instruments Co., Ltd., China).

To obtain a quantitative index of soil fertility for each soil sample, the evaluation method based on the soil management assessment framework (SMAF) was used as previously described (Andrews *et al.*, 2004; Shang *et al.*, 2014). Briefly, all the measured physicochemical properties were considered in a total data set (TDS). After the TDS was established, factor analysis (FA) was performed, and the commonality explained by each soil fertility parameter based on the load matrix was calculated. Each parameter of soil fertility was weighted by the ratio of its communality with the sum communalities of all parameters in the TDS (Shukla *et al.*, 2006). Each soil parameter in the TDS was transformed and normalized to a value ranging from 0.1 to 1.0 using the standard scoring function (SSF) method. Three types of SSF equation were chosen to standardize the TDS parameters (Karlen *et al.*, 2003). If, when increasing the level of the parameter, the fertility of the soil increases, the "more is better" curve equation is used. Conversely, the "more is worse" curve equation is suitable for decreasing soil fertility with a decreasing parameter level. Lastly, the "optimum" curve equation scores those parameters that are positively associated with soil fertility up to an optimal level, but beyond that point, they are negatively related to soil fertility. Descriptions of detailed scoring function values and weights assigned to the selected soil fertility parameters are available in Table S1. Following this, the soil fertility index was determined as follows:

$$Soil\ fertility\ index = \sum_{i=1}^{n} Wi \times Si$$

where W is the assigned weight of each parameter, S is the parameter score, and n is the number of parameters in TDS (Shang *et al.*, 2014). In the model, a higher index score indicates greater soil

fertility. This soil fertility index corresponds to a proxy for the fertility. All physicochemical properties and the soil fertility index are given in Table S2.

Geographic information and climatic data collection

The longitude and latitude of each sampling site were recorded at the time of soil sampling. Mean annual temperature (MAT) and mean annual precipitation (MAP) (average of the three years from 2013 to 2015) were obtained from the National Meteorological Information Center (http://data.cma.cn). The geographic and climatic data are also shown in Table S2.

Soil DNA extraction, fungal ITS1 sequencing, and bioinformatic analysis

DNA was extracted from 250 mg of homogenized fresh soil according to the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) procedure. The quality and quantity of soil DNA were analyzed with a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). Isolated DNA was stored at −20 °C for further analyses.

The broad-spectrum fungal primer set ITS5 (5′- GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS2 (5′- GCT GCG TTC TTC ATC GAT GC-3′) with adaptors and barcodes was used to amplify the first internal transcription spacer (ITS1) region (White *et al.*, 1990). For each sample, three replicated PCR amplifications were performed. The 25-μl PCR mixture contained 5 μl of 5× reaction buffer, 5 μl of GC buffer (5×), 2 μl of dNTPs (2.5 mM), 1 μl of both forward and reverse primers (10 μM), 2 μl of DNA template, 0.25 μl Q5 DNA polymerase, and 8.75 μl of ddH_2O. Amplification was performed with an initial denaturation of 2 min at 98 °C, followed by 30 cycles of 15 s at 98 °C (denaturation), 30 s at 55 °C (annealing), 30 s at 72 °C (extension), and a final extension at 72 °C for 5 min. Paired-end (2×250) sequencing was performed on the MiSeq platform (Illumina, USA) by Personal Biotechnology Co., Ltd. (Shanghai, China).

Raw paired–end sequences were processed and analyzed using USEARCH (version 10 for Windows 32 bit) according to the UPARSE pipeline (Edgar, 2013). After forward and reverse reads were merged, low quality sequences (quality score < 20, length < 150 bp, total expected errors > 0.5) were filtered. After exclusion of chimeras and singletons, the remaining sequences were clustered into operational taxonomic units (OTUs) by UPARSE with a 97% similarity threshold. Finally, representative sequences of each OTU were matched against the UNITE

database (Koljalg *et al.*, 2013) using the Ribosomal Database Project (RDP) classifier (Release version 11.5) (Wang *et al.*, 2007). Nonfungal OTUs and rare OTUs (with relative abundance < 0.005%) were removed from the dataset. Five fungal functional groups (*i.e.,* arbuscular mycorrhizal (AM) fungi, ectomycorrhizal (EcM) fungi, ericoid mycorrhizal (ErM) fungi, plant pathogens, and saprotrophs) were identified according to FUNGuild (Tedersoo *et al.*, 2014; Nguyen *et al.*, 2016). To obtain an equivalent sequencing depth for downstream analyses, all samples were rarefied to equivalent sequencing depth (mean 17,795 sequences per sample) using MOTHUR (Schloss *et al.*, 2009).

Fungal community diversity analyses

Both the Chao 1 (community richness) and Shannon (community diversity) index were calculated on the rarefied data using MOTHUR (Schloss *et al.*, 2009). Linear regression analyses were conducted on the relationship between Chao 1/Shannon and the soil fertility index when the Pearson correlations were significant (R Version 3.3.3, "lm" function).

In addition to a description of α- and γ-diversity (fungal community diversity within a given sample and global fungal diversity, respectively), a deep analysis of β-diversity has been performed to address the working hypotheses.

Dissimilarities in the fungal community between each pair of samples were calculated based on relative abundance data (Bray-Curtis distance) and presence/absence data (Sorensen distance). Moreover, dissimilarities resulting from species replacement (species turnover) and nestedness (nestedness-resultant) (Baselga, 2010), two patterns of total β-diversity based on presence/absence data (Sorensen dissimilarity), were computed using the betapart package in R. Nonmetric multidimensional scaling (NMDS) ordinations were performed to visualize the β-diversity (based on both Bray-Curtis and Sorensen distances) of the fungal communities between soil samples with different soil fertility index values or sample sites using the "metaMDS" function in the vegan package of R software. Given the stress values obtained we have chosen to build 3D-NMDS (*i.e.* 0.14-0.15). Permutational multivariate analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM) were conducted to test the significance of fungal community dissimilarity by using "adonis" and "anosim" functions (999 permutations) in the R package Vegan,

respectively. The relationships between the dissimilarity of the fungal community and the Euclidean distance of the soil fertility index and geographic distance were estimated based on Pearson correlations using the Mantel test in R (vegan package). Standard major axis regression (Model II regression) that usually used to estimate how one variable scales against another was also used to develop linear models relating soil fertility index dissimilarity and fungal community dissimilarity. The heterogeneity between the scaling regression slopes of different fungal guilds was assessed by using the likelihood ratio test (smatr package in R).

Network construction and analyses

Molecular ecological networks were constructed for soil fungal communities based on high-throughput sequencing data at each sample site using the random matrix theory (RMT)-based network approach (Zhou *et al.*, 2010; Zhou *et al.*, 2011; Deng *et al.*, 2012; Ling *et al.*, 2016). Briefly, covariations were measured across 10 soil samples at each sampled site to construct the networks. Only OTUs detected in 8 out of 10 samples were kept for network construction. This filter standard of OTUs (an occurrence of 80% per sample) could reduce the appearance of pseudo-correlation and enhanced the accuracy of network structure. Similarity matrices were measured by Spearman correlation coefficients. To compare network topologies, all networks were generated with a similar and appropriate similarity threshold (St = 0.81-0.84), identified based on RMT. For each created network, network topological properties such as network size (nodes), connectivity (links), average connectivity (avgK), average clustering coefficient (avgCC), and network modularity were characterized according to Deng et al. (2012). All the network analyses were performed using the Molecular Ecological Network Analyses (MENA) Pipeline (http://ieg2.ou.edu/MENA/). Networks were visualized using the Gephi 0.9.2-beta software (Bastian *et al.*, 2009). The correlations between parameters of network complexity (network size, connectivity, and average connectivity) and the average soil fertility index of each sample site were characterized by regression analyses when the Pearson correlations were significant. Similarly, the relationships between inter-/intra-module connections and the average soil fertility index were analyzed.

Quantifying the effects of environmental and spatial variables

Variation partitioning analysis (VPA) was used to evaluate the relative contribution of the environmental and spatial processes in shaping the soil fungal community with adjusted R^2 coefficients based on redundancy analysis (RDA) (Peres-Neto *et al.*, 2006). Before the RDA analysis, the fungal OTU data were Hellinger-transformed to make the data appropriate for analysis using the linear model (Legendre & Gallagher, 2001), and the environmental variables and spatial variables with significant explaining factors ($P < 0.05$) were selected by forward selection (Blanchet *et al.*, 2008). Here, spatial components were calculated by the principal coordinates of neighbor matrices analysis (PCNMs) based on the longitude and latitude coordinates of each sampling site (Borcard *et al.*, 2011). The relative contribution of deterministic and stochastic processes was explained by pure environmental variation (ENV | SPA), pure spatial variation (SPA | ENV), and the combined effects of both space and environment (SPA ∩ ENV). In the VPA analysis, the residual proportion represents the unexplained variance. Moreover, Mantel and partial Mantel tests were conducted to determine the relationships between soil fungal community dissimilarity and spatial/environmental variables. All analyses were performed in R (Vegan, PCNM, SpacemakeR, and Packfor package).

Niche-based and neutral model fitting

In order to confirm whether the niche-based or neutral mechanisms determined the soil fungal communities, fungal OTU rank relative abundance distributions were fitted to pre-emption, broken stick, log-normal, Zipf, Zipf–Mandlebrot, and zero-sum multinomial (ZSM) models, respectively. The ZSM model representing neutral theory was conducted using TeTame21 (Jabot *et al.*, 2008), and the other models representing niche theory were generated using the command "radfit" in the R package vegan. The fit qualities of the statistical models were compared based on the Akaike Information Criterion (AIC). The lowest AIC value indicated the best fit model (Dumbrell *et al.*, 2010).

Results

Overall fungal community characteristics

Analyses of the high-quality sequences from the 140 soil samples at 14 typical tea plantations

across South China (Figure 1a) revealed the presence of 1078 fungal OTUs. Across the soil sampling sites, the most abundant soil fungal phylum was Ascomycota, which varied in relative abundance from 29 to 72% (Figure S1a). At the class level, soil fungal communities consisted predominantly of the class Sordariomycetes, which accounted for an average relative abundance of 23% of the fungal sequences from the soils, followed by Tremellomycetes (18%), and Eurotiomycetes (10%) (Figure S1a). In terms of the fungal functional guilds which were identified by comparison of taxonomy to FUNGuild, saprotrophs, plant pathogens, ectomycorrhizal (EcM) fungi, ericoid mycorrhizal (ErM) fungi, and arbuscular mycorrhizal (AM) fungi accounted for 44.5%, 2.0%, 0.3%, 0.2%, and 0.1% of the sequences, respectively (Figure 1b). Fungal functional guilds showed variations among and within sites (Figure 1b and Figure S2, respectively).

Correlation of community diversity and soil fertility

The Chao 1 index of the fungal community was positively correlated with the soil fertility index ($R^2 = 0.14$, $P < 0.001$), and a weak relationship existed between the Shannon index and the soil fertility index ($r = -0.01$, $P = 0.907$) (Figure S3a-b). NMDS ordination plots based on both Bray-Curtis and Sorensen distances showed that the soil fungal community compositions among sites were significantly different (Bray-Curtis dissimilarity: PERMANOVA: $R^2 = 0.51$, $P = 0.001$; ANOSIM: $R = 0.83$, $P = 0.001$; Sorensen dissimilarity: PERMANOVA: $R^2 = 0.67$, $P = 0.001$; ANOSIM: $R = 0.97$, $P = 0.001$) (Figure S1b-c). Fungal communities from each site were clustered by geographic location. Additionally, analyses of β-diversity from both Bray-Curtis and Sorensen distances confirmed that the soil fungal community distribution is related to the soil fertility index gradient (Bray-Curtis dissimilarity: $R^2 = 0.06$, $P = 0.001$; Sorensen dissimilarity: $R^2 = 0.07$, $P = 0.001$) (Figure 2). Mantel tests corroborated this pattern, indicating a strong and positive relationship between soil fertility index dissimilarity and fungal community dissimilarity (Figure S3c, Figure 3). Partitioning the total β-diversity of the fungal community showed that differentiation among communities was primarily explained by OTU turnover (99.6%) instead of nestedness-resultant (0.4%) (Table S3). The association between the turnover component of β-diversity and the soil fertility index was significant ($P = 0.001$, Figure 3). Similar to the total fungi community, with increasing soil fertility index dissimilarity, the community dissimilarity

and turnover of fungal functional guilds (*i.e.,* AM fungi, EcM fungi, ErM fungi, plant pathogens, and saprotrophs) significantly increased (Figure 3). The slopes of dissimilarity and turnover for AM fungi, EcM fungi and ErM fungi were remarkably steeper than those for plant pathotrophs and saprotrophs ($P < 0.001$, Figure 3 and Table S4).

Characteristics of constructed networks

The curves of network connectivity distributions followed the power-law model (R^2 values range from 0.729 to 1.00) (Table S5). Modularity values (M) were higher than those in the corresponding randomized networks, and the metrics of the empirical networks were different from those in the random networks (Table S5). These overall topological properties indicated that all the network structures were scale-free, modular, and nonrandom. Distinct fungal co-occurrence patterns were detected at different sample sites, and the networks became more clustered as the soil fertility index increased (Figure 4a). Regression analyses showed that the size, connectivity, and average connectivity of the fungal networks were correlated with the average soil fertility index of each sample site (size, $R^2 = 0.38$, $P = 0.02$; connectivity, $R^2 = 0.51$, $P = 0.004$; average connectivity, $R^2 = 0.35$, $P = 0.03$) (Figure 4b-d). The intra-module connections of networks increased with increasing average soil fertility index ($R^2 = 0.39$, $P = 0.02$), whereas the inter-module connections showed a weak and non-significant relationship to average soil fertility index (Figure S4).

Relative importance of spatial processes and environmental factors

Correlation analysis showed that the dissimilarity in the fungal community was significantly and positively correlated with geographic distance (Bray-Curtis dissimilarity: Mantel $r = 0.33$, $P = 0.001$; Sorensen dissimilarity: Mantel $r = 0.58$, $P = 0.001$) (Figure 5a-b); that is, a strong distance decay in fungal community similarity existed. The RDA ordinations showed that five spatial factors (PCNM 1-5) and eight environmental variables (soil pH, EC, SOM, TN, AK, MAT, MAP) contributed to explaining the variation in the soil fungal community by using forward model selection ($P < 0.05$) (Figure S5). The variation partitioning analysis (VPA) based on relative abundance data revealed that the spatial and environmental variables taken together explained 35.8% of the variation in fungal communities, whereas the unexplained factor represented ~64% of the

variance (Figure 5c). In more details, the environmental selection accounted for ~14% of the variation in soil fungal community composition, the spatial processes were estimated to account for ~13.0%, and covariation related to both environmental constraints and spatial variation contributed ~9% of the community assembly (Figure 5a). When incidence-based data was used in VPA, trends were qualitatively similar (Figure 5b). The results of Mantel and partial Mantel tests also revealed that both environmental factors and spatial factors were significantly correlated with the fungal community (Table S6).

Theoretical species abundance distribution models

OTU relative abundance distributions in the fungal community were mainly fitted the lognormal, Zipf or Zipf-Mandelbrot models (Table S7). At the regional scale (*i.e.* all fields considered together), the proportion of the lowest AIC values for the Zipf-Mandelbrot model (53%) exceeded that of the other theoretical models (Table 1). Similarly, when the samples were examined based on local scale (*i.e.* fields clustered by tea plantation site), the Zipf-Mandelbrot model gave the closest fit for the soil fungal communities in the majority of cases (Table 1).

Discussion

Fungal assemblage complexity increased along the soil fertility gradient

A recent study by Delgado-Baquerizo *et al.* (2017) corroborated that soil fungal biodiversity was positively and strongly correlated with soil fertility at the continental scale. Similarly, there was a significant and positive relationship between fungal Chao 1 index (namely how many different species are present) and soil fertility index in the present study (Figure S3a). From β-diversity analyses, significant and positive linkages between the soil fungi community and the soil fertility index were demonstrated herein. Soil fertility was a structuring factor in fungal community composition, and the more dissimilar the soil fertility indexes value between the two soils were, the more dissimilarities in the fungal community composition took place (Figure 2-3, Figure S3c). It needs to note that the correlation coefficients of the above relationships were relatively lower. Unlike natural ecosystems, the strong linkage between soil fertility and fungal biodiversity may be weakened in ecosystems where soils (*e.g.* tea field) are subject to

anthropogenic pressure (Delgado-Baquerizo *et al.*, 2017). Therefore, it could still be considered acceptable and valid results (P = 0.001) in present study. Similarly, Sterkenburg *et al.* (2015) observed that there would be a trade-off between different species in the assembly of fungal communities along fertility gradients. Moreover, given the mutual causality between soil fungi and soil fertility, the relationships between fungal community composition and soil fertility also reflected the feedback of fungal community composition on soil fertility formation. Fungal communities play a central role in regulating soil fertility-related ecosystem services (Kyaschenko *et al.*, 2017) due to the ecological functions they perform, including carbohydrate-active enzyme production and organic matter breakdown, allowing the recycling and mobilization of mineral nutrients. Caution should be taken for the readers with an agronomic or plant science perspective that the soil fertility index used here is not intended to encapsulate the potential of the soil to sustain all plant growth. Even so, the use of the measured physicochemical properties for fertility index calculation allows us to decipher the effects of soil fertility on the fungal community in tea plantation soils.

Network analyses often reveal patterns of nonrandom covariation that reflect habitat heterogeneity and divergent selection regimes (Shi *et al.*, 2016). The soil fungal assemblages formed remarkably larger and more complex networks with increasing soil fertility index values (Figure 4), suggesting that more fertile soils have both a higher-order organization of the fungal community and greater potential for species interactions. Soil fertility plays a critical role in regulating the strength of interactions between fungal functional guilds (Fernandez & Kennedy, 2016). The increasing soil fertility creates more resource niches to be covered, thus, structuring higher community complexity and interactions, and also, community resilience. A more complex fungal network in which the soil fertility index is higher can also be interpreted as increased fungal complexity among fungal guilds (Nguyen *et al.*, 2016). In this case, higher diversity among fungal decomposers (*i.e.,* saprotrophs) is likely paired with higher functional complementarity (*i.e.,* higher niche complementarity) and/or redundancy among these decomposers. More widely, high soil fertility conditions could push ecosystems further towards even more fertile states by driving the interplay between fungal guilds (Kyaschenko *et al.*, 2017).

Both dissimilarity and turnover in each soil fungal functional guild community were positively correlated with soil fertility index dissimilarity. The same pattern of all the functional guilds means that soil fertility mainly regulated the total community rather than individual functional components. However, correlations between the soil fertility index and fungal communities differ according to the functional guilds (Figure 3). This trend along the soil fertility gradient was the strongest for the symbiotrophic fungal guilds (*i.e.,* AM fungi, EcM fungi, and ErM fungi) relative to that of both pathotrophic fungi and saprotrophic fungi (Figure 3, Table S4). Despite their low abundance (Figure 1B), mycorrhizal fungi are considered as keystone players in land ecosystems functioning (van der Heijden *et al.*, 2015; Powell & Rillig, 2018). Elsewhere, recent studies have demonstrated that rare taxa could serve as pool of functional specialists, and perform disproportionate types of functions (Jia et al., 2018). Virtually, the multiple interactions among mycorrhizal fungi and other organisms can lead to multitrophic feedback loops, which finally influence the synergy among biological, chemical, and physical processes that define soil fertility (Abbott & Johnson, 2017). It has to be underlined that functional classifications from FUNGuild are only a coarse description of fungal functional diversity relative to descriptions of known fungi. A second identified limit of the study is related to the primer bias which likely influences the resolution or coverage with respect to fungal functional guilds. Despite these limits, the interpretations and evidences obtained herein clarify and support the idea of the soil mycobiota as major component of the soil fertility-related ecosystem service.

Deterministic processes governed the assembly of soil fungal communities

In the present study, taxa turnover (*i.e.,* replacement of some species by others, 99.6%) almost entirely explained the fungal β-diversity rather than nestedness-resultant (*i.e.,* biotas at sites with smaller numbers of species are strict subsets of the biotas at richer sites, 0.4%) (Table S3), which implies that fungal β-diversity mainly arises from variation in OTU composition rather than differences in OTU richness. Fungal communities that exhibit a higher degree of taxa turnover across the soil fertility gradient may be the result of functional traits that govern physiological tolerance and competition for the resource requirements of fungi (Kranabetter *et al.*, 2015). The high proportion of OTU turnover suggests a possible dominance of deterministic processes of

fungal community assembly (Viana *et al.*, 2016). In fact, both environmental and spatial processes were responsible for the assembly of the fungal communities (Figure 5, Table S6) but the environmental variations were more influential contributors than the purely spatial components (Figure 5). A major fraction of the variation in the fungal community was nonetheless unexplained by the spatial and environmental variables (Figure 5). These results were consistent with other studies on fungal communities (Sterkenburg *et al.*, 2015; Rasmussen *et al.*, 2018; Yang *et al.*, 2019), which could be ascribed to possible unmeasured factors, species interactions or methodological issues. Elsewhere, to confirm the assembly mechanism of fungal community, a model-based approach was used. The analyses point to the fungal community assemblage in the soil best fitting with the Zipf-Mandelbrot model (niche model and related deterministic processes) rather than the ZSM model (neutral model embedding dispersal and drift, *i.e.,* stochastic processes) (Table 1). The result of the best model fit illustrates that the presence of fungal species is dependent on many environmental factors acting sequentially and suggests that deterministic processes are indeed important in driving fungal community variation in the study region. However, some studies have demonstrated an effect of dispersal limitation, such as distance–decay relationships (Talbot *et al.*, 2014; Gumiere *et al.*, 2016), which attempted to support the dominant role of stochastic processes. Nonetheless, it is important to emphasize that dispersal limitation alone cannot be used as the sole evidence for stochastic processes (Hanson *et al.*, 2012; Peay & Bruns, 2014; Zhou & Ning, 2017). Our findings are in line with previous works from various ecosystems that suggest that fungal community assemblages were predominantly influenced by deterministic processes (Table 1), even though stochastic processes also play an important role (Hazard *et al.*, 2013; Morrison-Whittle & Goddard, 2015). Tedersoo et al. (Tedersoo *et al.*, 2014) reported that soil fungal community composition could be best predicted by climatic, edaphic, and floristic factors at the global scale. Similarly, Yang et al. (Yang *et al.*, 2019) proposed that abiotic environmental filtering was the dominant driving force on soil fungal biogeography even after considering the effects of biotic interaction.

Conclusions

Fungal communities were influenced by both environmental variations and spatial factors,

and their assemblage complexity responded in a deterministic manner to changes in soil fertility. A soil with a high fertility exhibited a diverse fungal community and supported a high number of close interactions between fungal species/modules that promoted the formation of more complex fungal community assemblages. Moreover, the fungal functional guilds were all positively correlated with the gradient of soil fertility. This supports the idea that soil fertility is closely related to the total community of soil fungi and that the soil mycobiota is an indispensable component of the soil fertility-related ecosystem service.

Understanding the relationships between soil fertility-related ecosystem services and the complexity of the soil fungal community could enhance our capacity to predictably manipulate microbial community assemblages and improve ecosystem functions. The linkage between soil mycobiota and soil fertility implied that differences in fungal assemblages, for example fungal species diversity or co-occurrence patterns, may result in large contrasts in terms of soil fertility, even on a regional scale. Our results call for a shift in focus from abiotic factors to living entities in soil in deciphering soil fertility-related ecosystem services. This shift would likely also offer new opportunities to improve sustainable agriculture by developing soft strategies to shape the soil mycobiota in the expected direction (Wall et al., 2019). Meanwhile, a better understanding of the underlying relationship between microbial assemblage complexity and ecological processes requires further research.

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Availability of data and materials

All the raw sequence data for ITS gene amplicons were submitted to the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/Traces/sra/) database under the accession number SRP136886. Scripts employed in the analyses are available upon request.

Authors' contributions

N.L. and S.W.G. conceived and designed the study; J.J.G., Z.J.C., L.S.L and L.M.G. carried out the experiments and collected the data. C.X., L.L. and M.W. assisted with the analytic tools. J.J.G., N.L. and P.V. performed the analyses and wrote the first draft of the manuscript. J.Y.R., S.W.G. and Q.R.S. made plentiful valuable comments for the manuscript. All the authors reviewed and approved the manuscript.

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Supporting Information

Figure legends:

Figure 1 (a) Location of sampling sites in South China. CQ, Chongqing; PJ, Pujiang; QY, Qiyang; CZ, Chaozhou; NC, Nanchang; DDG, Dadugang; FA, Fu'an; YH, Yuhang; CS, Changsha; XN, Xianning; XY, Xinyang; PE, Pu'e; JR, Jurong; MH, Menghai. (b) Functional composition of the soil fungal community at different soil sample sites.

Figure 2 Nonmetric multidimensional scaling (NMDS) ordination plots depict the distribution of soil fungal communities across a soil fertility gradient (n = 140). The distance between samples (points) represents distinctness in community composition, calculated as dissimilarity based on abundance data (Bray-Curtis, a) or presence/absence data (Sorensen, b). Each point corresponds to a different sample colored by the soil fertility index.

Figure 3 Relationship between soil fertility index dissimilarity and soil fungal beta diversity and its turnover and nestedness-resultant component (based on Sorensen distance) for total fungi and each functional guild. The solid line indicates the fitted linear regression model. Gray shadows represent 95% confidence intervals. The results of Mantel tests and the slopes of standardized major axis fit are shown in the diagram.

Figure 4 (a) Co-occurrence networks of fungal communities at 14 studied sites with different fertility. Networks are randomly colored by modules. CQ, Chongqing; PJ, Pujiang; QY, Qiyang; CZ, Chaozhou; NC, Nanchang; DDG, Dadugang; FA, Fu'an; YH, Yuhang; CS, Changsha; XN, Xianning; XY, Xinyang; PE, Pu'e; JR, Jurong; MH, Menghai. (b) Regressions between the average soil fertility index and the parameter of network complexity (size, connectivity and average connectivity) of fungal communities in soil samples. The solid line indicates the fitted linear regression model. Gray shadows represent 95% confidence intervals. All regression lines are significant at $P < 0.05$.

Figure 5 (a, b) Relationship between geographic distance and fungal community dissimilarity (based on Bray–Curtis distances or Sorensen distances) in soil samples. The solid line indicates the fitted linear regression model. Gray shadows represent 95% confidence intervals. (c, d) The relative importance of space and the environment in structuring the soil fungal community based on variation partitioning analysis. The community dissimilarity was calculated based on abundance data (Bray-Curtis, a) or presence/absence data (Sorensen, b). All values are presented using the adjusted coefficient of determination (adjusted R^2). The variation is partitioned into four fractions: pure environmental variation (ENV | SPA), pure spatial variation (SPA | ENV), shared environmental and spatial variation (SPA ∩ ENV) and unexplained variation (residuals). Asterisks indicate that a significant amount of variation is explained by the given fraction (***, $P < 0.001$).

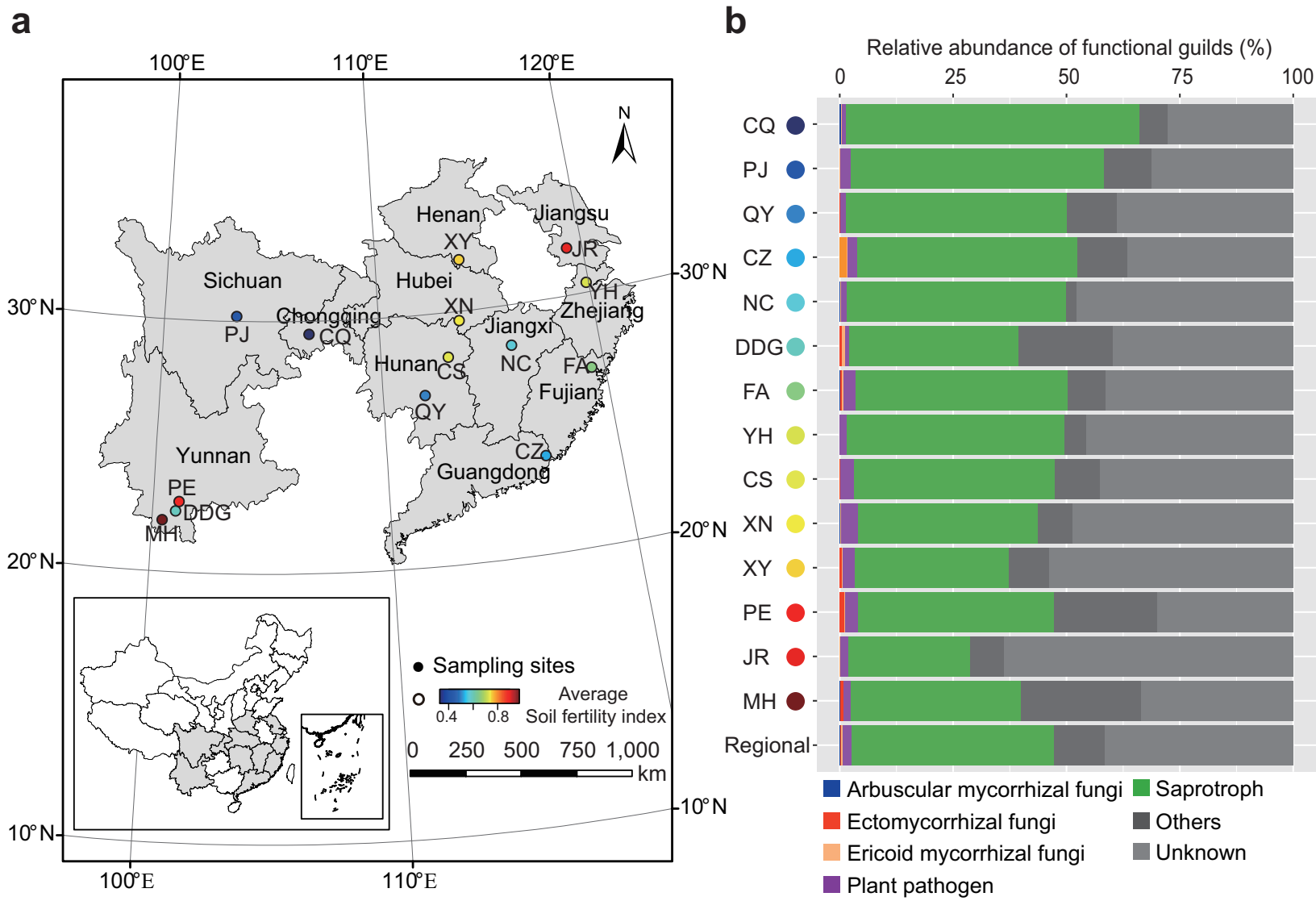
Table 1 The proportion of the lowest Akaike information criterion (AIC) value for six species rank abundance distribution models of fungal communities on the local scale and regional scale.

		Break Stick	Pre-emption	Lognormal	Zipf	Zipf-Mandelbrot	ZSM
Local	CQ	-	-	20%	10%	**50%**	20%
	PJ	-	-	10%	10%	**80%**	-
	QY	-	-	-	-	**90%**	10%
	CZ	-	-	40%	-	**60%**	-
	NC	-	-	30%	-	**60%**	10%
	DDG	-	-	**50%**	40%	-	10%
	FA	-	-	10%	-	**90%**	-
	YH	-	-	30%	10%	**60%**	-
	CS	-	-	**70%**	-	30%	-
	XN	-	-	40%	10%	**50%**	-
	XY	-	-	30%	10%	**50%**	10%
	PE	-	-	10%	**30%**	**30%**	**30%**
	JR	-	-	20%	-	**70%**	10%
	MH	-	-	30%	**50%**	20%	-
Regional		-	-	28%	12%	**53%**	7%

CQ, Chongqing; PJ, Pujiang; QY, Qiyang; CZ, Chaozhou; NC, Nanchang; DDG, Dadugang; FA, Fu'an; YH, Yuhang; CS, Changsha; XN, Xianning; XY, Xinyang; PE, Pu'e; JR, Jurong; MH, Menghai.

ZSM, zero-sum multinomial.

Bold values indicated the highest proportion of fitted models in each site or entire region.



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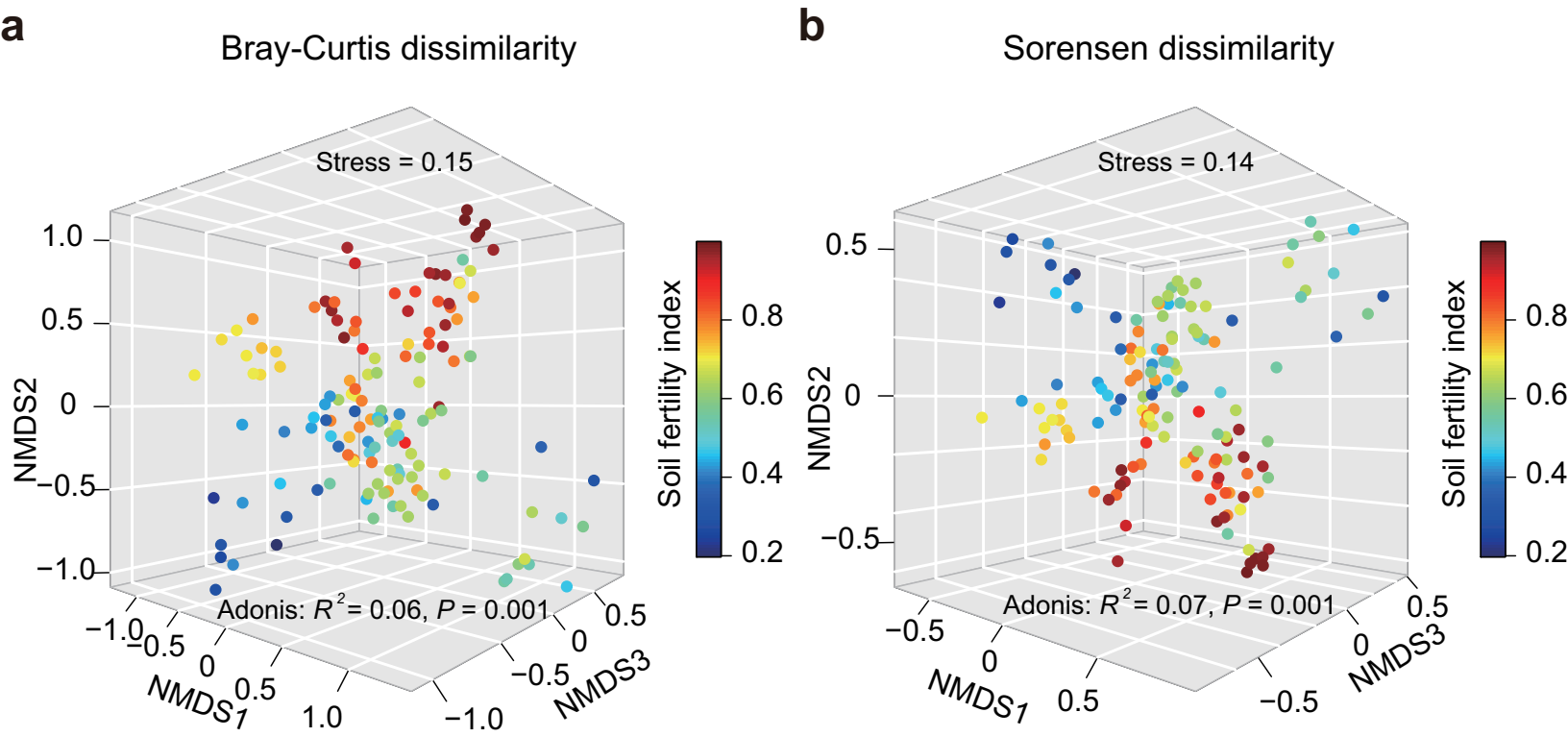

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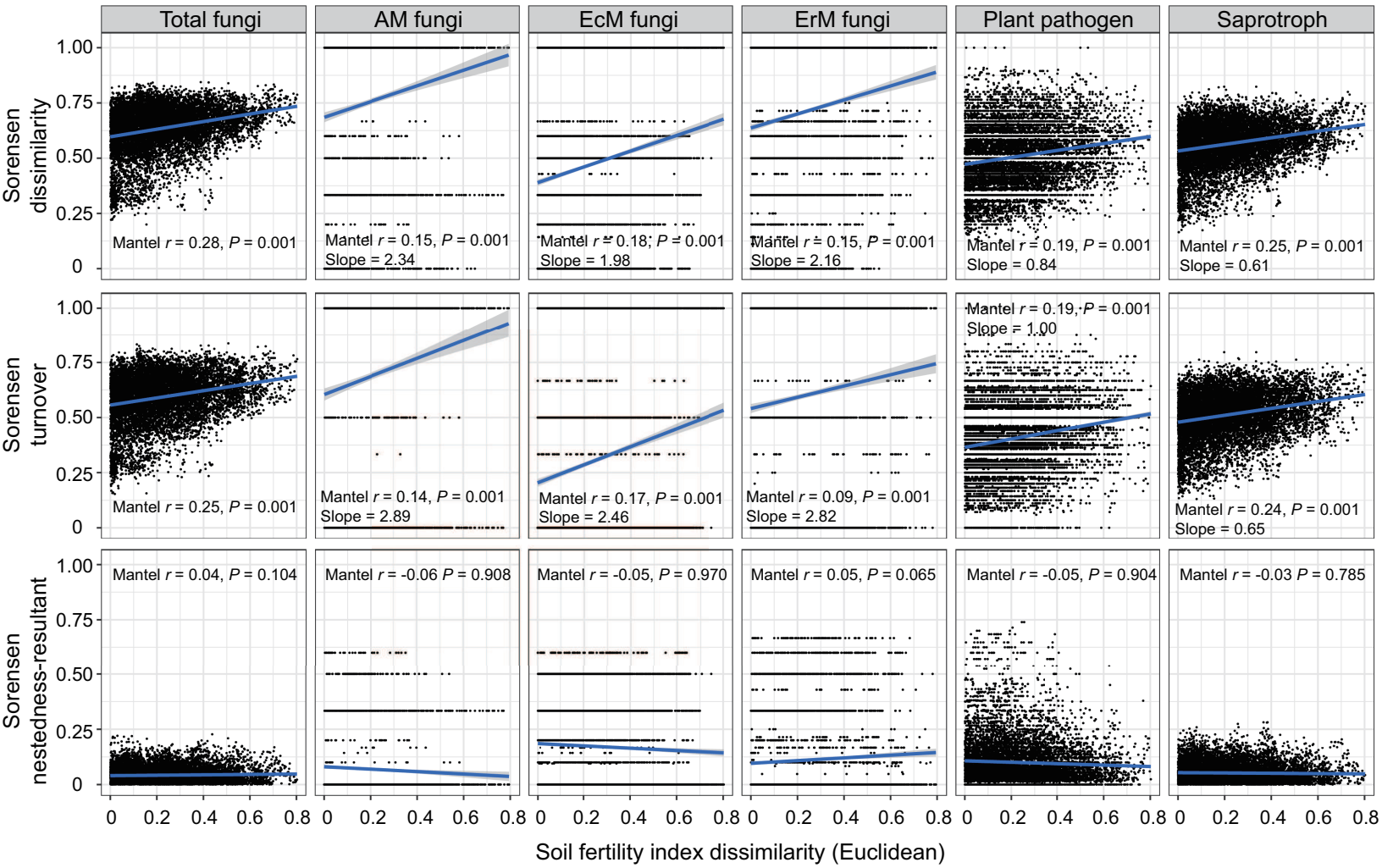

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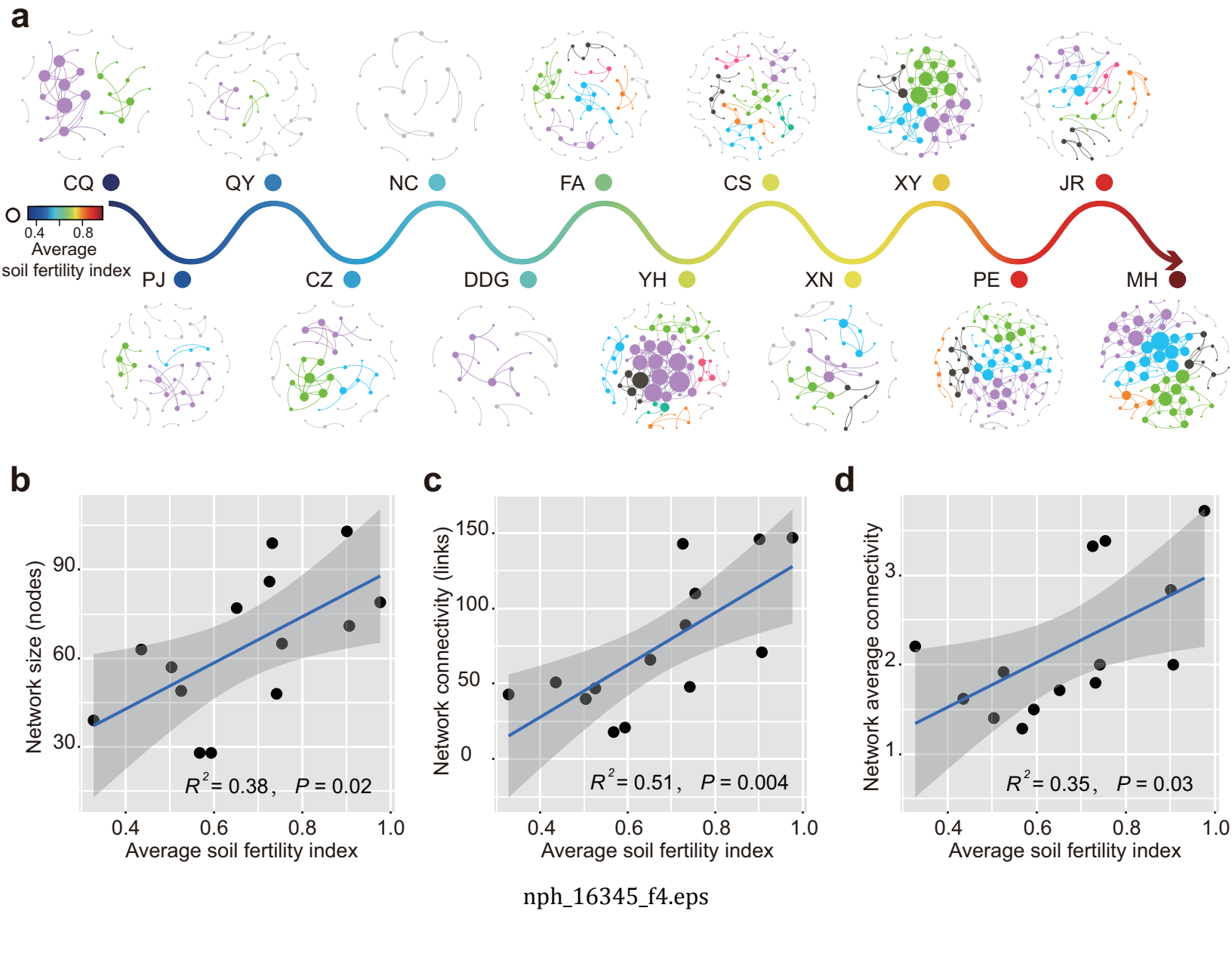

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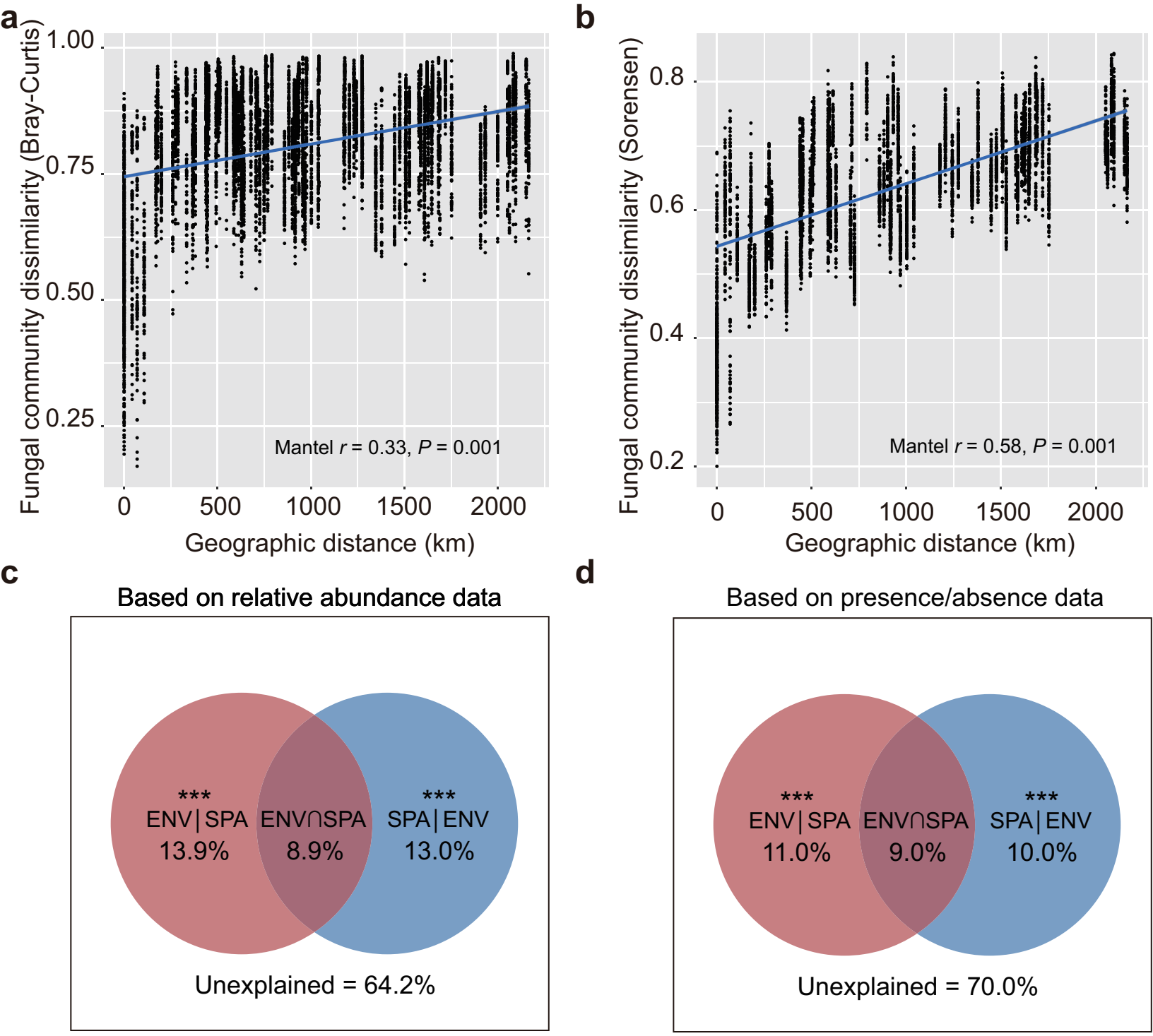

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