Soil fungal diversity and community assembly: Affected by island size or type?

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ABSTRACT

Fungi have a huge biodiversity and play important roles in soil biogeochemical cycling in island ecosystems. Although island biogeography has been widely studied for macroorganisms, fungal community assembly in true islands and its relationship with island area are less documented. We examined soil fungal communities in 18 oceanic islands of two types (eight general islands in Wanshan and 10 coral islands in Xisha) using the Illumina MiSeq sequencing technique. Our results showed that fungal α-diversity (species richness) was substantially different among the oceanic islands, with a higher value in Wanshan islands than in Xisha islands. Fungal α-diversity was significantly affected by soil potassium and magnesium (Mg) and plant communities in Wanshan islands, whereas only soil Mg significantly affected it in Xisha islands. Soil fungal community composition was significantly different in the Wanshan and Xisha islands and was influenced by soil property, plant community, and spatial distance. The ecological stochasticity model showed that the fungal community assembly was mainly governed by deterministic processes regardless of island type. Fungal β-diversity, but not α-diversity, increased significantly with increasing island area. These findings have implications for the better prediction of soil fungal community dynamics in island systems and biodiversity conservation in fragmented habitats.

Keywords: α-diversity; β-diversity; community composition; deterministic process; oceanic island; species-area relationship

INTRODUCTION

Islands are characterized by their geographical isolation and simple biotas and have served as a model system for developing and testing basic evolutionary and ecological theories or hypotheses (Warren et al. 2015; Whittaker et al. 2017). Island-related research has emerged primarily since the pioneering work of the theory of island biogeography (MacArthur and Wilson 1967), which was considered the dominant symbol of a transition from descriptive to analytical approaches in ecology and biogeography (Losos and Ricklefs 2010). Subsequently, there is a large body of observational and empirical studies on the biogeography of island-dwelling macroorganisms (Stuart et al. 2012; Cabral et al. 2019; Rojas-Sandoval et al. 2020). Soil fungi, which are important components of belowground microorganisms, have remarkable biodiversity and are major ecological players in biogeochemical (organic matter and nutrients) cycling and other ecosystem functioning on Earth (Philippot et al. 2013; Nilsson et al. 2019). Compared with island studies of animals and plants, however, little is known regarding the diversity and biogeography of soil microorganisms in island ecosystems (Li et al. 2020).

In general, a positive species–area relationship (SAR) leads to higher species richness on large islands according to the classic theory of island biogeography (MacArthur and Wilson 1967). The SAR has been well tested with insular animals and plants (MacArthur and Wilson 1963; Lomolino 1984; Kohn and Walsh 1994; Losos and Ricklefs 2010; Rojas-Sandoval et al. 2020). The usefulness of SAR in explaining species richness patterns of microorganisms has also been investigated in some ‘virtual island’ systems. For example, SAR was observed for bacterial α-diversity in water-filled tree holes of large European beech (Bell et al. 2005), and smaller sump tanks (analogous to islands) contained lower diversity (van der Gast et al. 2005), indicating that island biogeography theory holds for the bacterial communities. Similarly, ectomycorrhizal fungal species richness was found to increase with the size of tree islands (Peay et al. 2007; Glassman et al. 2017). In a recent study, Li et al. (2020) did not observe significant SAR for soil fungi on inland islands. However, to our knowledge, there is no study regarding soil fungal SAR from oceanic islands.

Besides α-diversity, microbial β-diversity (community turnover) has also been attracting great attention. Exploring microbial β-diversity can provide insight into the mechanisms that generate and maintain microbial biodiversity in ecosystems (Ettema and Wardle 2002; Beck et al. 2015; Mori et al. 2018). A positive relationship between microbial β-diversity and area, as a universal biogeographic pattern, was observed in mainland ecosystems using the distance-decay approach (Horner-Devine et al. 2004; Beck et al. 2015; David et al. 2016; Zheng et al. 2020). However, there is less available knowledge on the relationships between microbial β-diversity and area in island systems. For example, a significantly positive relationship was observed between soil fungal β-diversity and area, and habitat heterogeneity and/or dispersal limitation were considered as potential mechanisms influencing soil fungal communities in inland islands (Li et al. 2020).

Exploring the microbial community assembly patterns and the processes resulting in these patterns is pivotal to gaining a more mechanistic understanding of biodiversity maintenance and community stability (Chase 2010; Nemergut et al. 2013). The relative importance of deterministic and stochastic processes in structuring soil fungal community assembly is dependent on scales (Caruso et al. 2012; Kivlin et al. 2014; Schröter et al. 2019) and habitats (Powell et al. 2015; Zhao et al. 2019). For instance, soil fungal communities were more strongly affected by deterministic processes in systems with less disturbance (bogs, moors, etc.) than in more intensive land use systems such as managed grasslands and arable systems (Powell et al. 2015). In general, stochasticity plays a dominant role at relatively smaller geographic scales, whereas determinism dominates at larger scales (Zhao et al. 2019). Although community assembly of soil fungi on mainland ecosystems has been well studied, little is known about the assembly mechanisms of fungal communities in the oceanic islands.

To understand the biogeography of soil fungi in different island systems, we examined the soil fungal communities in 18 oceanic islands belonging to two types of islands (including eight general islands and 10 coral islands) in the South China Sea using Illumina MiSeq sequencing techniques. There are great differences between the two types of islands, such as plant community structure and soil properties. We analyzed the relationships between fungal α- and β-diversities and island area. Moreover, we tried to quantify the strength of stochasticity of fungal community assembly and detect whether any differentially potential drivers influenced the fungal community diversities between the two island types. We propose two hypotheses: (H_1) fungal β-diversity rather than α-diversity increases with island area and (H_2) fungal α- and/or β-diversities are structured by different factors in general and coral islands.

MATERIALS AND METHODS

Sites and sampling

This study was conducted at 18 oceanic islands located in the South China Sea **(Fig. S1)**, which were categorized into the Wanshan islands (incl. eight general islands with distances of 1.5–42.8 km and 30.5–64.5 km between the islands and from the mainland, respectively) and Xisha islands (incl. 10 coral islands with distances of 0.5–95.0 km and 372–590 km between the islands and from the mainland, respectively) **(Table S1)**.

The distance between the Wanshan and Xisha islands is *ca.* 685 km **(Fig. S1)**. We established 3–20 plots (20 m × 20 m, > 50 m apart) at each site according to the island size **(Table S1)**. Within each plot, 10 soil cores (3.5 cm in diameter and 10 cm in depth) were randomly collected and combined into one composite sample, which resulted in a total of 203 soil samples. The samples were transported in an ice box to the laboratory and sieved through a 2-mm sieve to remove roots and debris. One portion of the samples was stored at –80 °C until DNA extraction; another portion was air-dried for analysis of the soil properties.

Plant and soil properties

Plant species richness was investigated in each plot **(Table S1)**. Soil pH was determined with a soil-to-water ratio of 2:5 (w/v) using a glass electrode (FE20, Mettler Toledo). The metal ions of potassium (K), magnesium (Mg), and total phosphorus (P) were measured using an inductively coupled plasma-atomic emission spectrometer (ICP-AES, iCAP 6300, Thermo Jarrell Ash Co. USA). Soil total carbon (C) and total nitrogen (N) were determined using an Elementar Vario EL III (Elementar Analysensysteme GmbH, Germany). Information about soil properties among the 18 islands is given in **Table S2**.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from 0.25 g frozen soil using a PowerSoil DNA isolation kit (MoBio Laboratories, Inc. USA) according to the manufacturer’s instructions. The concentration of DNA was measured using a TBS 380 fluorescence spectrophotometer (Promega, USA). The fungal ITS2 region was amplified using the primers fITS7 (5’-

GTGARTCATCGAATCTTTG-3’) and ITS4 (5’- TCCTCCGCTT-ATTGATATGC-3’) linked with a 12-base barcode to distinguish the sample origin. The thermocycling conditions were as follows: 94 °C for 5 min, followed by 32 cycles of 1 min at 94 °C, 50 s at 56 °C, and 1 min at 68 °C. Each DNA sample was amplified in triplicate. The PCR products were purified using a PCR product gel purification kit (Axygen, Union City, CA, USA) and were pooled with equimolar amounts (50 ng) from each sample and adjusted to 10 ng μL^{-1}. The pooled DNA was subjected to sequencing on an Illumina MiSeq PE250 platform for sequencing using the paired end (2 × 250 base pair (bp)) option at the Environmental Genome facilities of Chengdu Institute of Biology, Chinese Academy of Sciences, China.

Sequence processing

The raw sequences were filtered using ‘Quantitative Insights into Microbial Ecology’ (QIIME v. 1.7.0) to remove low-quality reads with an average quality score < 20, without valid primer sequences or barcode sequences, containing ambiguous bases or length < 250 bp. Chimeric sequences were detected using the ‘chimera.uchime’ command in Mothur 1.32.2 (Schloss et al. 2009) by comparison with the classification (UNITE) database (Kõljalg et al. 2013). Nonchimeric ITS2 sequences were clustered into different operational taxonomic units (OTUs) at a 97% sequence similarity level based on the UPARSE pipeline using the USEARCH v8.0 after discarding replicated sequences and singletons (Edgar 2013). The representative sequence (i.e. the most abundant sequence) from each OTU was selected through the ‘get.oturep’ command and classified using the SINTAX algorithm (Edgar 2016) against the UNITE database with a confidence threshold of 65%. To eliminate the potential effects of uneven sequence depths across samples on fungal community analysis, the number of sequences per sample was rarefied to the smallest sample size using the ‘sub.sample’ command in Mothur for further analysis. The raw sequences (files as .fastq format) are available at the Microbiome Database (http://egcloud.cib.cn): PRJ-AMPLI-5046071a7a38a3cf9afb665a3e2118d4.

Statistical analysis

All statistical analyses were conducted using R-4.0.2 (R Core Team 2020). Fungal α-diversity was defined as the observed OTU richness of each soil sample. Pairwise comparisons of group means of OTU richness among the 18 oceanic islands were carried out after the nonparametric Kruskal-Wallis test, where data did not satisfy homogeneity of variance, at $P < 0.05$. The difference in α-diversity and the relative abundance of dominant OTUs between two island groups (Wanshan vs. Xisha islands) was analyzed using the independent two-sample T-test. Plant community composition data were subjected to principal component (PC) analysis using the ‘rda’ command in the ‘vegan’ package (Oksanen et al. 2013). Subsequently, to quantify the effects of soil variables, plant species richness and composition (i.e. plant PC), and their interactions on soil fungal α-diversity, we employed linear mixed-effects models to control the random effect of site using the ‘lme4’ package (De Boeck et al. 2011).

Fungal β-diversity was defined as community turnover (dissimilarity) among samples using the Sørensen index (presence/absence data) (Legendre and Legendre 1998). The fungal community composition was ordinated using nonmetric multidimensional scaling (NMDS) with dissimilarity matrices using the ‘metaMDS’ function in the ‘vegan’ package. To evaluate the effect of the sites on fungal β-diversity at scales of two types of islands and 18 islands, the permutational multivariate analysis of variance (PerMANOVA) was conducted based on distance matrices using ‘adonis’ function in the ‘vegan’ package with 999 permutations. Principal coordinates of neighbor matrices (PCNM) vectors with positive eigenvalues were calculated based on geographical coordinates (latitude and longitude) using the ‘PCNM’ command in the ‘PCNM’ package (Dray et al. 2006). The ‘varpart’ function in the ‘vegan’ package was used to partition the variation of soil fungal β-diversity by the individual and interactive effects of spatial distance (i.e. PCNM) as well as soil and plant (richness and community composition) parameters. To test the homogeneity of the fungal community among different islands (Anderson and Walsh 2013), beta dispersion of Simpson dissimilarity (free from richness variance) was explored by the ‘betadisper’ function in the ‘vegan’ package.

The normalized stochasticity ratio (NST) was tested with simulated communities by considering abiotic filtering, competition, environmental noise, and spatial scales (Ning et al. 2019). The modified stochasticity ratio (MST) index was calculated using the ‘tNST’ function in the ‘NST’ package to evaluate the contribution of stochasticity to community assembly (Ning et al. 2019). The MST index, a special transformation/case of NST, ranges from 0% to 100%, where 0% indicates no contribution of stochasticity, and 100% indicates that the community assembly is completely stochasticity driven. Particularly, this index was developed with 50% as the boundary point between more deterministic (< 50%) and more stochastic (> 50%) assemblies. Moreover, a neutral community model was used to determine the contribution of stochastic process to fungal community assembly by predicting the relationship between the occurrence frequency of OTUs (the proportion of local communities in which each OTU is detected) and their abundance (the mean relative abundance across all local communities) (Sloan et al. 2006). This model emphasizes the effect of stochastic dispersal and drift but ignores the ecological difference between species and their response to the surrounding environment. In this model, the estimated migration rate (*m*) was a parameter for evaluating the probability that a random loss of an individual in a local community would be replaced by dispersal from the metacommunity. The parameter R^2 represented the overall fit to the neutral model. An R^2 value closer to 1 implied that the community was consistent with the neutral process of dispersal and ecological drift, whereas $R^2 < 0$ was not fit. These parameters were determined using non-linear least squares fitting in the 'minpack.lm' package (Elzhov et al. 2016). Calculation of 95% confidence intervals around the model prediction was conducted by bootstrapping with 1,000 bootstrap replicates in the 'HMisc' package (Harrell et al. 2020). The relationships between fungal α-diversity or β-diversity and the island sizes were analyzed using type II linear regression (ordinary least squares) in the 'lmodel2' package (Legendre 2011). Akaike information criterion values were employed to judge whether the quadratic model was better than the linear model (Burnham and Anderson 2002).

RESULTS

Identification of fungi

After quality control, we obtained 5 154 835 quality-filtered ITS2 sequences from 6 790 156 raw sequences, in which 3 968 623 sequences were identified into 9852 fungal OTUs. We performed normalization using 3373 sequences (3373–60 467 sequences among soil samples) and obtained 7629 fungal OTUs (684 719 reads), which were distributed in 10 phyla (relative abundance 92.26%) and 644 unidentified fungal OTUs (7.74%). The fungal community was dominated by Ascomycota (3980 OTUs, 46.9%) and Basidiomycota (2427 OTUs, 26.4%) **(Fig. S2)**. The rarefaction curves of 18 islands roughly tended to reach an asymptote, reflecting that the majority of distinct fungal OTUs could be recovered **(Fig. S3)**.

Fungal α-diversity

Fungal α-diversity (OTU richness) was significantly different among the 18 islands ($P < 0.001$**, Fig. 1A**). The average fungal α-diversity was significantly higher in the Wanshan islands than in the Xisha islands **(Fig. 1B)**. We did not observe any significant relationships between fungal α-diversity and island area in Wanshan, Xisha, and total islands **(Fig. 2A)**. The linear mixed-effects models showed that the fungal α-diversity was significantly affected by soil K, Mg, and N in the total islands, soil K and Mg and plant community composition (PC1 and PC2) in the Wanshan islands, and soil Mg in the Xisha islands **(Table 1)**.

Fungal β-diversity

We observed a higher relative abundance of Mortierellomycota but lower relative abundance of Ascomycota in the Wanshan islands compared to the Xisha islands **(Fig. S2)**. OTU5749, OTU1295, OTU5468, OTU358, and OTU6298 were abundant in Wanshan islands, whereas OTU5355, OTU5897, OTU18, and OTU4867 were rich in the Xisha islands **(Fig. S4)**. Moreover, PerMANOVA analysis showed that the fungal community composition was significantly distinct in eight islands in Wanshan ($R^2 = 0.187$; $P < 0.001$), 10 islands in Xisha ($R^2 = 0.261$; $P < 0.001$), and between two island types ($R^2 = 0.094$; $P < 0.001$). The NMDS analysis also showed similar results (**Fig. 3**). Interestingly, we found significant exponential relationships between fungal β-diversity and island area in Wanshan, Xisha, and total islands (all $P < 0.05$, **Fig. 2B**). These results were further supported by the beta dispersion of the Simpson dissimilarity, which indicated significantly stronger fungal β-diversity among larger islands than among smaller islands in both the Wanshan islands ($P = 0.005$, **Fig. 4A**) and Xisha islands ($P < 0.0001$, **Fig. 4B**). Moreover, the results of the variation partitioning analysis showed that the fungal community was explained by plant community composition (17.92%), space (8.96%), and soil properties (6.11%) in the Wanshan islands, by spatial distance (11.63%), soil properties (10.96%), and plant community composition (8.87%) in the Xisha islands, and by plant community composition (18.06%), soil properties (12.82%), and spatial distance (7.11%) in total islands **(Fig. 5)**.

The MST model showed that the MST value of each island ranged from 24.4 to 43.3% in Wanshan, 11.7 to 46.9% in Xisha, and 11.7 to 46.9% in total islands, indicating that fungal community assembly was mainly shaped by deterministic process **(Fig. 6A)**. Furthermore, a lower MST value was observed in the Wanshan islands (23.9%) than in the Xisha islands (31.7%), suggesting a stronger deterministic effect in the Wanshan islands compared to the Xisha islands **(Fig. 6B)**. The neutral community model showed that the goodness of fit was similar among Wanshan (39.7% explained), Xisha (38.9% explained), and total (41.2% explained) islands **(Fig. 7)**. The migration rate (m) was consistently low (0.016 to 0.024, compared to 1), reflecting strong deterministic processes driving the fungal community assemblies of the three datasets **(Fig. 7A-C)**. Moreover, this parameter was lower in Wanshan ($m = 0.018$) than in Xisha ($m = 0.024$), indicating that the deterministic process was even more important in shaping the fungal community assembly in Wanshan compared to the Xisha islands.

DISCUSSION

We found that fungal α-diversity was relatively higher in Wanshan than in Xisha islands. This may be because plant species richness is higher in Wanshan than in the Xisha islands **(Table S1)**, which is a well-known tight interaction of plants contributing C to fungi (Mueller et al. 2014; Chen et al. 2017; Hiiesalu et al. 2017). In addition, according to the theory of island biogeography, a smaller isolation degree (distance) from the Wanshan islands to the mainland (potential source communities), compared to the Xisha islands, could be interpreted as another evidence for higher α-diversity in the Wanshan islands. Furthermore, no correlation was observed between fungal α-diversity (species number) and island area, regardless of island type **(Fig. 2A)**. Similarly, soil fungal α-diversity was also unaffected by island area in a land-bridge island system (Li et al. 2020).

The fungal community composition was significantly different in the Wanshan and Xisha islands in this study. This might be caused by substantial differences in the relative abundances of some fungi, including phyla of Ascomycota and Mortierellomycota, and some fungal OTUs between the Wanshan and Xisha islands. In addition, the significant difference in plant species richness between the two island types mentioned above may also be attributed to the observed differentiation of fungal community compositions. Furthermore, we found that soil fungal β-diversity was significantly and positively correlated with island area, irrespective of total, Wanshan, and Xisha islands, thus supporting our first hypothesis (H_1). Likewise, fungal β-diversities increased with island area (Li et al. 2020). Given that fungal β-diversity rather than α-diversity increased with island area, it was interpreted that this positive relationship between β-diversity and island area would be due to greater habitat heterogeneity on larger islands as compared with smaller islands, according to a conceptual diagram proposed by Li et al. (2020).

We found that fungal community assembly was mainly driven by a deterministic process rather than a stochastic process. Similarly, soil fungal community assembly was strongly shaped by the deterministic process, which was mainly filtered from great environmental heterogeneity, including host plants and soil organic matter from dry sclerophyll forest to coastal heathlands (Beck et al. 2015) and soil fertility in tea plantation ecosystems (Guo et al. 2020). Indeed, we found that the plant and soil (selection relevant) had a stronger effect on fungal community compared to geographic distance (concerning dispersal limitation) **(Fig. 5)**. In addition, before the stable fungal community was formed, species could be further added through speciation, long-distance dispersal, and migration over past land bridges and global trade (Tedersoo et al. 2014). Once the community composition became relatively stable, the niche-based (selection) process dominated the soil fungal community assembly, because the neutral-related processes became much less important (Zhou et al. 2014).

Furthermore, we found that fungal community assembly in the Wanshan islands seemed to be more deterministic driven than the Xisha islands **(Fig. 6B)**. Indeed, we found that plants and soil exerted much stronger impacts on fungal community composition in the Wanshan islands than in the Xisha islands, according to the variation partitioning results **(Fig. 5)**, and thus our second hypothesis (H_2) 'β-diversity is affected by different factors in two types of islands' was supported. In addition, we found that geographic distance also affected fungal community composition. The distance effect is often considered to represent the influence of dispersal, and dispersal could be stochastic to a large extent (Lowe and McPeek 2014; Zhou and Ning 2017). The effect of spatial distance on the fungal community suggests that fungal community assembly is also affected by stochastic processes; however, it is not the major force, as reported in previous studies (Beck et al. 2015; Guo et al. 2020). We thus could not completely exclude the potential effect of stochastic processes on fungal community assembly.

In summary, we investigated soil fungal diversity among 18 oceanic islands belonging to the general (Wanshan) and coral (Xisha) islands in the South China Sea. Fungal α-diversity was higher in Wanshan than in Xisha islands. In addition to soil Mg, plant community and soil K significantly affected fungal α-diversity in the Wanshan islands. Soil fungal community composition was different in the Wanshan and Xisha islands. The deterministic process mainly governed the fungal community assembly, with a higher force in Wanshan than in the Xisha islands. Fungal β-diversity, but not α-diversity, was significantly and positively related to island area. Our findings have implications for enhancing the predictability of soil fungal community dynamics and biodiversity conservation in discrete habitats and fragmented landscapes.

SUPPLEMENTARY DATA

Supplementary data are simultaneously submitted.

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Figure legends

Figure 1. Fungal α-diversity (mean OTU richness) in 18 oceanic islands **(A)** and in the Wanshan and Xisha islands **(B)**. Bars without shared letters indicate significant differences among 18 islands and between two island types according to the nonparametric Kruskal-Wallis test and two-sample T-test ($P < 0.05$), respectively.

Figure 2. The relationships between the observed fungal α-diversity **(A)** and β-diversity **(B)** and island area at Wanshan (black), Xisha (red), and total (blue) islands. Fungal β-diversity (**B**, $P < 0.05$), but not α-diversity (**A**, $P > 0.05$) increased with increasing oceanic island size.

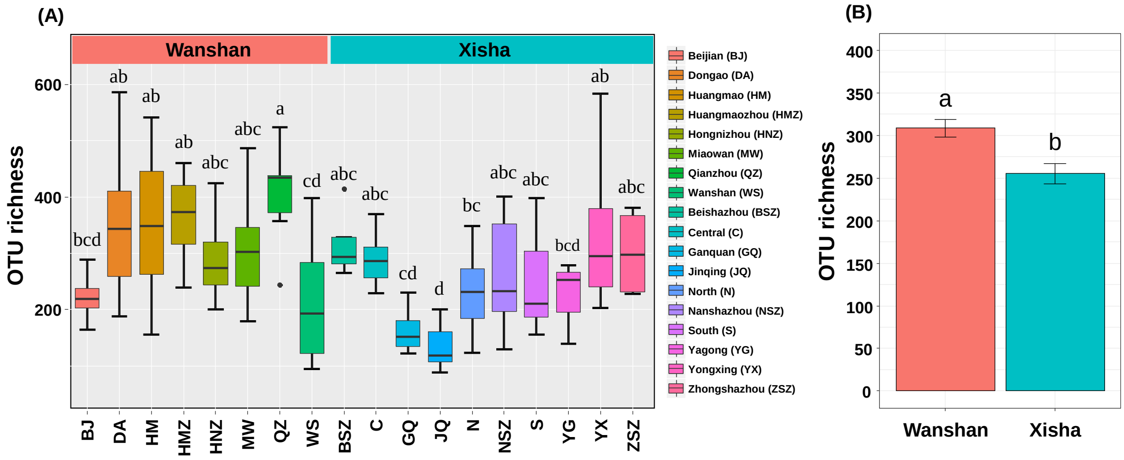
Figure 3. Non-metric multidimensional scaling (NMDS) of the fungal community compositions in 18 islands. Ellipses indicate 95% confidence intervals around centroids of different islands. The stress value in this 2-dimensional NMDS is 0.198.

Figure 4. The fungal community turnover analyses for the Wanshan islands **(A)** and the Xisha islands **(B)** based on the dispersion index (Simpson dissimilarity). Within the legend, the colored points representing eight (Wanshan, **A**) and 10 (Xisha, **B**) islands are arranged according to increasing island area from top to bottom.

Figure 5. Variation partitioning analyses showing the pure and shared effects of plant, soil, and space factors on fungal community compositions in the total **(A)**, Wanshan **(B)**, and Xisha **(C)** islands. Numbers indicate the proportion of explained variation.

Figure 6. Modified stochasticity ratio (MST) analysis showing the fungal community assembly pattern. The MST values amongst 18 islands **(A)** and the average MST values in Wanshan, Xisha, and total islands **(B)**.

Figure 7. The neutral community model showed that neutral interpretation has a good fit to fungal community distribution for the total **(A)**, Wanshan **(B)**, and Xisha **(C)** datasets. The predicated occurrence frequency is shown as a solid blue line, and dashed blue lines represent 95% confidence intervals around the model prediction; red and light blue dots indicate the fungal operational taxonomic units that occur less and more frequently than given by the model; R^2 and m indicate the degree of fitting to the neutral community model and the immigration rate, respectively.



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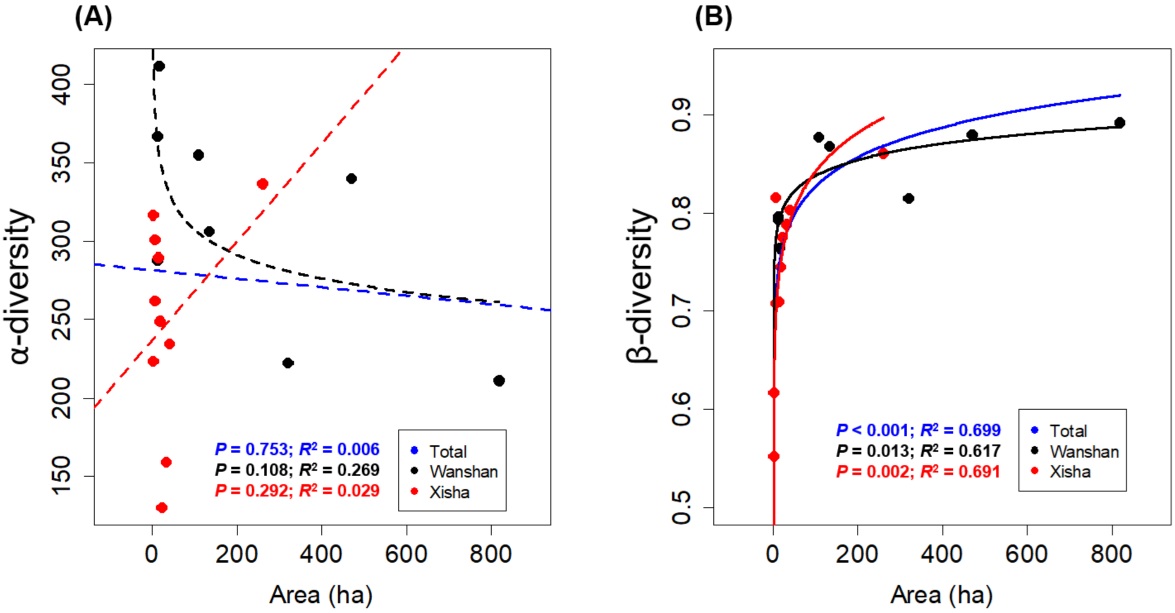

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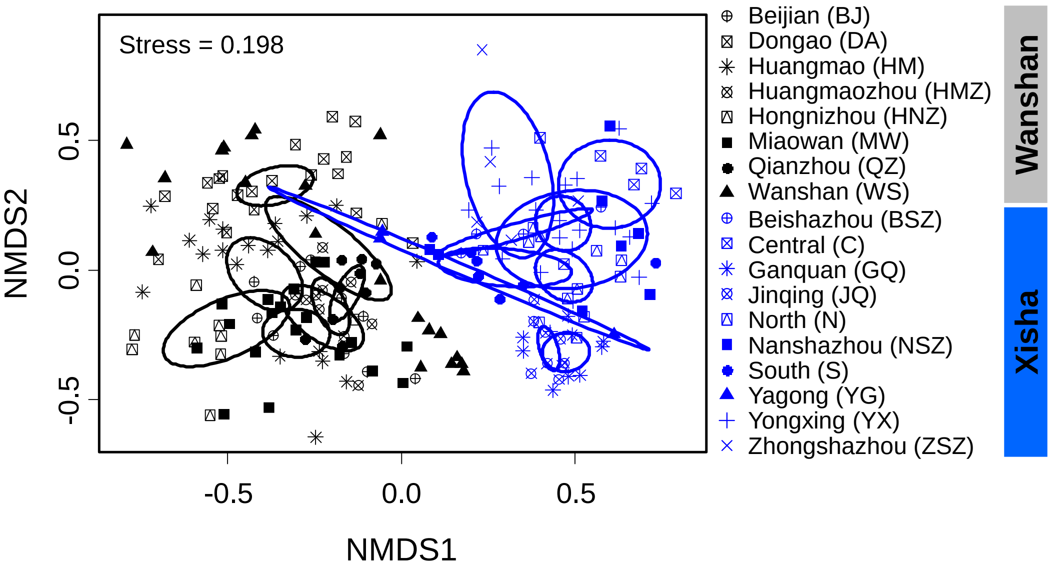

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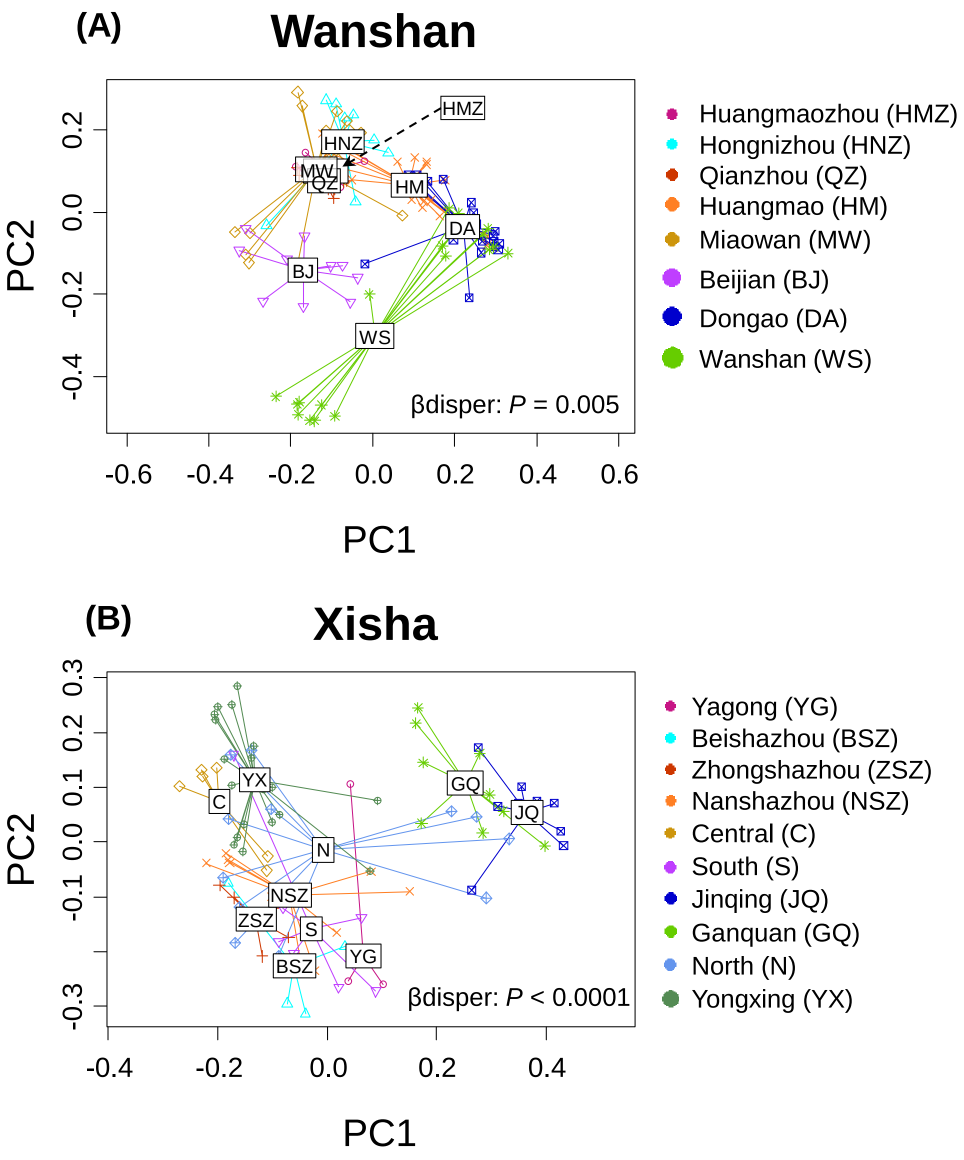

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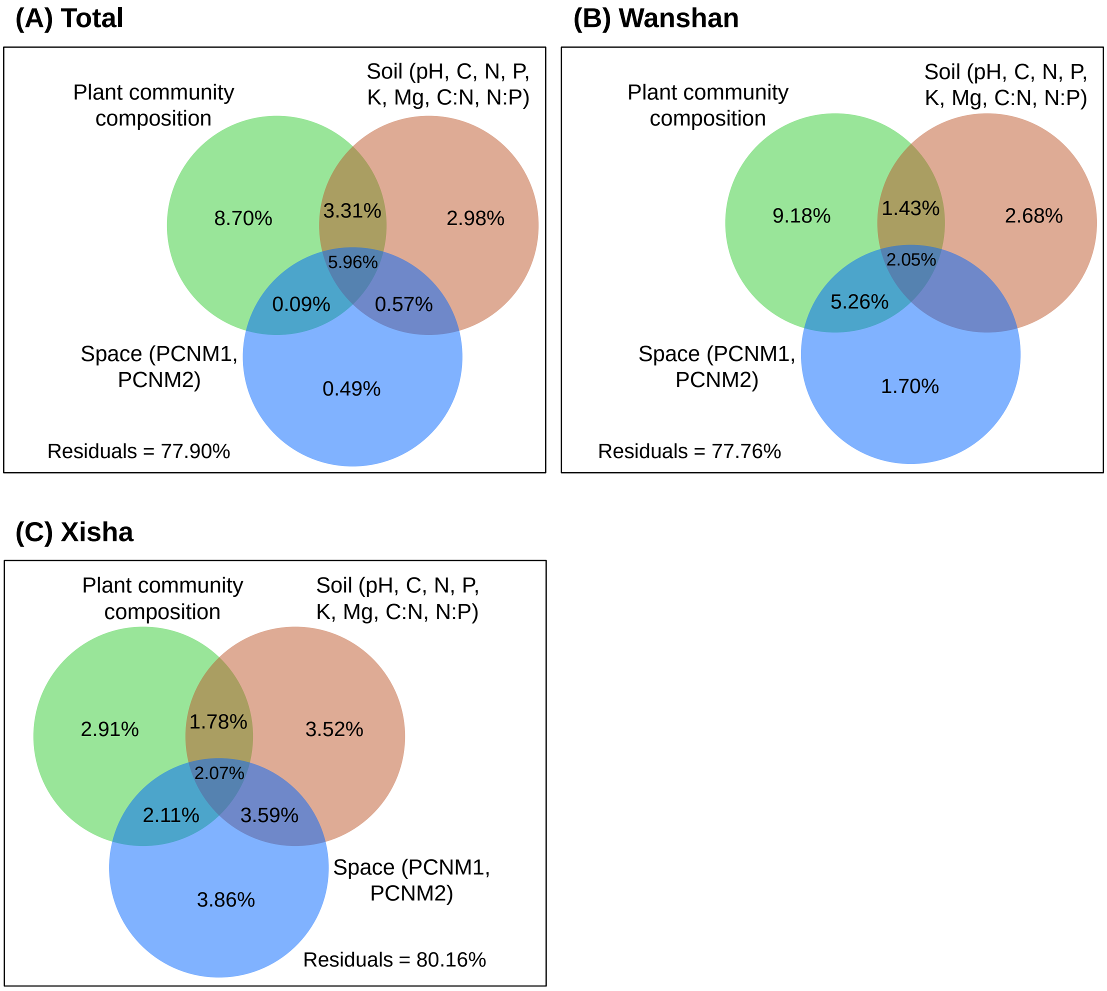

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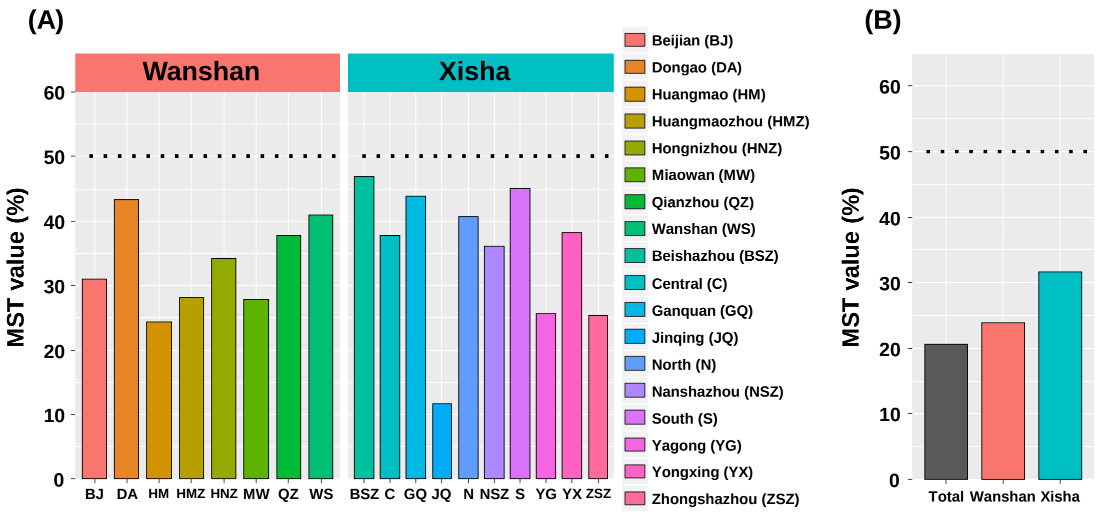

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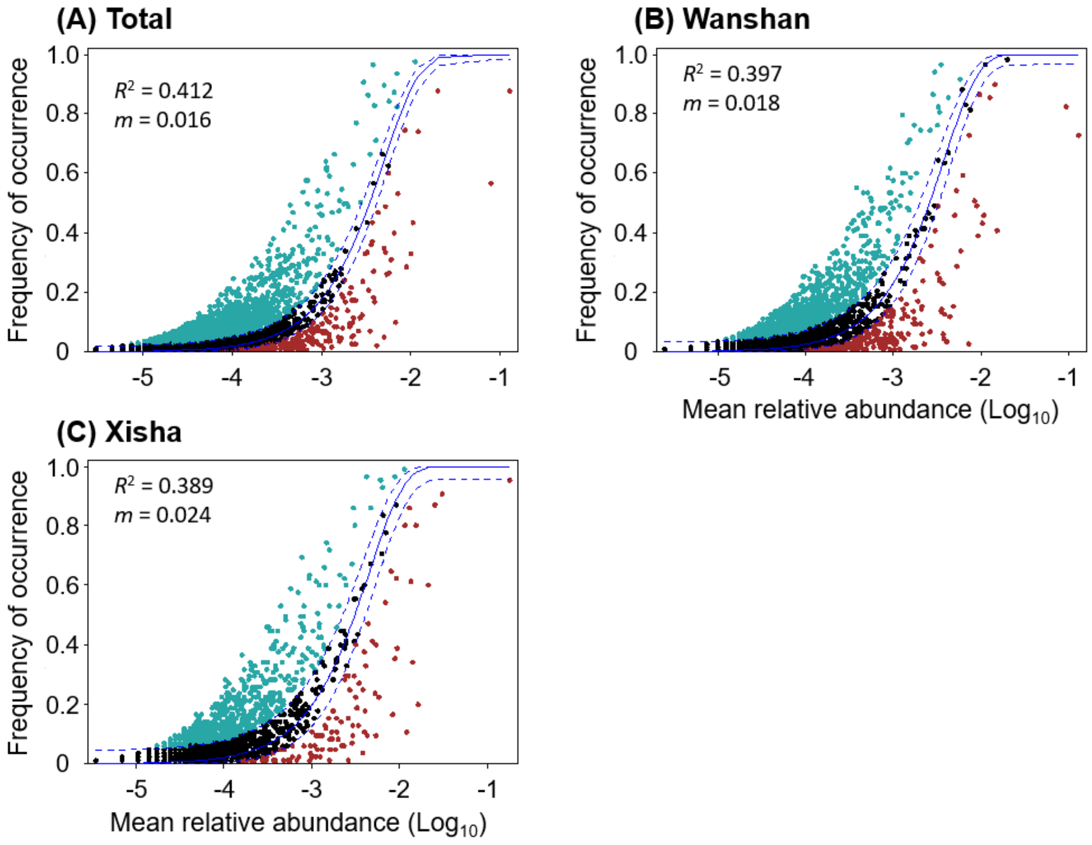

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Table 1.

Soil fungal α-diversity predicted by plant and abiotic variables as explored by linear mixed-effects models controlling random effect of site

Fungal richness	**Independent variable**	**Slope**	***SE***	***df***	***t***	P_{adj}
Total data	K	3.279	0.880	176	3.727	< 0.001
	Mg	12.07	4.189	176	2.882	0.004
	N	11.68	5.312	176	2.198	0.029
Wanshan	K	4.634	1.003	105	4.621	< 0.001
	Plant PC1	37.06	9.176	105	4.038	< 0.001
	Plant PC2	-26.53	7.378	105	-3.596	< 0.001
	Mg	53.01	20.16	105	2.630	0.010
Xisha	Mg	8.084	3.688	70	2.192	0.032