# Regional microbial biogeography linked to soil respiration

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## HIGHLIGHTS

- Microbial diversities were investigated along an elevational gradient in southeastern Tibet.
- Stochastic processes played a dominant role in shaping bacterial and fungal communities.
- Significantly positive relationships between microbial β-diversities and Rs were observed.
- Soil temperature, soil moisture, and total nitrogen were the most important edaphic properties in explaining Rs.
- Stochastic processes significantly mediated the effects of microbial β-diversities on Rs.

## GRAPHICAL ABSTRACT
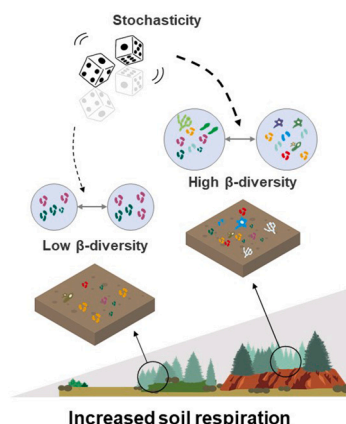


## ARTICLE INFO

Editor: Wei Shi

## ABSTRACT

The relationships between α-diversity and ecosystem functioning (BEF) have been extensively examined. However, it remains unknown how spatial heterogeneity of microbial community, i.e., microbial β-diversity within a region, shapes ecosystem functioning. Here, we examined microbial community compositions and soil respiration (Rs) along an elevation gradient of 853–4420 m a.s.l. in the southeastern Tibetan Plateau, which is renowned as one of the world's biodiversity hotspots. There were significant distance-decay relationships for both bacterial and fungal communities. Stochastic processes played a dominant role in shaping bacterial and fungal community compositions, while soil temperature was the most important environmental factor that affected microbial communities. We evaluated BEF relationships based on α-diversity measured by species

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richness and β-diversity measured by community dispersions, revealing significantly positive correlations between microbial β-diversities and Rs. These correlations became stronger with increasing sample size, differing from those between microbial α-diversities and Rs. Using Structural Equation Modeling (SEM), we found that soil temperature, soil moisture, and total nitrogen were the most important edaphic properties in explaining Rs. Meanwhile, stochastic processes (e.g., homogenous dispersal and dispersal limitation) significantly mediated effects between microbial β-diversities and Rs. Microbial α-diversity poorly explained Rs, directly or indirectly. In a nutshell, we identified a previously unknown BEF relationship between microbial β-diversity and Rs. By complementing common practices to examine BEF with α-diversity, we demonstrate that a focus on β-diversity could be leveraged to explain Rs.

## 1. Introduction

Biodiversity is important for providing and stabilizing ecosystem functions, which ensures the provision of various ecosystem services to our society (Isbell et al., 2017). Owing to its importance, the relationship between biodiversity and ecosystem functioning, known as the biodiversity-ecosystem functioning (BEF) relationship, has been widely studied (Loreau et al., 2001). However, those studies have primarily focused on α-diversity, which addresses species gain or loss within a local site. For example, positive effects of species richness have been observed on root biomass accumulation, plant gross primary productivity, and ecosystem stability in the forest, grassland, and wetland ecosystems (Qiu et al., 2021; Wu et al., 2022). Unlike α-diversity, β-diversity – a hitherto underexplored facet of biodiversity – describes the spatial heterogeneity of ecological communities. Loss of β-diversity (i.e., homogenizations of community compositions in a given area) could affect ecosystem functioning as strongly as, or even more strongly than, the effects of local species losses (Loreau et al., 2003). Therefore, focusing on α-diversity is not sufficient to capture the consequences of environmental disturbance to ecosystems for their functioning. Examining β-diversity has the potential to improve process-level understanding of biodiversity, which provides valuable insights for better predictions of natural and anthropogenic influences on diversity and ecosystem functioning.

In nature, community assemblies are influenced by both deterministic processes (e.g., abiotic and biotic selection) and stochastic processes (e.g., ecological drift, dispersal limitation, and homogeneous dispersal), whose relative importance varies in space (Ning et al., 2020; Yang, 2021). The β-diversities of elevational or latitudinal gradients often result from environmental sorting or historical constraints to infer ecological processes underlying microbial community assemblies (Wang et al., 2020). Compared to studies in plant ecology, the β-diversities of microbial communities are less understood. Stochastic processes, such as dispersal limitation, homogeneous dispersal, and ecological drift, were dominant in structuring spatial variations in bacterial communities in global wastewater treatment plants (Wu et al., 2019), but were less influential than deterministic processes such as environmental selection in other habitats, including soil, stream, and lake (Wang et al., 2013).

In this study, we examined whether a positive BEF relationship existed between microbial β-diversity and soil respiration (Rs), a crucial soil function of carbon cycling closely related to microorganisms in bulk soil or rhizosphere (Lei et al., 2021). High variability of Rs within a small area is common due to differences in edaphic properties and microbial community composition (Rodeghiero and Cescatti, 2008). Therefore, we conducted an intensive sampling campaign across a range of elevations in the southeastern Tibetan Plateau, including the Hengduan Mountains. This campaign spanned from 853 m to 4420 m and included 12 elevations, including dry-hot valley, evergreen forest, mixed forest, coniferous forest, and alpine meadow (Table S1). The total region was over 25,000 km$^2$, which was chosen because it is a biodiversity hotspot but has been seldom sampled due to its remoteness (Hughes, 2017). Using a hierarchically nested, spatially explicit design (Fig. S1), we collected 11 soil samples per elevation in July 2019. Elevations were treated as continuous patches with similar environmental conditions (Fig. S2). To

assess the community heterogeneity within each elevation, we computed the distance of each sample to the centroid of 11 samples, representing the community dispersion (Anderson et al., 2006). This measure can serve as an alternative to pairwise sample distances for characterizing the β-diversity of the individual sample within the same elevation (Anderson et al., 2011). We hypothesized that microbial β-diversity had a positive effect on Rs. Since microbial communities within a short distance might be highly dissimilar even though environmental conditions were similar (O'Brien et al., 2016), we also hypothesized that stochastic processes played a significant role in mediating microbial β-diversity - Rs relationship.

## 2. Materials and methods

### 2.1. Site description and a hierarchical sampling scheme

Sampling sites are distributed along the Nujiang and Lancang River Basins (96–99°E, 25–29°N) in the Tibet Autonomous Region and Yunnan Province, China (Fig. S1 and Table S1), with the mean annual air temperature ranging from 4.5 to 25 °C. We collected 11 soil cores in a 100 × 100 m$^2$ L-shape at 12 elevations, resulting in a total of 132 samples. To collect the soil cores, we established a grid origin with 100 m x- and y-axes, and then collected five samples along each axis at 5 m, 10 m, 25 m, 50 m, and 100 m from the grid origin. For each sample, three soil cores were collected with a sterilized soil auger from a 0–20 cm depth and 5.0 cm diameter and then mixed completely to make a composite sample. Soil samples were stored at 4 °C in a portable refrigerator when transporting them to the laboratory.

### 2.2. Measurements of Rs and edaphic properties

$CO_2$ effluxes were measured for each soil core before sampling, resulting in a total of 132 $CO_2$ efflux data. To measure soil $CO_2$ efflux, we used the portable system LI-8100 (LI-COR Inc., Lincoln, NE, USA) and conducted in situ measurements from 9:00 a.m. to 2:00 pm during our sampling period in July 2019. The soil chamber was set up by inserting PVC collars (20 cm in inner diameter and 11 cm in height) into the soil at 2.5 cm depth, after removing aboveground vegetation manually without disturbing the soil. Rs was measured for 2 min at flux-balance status in the chamber. Soil temperature at a depth of 10 cm was recorded using probes attached to the soil chamber (LI-COR Inc., Lincoln, NE, USA). Soil moisture at a depth of 10 cm was recorded using Shunkeda TR-6 soil moisture sensor (Shunkeda Inc., Beijing, China).

Soil pH was measured in a soil: water (1:5) extract with a Mettler Toledo S220 pH meter (Mettler Toledo Inc., Colombus, OH, USA). Soil organic carbon and total nitrogen were measured using the Walkley-Black and Kjeldahl method. Soil $NH_4^+$ and $NO_3^-$ were measured using a continuous flow analyzer (AutoAnalyzer-AA3, Seal Analytical, Norderstedt, Germany) after extraction with 1 mol L$^{-1}$ KCl. The values of Rs and edaphic properties are shown in Fig. S2.

### 2.3. DNA extraction, Illumina sequencing, and raw data processing

DNA was extracted from each soil sample by Mobio Powersoil DNA

extraction Kits (Qiagen Inc., Hilden, Germany), whose quality and quantity were examined by the A260/A280 and A260/A230 ratios. The ribosomal marker genes were amplified and sequenced under the protocol of the Earth Microbiome Project (EMP) (Gilbert et al., 2018). Two rounds of PCRs were performed before sequencing, with a common primer pair to target the V4 hypervariable region of the bacterial 16S rRNA gene (515f: 5-GTGYCAGCMGCCGCGGTAA-3′; 907r: 5-CCGY-CAATTYMTTTRAGTTT-3′) and internal transcribed spacer (ITS) region of fungal genomes (ITS1F: 5-CTTGGTCATTTAGAGGAAGTAA-3′/ITS2: 5-GCTGCGTTCTTCATCGATGC-3′). The 2 × 250 bp paired-end sequencing of PCR products was performed on an Illumina HiSeq platform (Illumina Inc., San Diego, CA, USA). Raw sequences of pair-end reads were merged and dereplicated using USEARCH11, with the maximum expected error set to 1.0 for quality control. To achieve fine taxonomic resolution, the error-correcting algorithm UNOISE was used to identify amplicon sequence variants (ASV) (Edgar, 2016). Sequence-clustering algorithm UPARSE at the 97 % sequence identity level was also used for comparison analysis (Edgar, 2013). Bacterial taxonomic assignments were annotated by using the RDP classifier trained on the Silva database of version 132 (Quast et al., 2013), while fungal taxonomic assignments were annotated by the Unite database of version 8 (Nilsson et al., 2019). Only 0.3 % of ASVs belonged to archaea. Therefore, they were removed without further analyses. To investigate the effects of taxonomic resolutions, we used ASV as the finest taxonomic resolution in our study, followed by the OTU. Then, ASV tables were conglomerated from genus to phylum based on the annotations, which allowed us to generate coarser taxonomic resolutions than ASV and OTU. We amalgamated ASV read abundances, taxonomic assignments, and sample meta-data using the R package *phyloseq* (McMurdie and Holmes, 2013).

## 2.4. Statistical analyses

To examine distance-decay relationships of microbial communities, we calculated the dissimilarity matrix of microbial communities based on Bray-Curtis distance and edaphic properties based on the Euclidean distance and then used multivariate-distance regression matrix (MRM) modeling to quantify slopes (i.e., species turnover rate) and explained variance of these models (Martiny et al., 2011). We validated model results by 999 permutations of the dissimilarity matrix, as described in a previous study (Gao et al., 2019). The β-diversities of each sample were evaluated by community dispersions with multiple β-diversity distances and taxonomic resolutions (Anderson et al., 2006). We examined their positive relationship with Rs by using the linear regression model and non-parametric Spearman correlation analysis. The robustness of those relationships was examined by bootstrap resampling examination (Fieberg et al., 2020). Since sample size might affect our results (McGill et al., 2007), we randomly selected samples for 1000 times using a sample size from 3 to 9 per elevation. All sub-datasets resulting from resampling were used to calculated correlations between microbial diversities and Rs, thereby generating distributions of *P*-values that could be used for testing the robustness of correlations.

To measure the proportions of the stochasticity of community assembly, we used normalized stochastic ratio (NST) calculated by Bray-Curtis distance (Ning et al., 2019). After generating a randomly distributed matrix of distance as the null model, NST values were pairwise calculated among samples, whose averages were then assigned to each sample or each elevation. As NST values ranged between 0 and 1, we set 0.5 as the cutoff, with 0.5–1.0 being more stochastic and 0–0.5 being more deterministic. The deviation of NST values was examined by 1000 times bootstrap examination on the level of elevations against the default "PF" null model. We used phylogenetic binning-based iCAMP methods to determine the contributions of different ecological processes of community assemblies (Ning et al., 2020). Proportions of ecological processes were calculated for each sample within each elevation. All parameters of iCAMP were set by default (distance measurement, Bray-

Curtis; minimum taxa number of phylogenetic bins, 48; distance threshold for binning, 0.20; Spearman correlation to estimate phylogenetic signals). To examine the effect paths of β-diversity on Rs, Structural Equation Modeling (SEM) was used to examine the direct effect of β-diversity and indirect effects via edaphic properties and stochastic processes of community assemblies. We constructed a priori model that included the following types of hypothesized relationships. (i) Edaphic properties were correlated with microbial diversities. (ii) Edaphic properties and microbial diversities were correlated with stochastic ecological processes. (iii) Edaphic properties, microbial diversities, and stochastic ecological processes were correlated with Rs. We pruned links to optimize modeling performance by stepwise selection of Akaike information criterion (AIC) (Yamashita et al., 2007). The SEM analysis was carried out by the R package *lavaan* (Rosseel, 2012). All data processing and statistical analyses were performed in the R statistical software version 3.6.3 (R Development Core Team, 2018).

## 3. Results and discussions

### 3.1. Microbial biogeographical patterns along the elevational gradient

We obtained 56,320 bacterial ASVs and 17,580 fungal ASVs after rarefying 40,000 high-quality reads per sample. The α-diversities of both bacterial and fungal communities decreased with increasing elevation ($P < 0.050$, Fig. 1A and B), as measured by species richness and Shannon indices. The most abundant bacterial phylum was Proteobacteria (27.4 % ± 8.3 %, Fig. S3), followed by Acidobacteria (24.7 % ± 6.4) and Bacteroidetes (11.8 % ± 5.4 %). The most abundant fungal phylum was Ascomycota (49.0 % ± 17.0 %, Fig. S4), followed by Basidiomycota (26.4 % ± 22.2 %).

We estimated β-diversities of microbial communities by measuring community dispersions within each elevation. The β-diversities in fungal communities exhibited a significantly decreasing trend ($P < 0.050$, Fig. 1C), while no significant trend was detected in bacterial communities. The largest dispersion for bacterial communities was detected at the elevation of 2810 m (0.463 ± 0.77, Fig. 1C), while that for fungal communities was detected at the elevation of 2629 m (0.591 ± 0.62, Fig. 1C). There were significant distance-decay relationships for bacterial ($R^2 = 0.218$, $P < 0.001$, Fig. 1D) and fungal communities ($R^2 = 0.151$, $P < 0.001$, Fig. 1D). The slope was steeper for bacterial communities (slope = −0.088, $P < 0.001$, Fig. 1D) than fungal communities (slope = −0.067, $P < 0.001$, Fig. 1D), suggesting a faster bacterial community turnover. Due to the spatial hierarchy design of the sampling scheme, we also analyzed the distance-decay relationship for samples within each elevation as the local scale, which contrasted with the regional scale that pooled samples from all elevations (Fig. 1D). Significant distance-decay relationships ($P < 0.050$) were detected at the local scale in 8 elevations for bacterial communities (with slopes ranging from −0.041 to −0.144, Fig. S5) and in 4 elevations for fungal communities (with slopes ranging from −0.080 to −0.172, Fig. S6).

### 3.2. Deterministic and stochastic processes underlie microbial biogeographic patterns

Both geographic distance and the measured edaphic properties jointly explained 42.6 % of the bacterial community variations and 31.7 % of the fungal community variations (Table 1). Among all measured edaphic properties, soil temperature was the most important driver for both bacterial (13.7 %) and fungal communities (8.6 %), followed by soil moisture (bacterial community: 4.2 %; fungal community: 1.5 %, Table 1).

Geographic distance remained significant after controlling edaphic properties, explaining 20.9 % of bacterial community variations and 13.9 % of fungal community variations (Table 1). As geographic distance is an important factor relating to stochastic processes (Gao et al., 2019), we measured the stochastic process of the community assembly
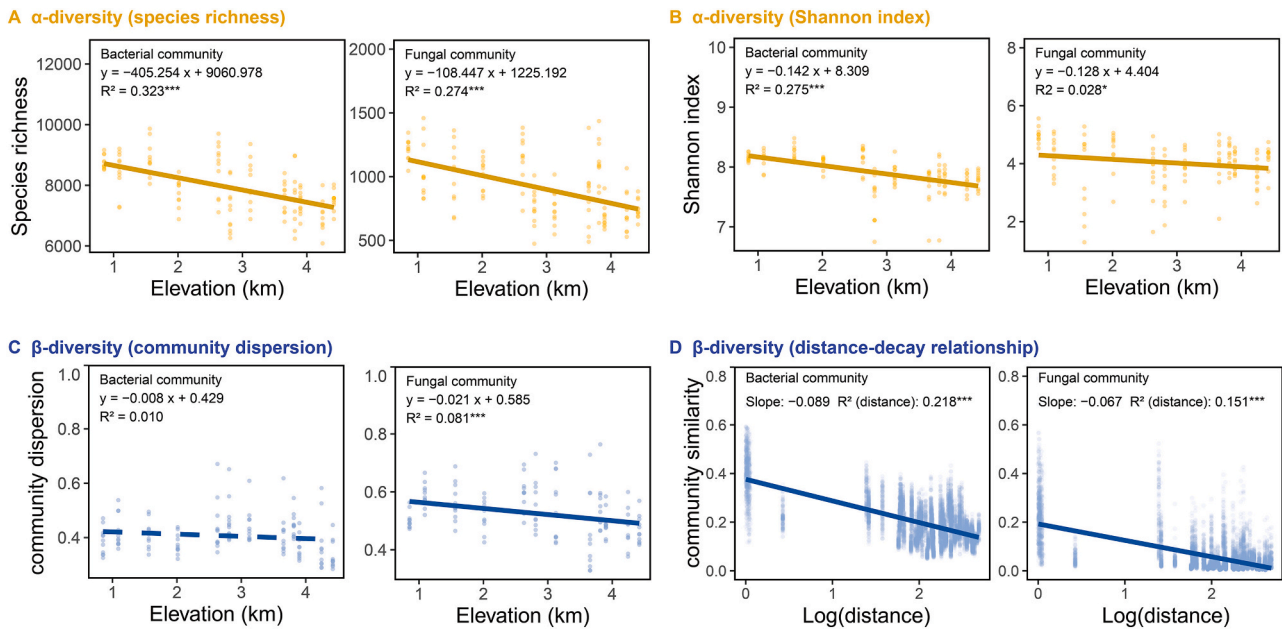
**Fig. 1.** Microbial geographic patterns along the elevational gradient of the southeastern Tibetan Plateau. Patterns of α-diversities were characterized by (A) species richness and (B) Shannon index. Patterns of β-diversities were characterized by (C) community dispersions of Bray-Curtis dissimilarities within each elevation and (D) distance-decay relationships. Elevational trends were examined by the linear fitting model and noted with the fitting formula and $R^2$. Slopes (i.e., the decay rate) and $R^2$ accounting for geographic distance were calculated by the multivariate distance-matrix regression model. The significance level is labeled by: *, $P < 0.050$; **, $P < 0.010$; ***, $P < 0.001$. The dashed line represents an insignificant trend ($P > 0.050$).

**Table 1**
Coefficients and partial $R^2$ of geographic distance and edaphic properties for community dissimilarities.

|  | Bacterial community | | Fungal community | |
|---|---|---|---|---|
|  | Coefficient | Partial $R^2$ | Coefficient | Partial $R^2$ |
| Distance | 0.410 | 0.209 | 0.351 | 0.139 |
| Soil temperature | 0.321 | 0.137 | 0.270 | 0.086 |
| pH | 0.068 | 0.007 | 0.036 | 0.002 |
| Soil moisture | 0.163 | 0.042 | 0.105 | 0.015 |
| Soil organic carbon | 0.040 | 0.001 | 0.121 | 0.008 |
| Total Nitrogen | 0.128 | 0.009 | 0.067 | 0.002 |
| $NO_3^-$ | 0.088 | 0.010 | 0.102 | 0.012 |
| $NH_4^+$ | −0.022 | <0.001 | \ | \ |
| Full model | Total $R^2$: 0.426 | | Total $R^2$: 0.317 | |

*Note:* Coefficients were standardized in the multivariate distance-matrix regression model. Only significant ($P < 0.050$) variables are shown.

by taxonomic-based normalized stochasticity ratios (NST). The stochastic process dominated bacterial community assembly (61.7–85.8 %) within each elevation, while there was a trend of increased stochasticity for fungal community assembly along the elevation gradient, reaching 95.1 % at the highest elevation of 4420 m (Fig. 2A).

To disentangle ecological processes that contribute to community assemblies, we adopted a recently developed algorithm named iCAMP (Ning et al., 2019). For bacterial communities, dispersal limitation contributed to 24.7 % of total community variations, drift contributed to 20.7 % of total community variations, and homogeneous dispersal contributed to 10.2 % of total community variations (Fig. 2B). For fungal communities, dispersal limitation contributed to 37.9 % of total community variations, drift contributed to 13.4 % of total community variations, and homogeneous dispersal contributed to 5.2 % of total community variations (Fig. 2B).
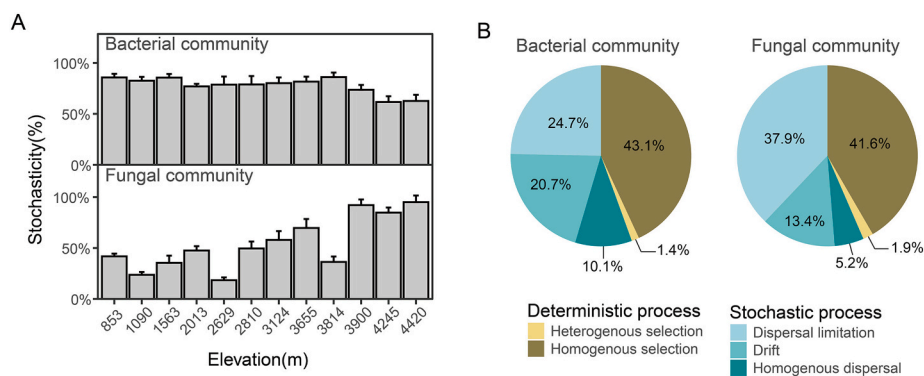


**Fig. 2.** The stochasticity of ecological processes of community assemblies along the elevational gradient. (A) Proportions of the stochasticity measured by the normalized stochastic ratio (NST). Bray-Curtis distances of samples were compared with the null model with random distributions of distances. Error bars refer to the standard deviation of 1000 times bootstrap examination. (B) Proportions of two deterministic processes and three stochastic processes quantified by the iCAMP method with phylogenetic distances.

### 3.3. The positive relationships between microbial β-diversity and Rs

We found that microbial β-diversities characterized by community dispersions were positively correlated with Rs (coefficient of bacteria: 3.489, coefficient of fungi: 3.615; $P < 0.050$ in linear regression models, Fig. 3). Positive correlations were also detected when changing the distance measures of dispersions from Bray-Curtis to Jaccard, Sorensen, and Morista-Horn ($P < 0.050$ in non-parametric Spearman test, Table S2), indicating that correlations were robust to measure types. In contrast, significant correlations between microbial α-diversity and Rs were only observed when using species richness as α-diversity instead of the Shannon index and inverse Simpson index (Table S2). When shifting taxonomic resolutions (from ASVs to Phyla) in correlations between microbial β-diversities and Rs, significant correlations were detected from ASVs to orders in bacterial communities and from ASVs to classes in fungal communities (Table S3). However, correlations between microbial α-diversities and Rs were only detected in ASVs, genera, and families of bacterial communities and families of fungal communities (Table S3). We also examined the effects of β-diversities and their components (turnover and nestedness) (Baselga, 2010; Podani et al., 2013) on Rs by partial Mantel test. Turnover, defined as the species replacement by other samples, showed a significant correlation with Rs

variation (Mantel $r = 0.066$–$0.084$, P < 0.050, Table S4). However, there were no significant correlations for nestedness, which was defined as nested degree against other species-richer samples (Baselga, 2010). To date, potential degradation of ecosystem functions and services has been observed in response to a decline in microbial α-diversity (Wu et al., 2022). Recently developed measurements of biodiversity (e.g., multivariate decomposition of the diversity matrix) or ecosystem functions (e.g., ecosystem multifunctionality) have significantly extended our understanding of BEF relationships in soil and aquatic environments (Qiu et al., 2021; Wang et al., 2022; Zhang et al., 2021). However, similar investigations are still widely lacking for disentangling the effects of community heterogeneity evaluated by microbial β-diversity. Since the loss of β-diversity is not equivalent to a loss of local species richness (Mori et al., 2015), herein we provide empirical evidence that β-diversities can reveal the role of ecological processes in explaining Rs (Fig. 5), which is an important ecosystem functioning closely related to climate change and soil fertility. Unlike α-diversity, β-diversity can reveal compositional changes, which provides a new facet in explaining BEF relationships (Wang and Loreau, 2014).

We used bootstrap resampling to assess whether the sample size at each elevation was sufficient for capturing correlations between microbial diversities and Rs. Among all sub-datasets generated from 1000
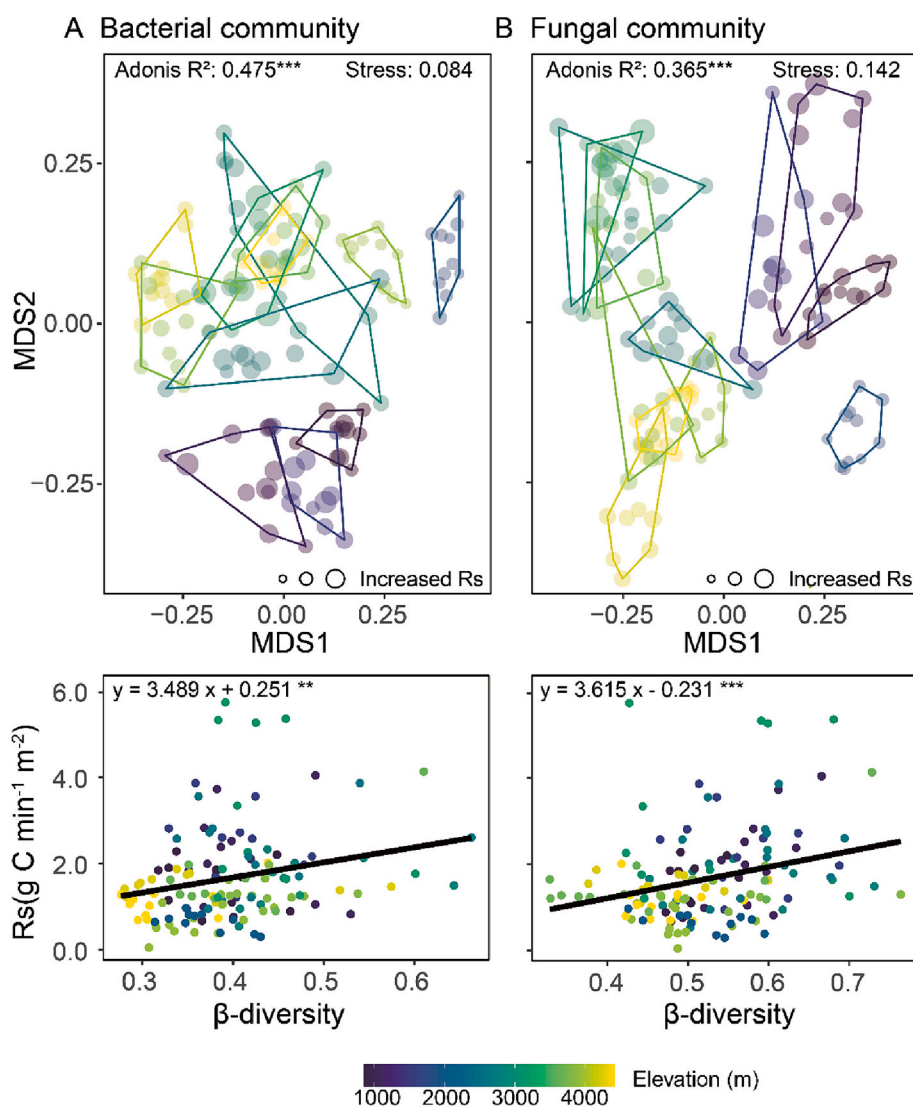


**Fig. 3.** Correlations between microbial β-diversity and soil Rs. Community dispersions of (A) bacteria and (B) fungi were examined by the Adonis test and visualized by Nonmetric multidimensional scaling (NMDS). Correlations between community dispersions and Rs were examined by the linear fitting model (**, P < 0.010; ***, P < 0.001). Larger circles correspond to increased Rs.

bootstrap resampling iterations, only 13.0 % to 47.0 % exhibited significant correlations ($p < 0.050$) with increasing sample sizes in terms of bacteria α-diversities (Fig. 4A). When considering fungal α-diversities, the recaptured correlations were consistently low, ranging from 6.0 % - 9.0 % and irrelevant with increasing sample size (Fig. 4B). In contrast, positive correlations between fungal β-diversity and Rs were detected in all sub-datasets when the sample size was >8, suggesting that the correlations were robust (Fig. 4D). When sample sizes exceeded 5, at least 50 % of correlations between β-diversities and Rs were re-captured, suggesting that the sample size was important. Together, we revealed the positive relationship between microbial β-diversity and Rs under different β-diversity measures and multiple taxonomic resolutions (Fig. 3 and Table S2 and S3), demonstrating the robustness of our findings. Real-world communities are subjected to patchy patterns with substantial species heterogeneity, which are influenced by chance events. As a result, species richness may not always be an accurate indicator of ecosystem functions. Moreover, positive α-BEF relationships may not be detectable due to functional redundancy (Louca et al., 2018). A region with a higher β-diversity can accommodate more heterogeneous communities, generating a form of 'spatial stability' of ecosystem functioning that is known as the spatial insurance effect (Yachi and Loreau, 1999). When different species are favored under different environmental conditions, they would show compensatory fluctuations in contributing to ecosystem function since some species' contributions may decrease while others' contributions may increase. Therefore, spatial complementarity between species, arising from the local selection of species that is best adapted to the local environmental conditions, can provide spatial insurance of ecosystem functioning. The β-diversity

plays a key role in preserving the spatial stability of ecosystem functioning by promoting niche complementarity, which allows the best-performing species to flourish in the right place.

### 3.4. Differences of microbial diversities in explaining Rs patterns

We used structural equation modeling as a path analysis to quantify the contributions of microbial diversities in explaining Rs patterns. The a priori model (Fig. 5A) hypothesized that Rs might be influenced by edaphic properties, microbial diversities, and ecological stochastic processes. Stochastic processes might mediate indirect effects between microbial diversities and Rs. After pruning non-informatic links (Table S5), we found that Rs exhibited significant correlations with stochastic processes (ρ of homogenous dispersal: −0.444; ρ of dispersal limitation: −0.199 - -0.370; Fig. 5B and C) in addition to the effects from edaphic properties (ρ of total nitrogen: 0.256–0.347; ρ of soil moisture: 0.197–0.220; ρ of soil temperature: 0.150–0.276; Fig. 5B and C). Bacterial α-diversity indirectly explained Rs through dispersal limitation (ρ = −0.342, Fig. 5B), while fungal α-diversity had no significant effect. Bacterial β-diversity explained Rs by two significantly indirect effects via stochastic processes, i.e., homogenous dispersal (ρ = −0.609, Fig. 5A) and dispersal limitation (ρ = 0.594, Fig. 5A). Fungal β-diversity had a significant direct effect in explaining Rs (ρ = 0.276, Fig. 5B). Fungal β-diversity also had an indirect effect on Rs via dispersal limitation (ρ = 0.339, Fig. 5B). In addition, our models also estimated the effects of edaphic properties on microbial diversities. Total nitrogen was the most important factor for both bacterial α-diversity (ρ = 0.634, Table S6) and fungal α-diversity (ρ = 0.704, Table S6). In contrast, bacterial β-diversity
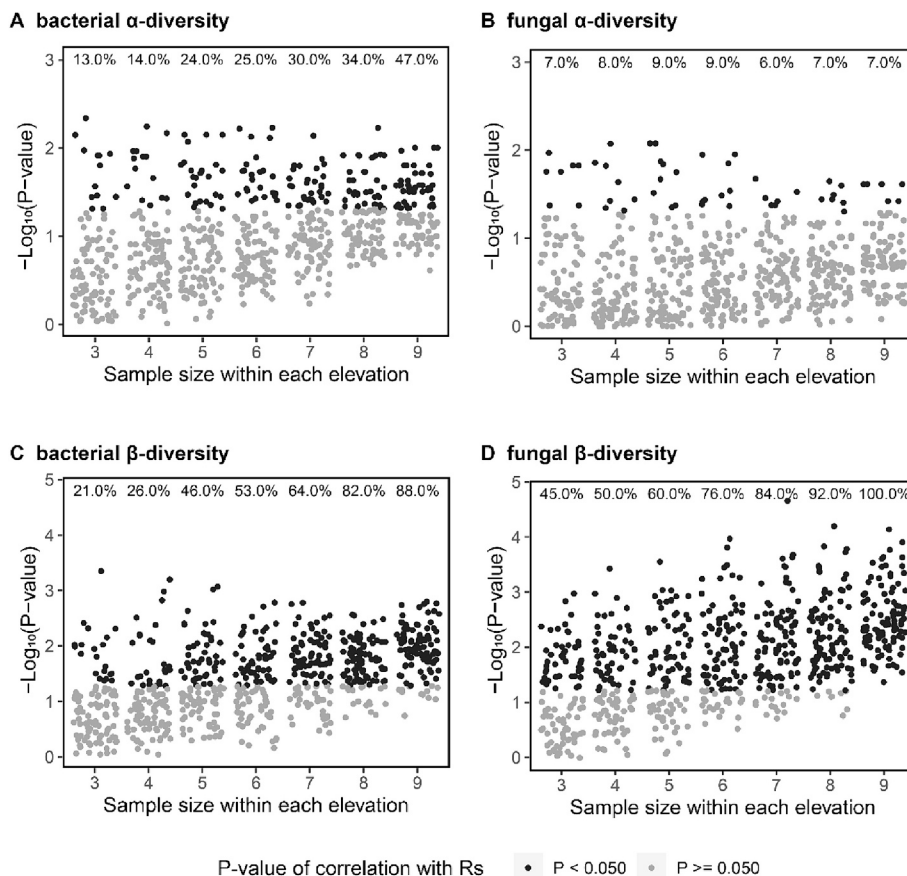


**Fig. 4.** Bootstrap resampling examinations showing the robustness of correlations between microbial diversities and Rs. Sub-datasets were randomly resampled 1000 times without replacement at a given sample size within each elevation. *P*-value distributions were generated by linear regression correlations between Rs and microbial diversity of each sub-dataset. (A and B) Taxon richness was used as the metric of α-diversity, while (C and D) community dispersion was used as the metric of β-diversity. Proportions of significant correlations (P < 0.050) in all iterations are shown at the top of the panels.
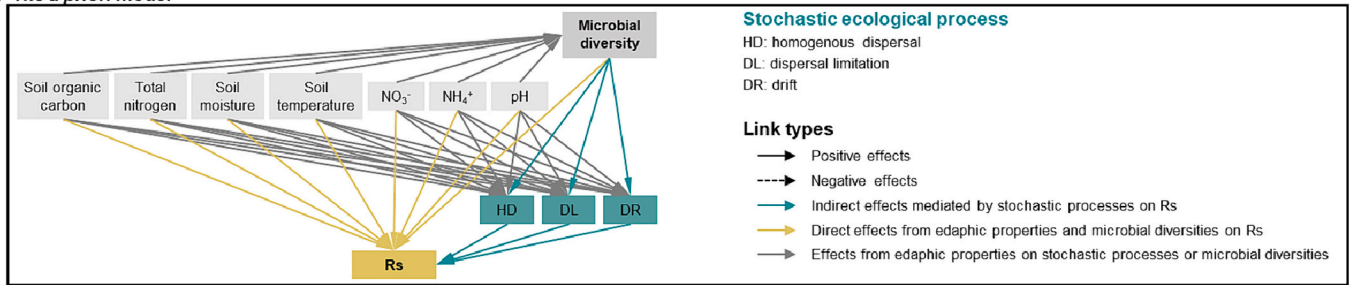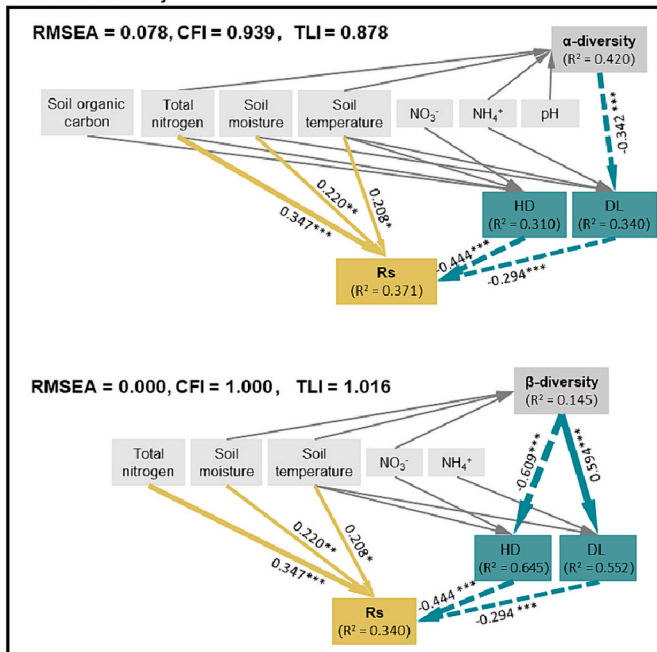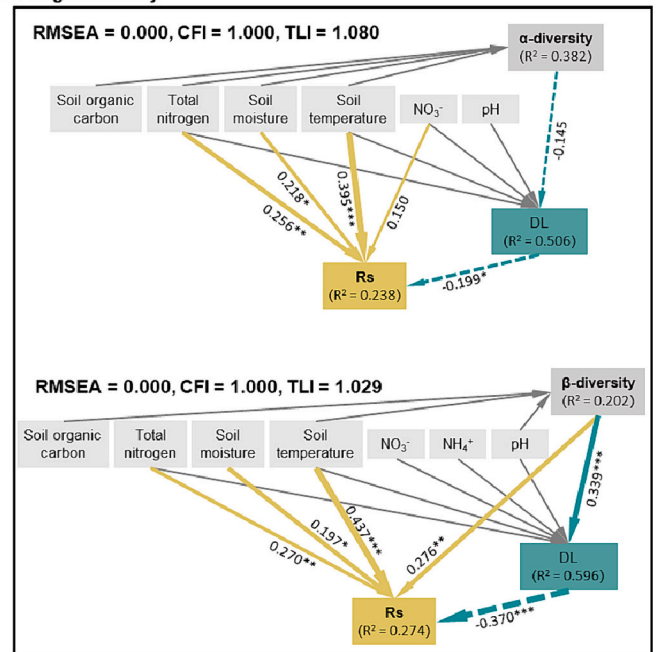
**Fig. 5.** Structural equation modeling (SEMs) showing the effects of microbial diversities on Rs. (A) The a priori model was pruned to remove non-informative links by the Akaike information criterion (AIC) for (B) bacterial diversities and (C) fungal diversities. The effects of α-diversity (measured by species richness), β-diversities (measured by community dispersions), stochastic processes, and edaphic properties were examined in the final model. The coefficient (ρ) on the arrow indicates the standardized effect size (*, P < 0.050; **, P < 0.010; ***, P < 0.001), which is proportional to the arrow width. Solid lines represent positive effects and dashed lines represent negative effects. The root mean square error of approximation (RMSEA), comparative fit index (CFI), and Tucker–Lewis index (TLI) are displayed as performance parameters (Table S5). For simplicity, coefficients of edaphic properties on microbial diversities and stochastic processes are shown in Table S6 instead of this figure.

exhibited a stronger correlation with soil moisture (ρ = 0.238, Table S6), while fungal β-diversity was more correlated to soil organic carbon (ρ = 0.512, Table S6). These patterns aligned with the distinct life strategies of bacteria and fungi (Ho et al., 2017) and the high preference of fungi for carbohydrates as a food source (Liu et al., 2015).

Fungal correlation with Rs was detected in the most abundant phylum of Ascomycota (Table S2), which dominates soil fungal communities worldwide and is enriched in genes related to carbohydrate metabolism (Egidi et al., 2019). Considering environmental heterogeneity, species interactions, and stochastic processes that affect species assembly, microbial β-diversity could be essential in understanding how naturally diverse communities are organized, and how such ecological processes underlie ecosystem functioning. Both deterministic and stochastic processes of community assemblies underlie the positive relationship between microbial β-diversity and Rs (Fig. 4). Niche theory postulates that competing species within guilds will undergo divergence, resulting in a reduction of niche overlap (MacArthur, 1970). Otherwise, all but one of the competing species will be driven extinct, i. e., competitive exclusion. The ubiquity of ecological niches explains the positive relationship between β-diversity and ecosystem functioning: competitive divergence leads to the niche partitioning of species in a community, collectively contributing to ecosystem functioning (Craven et al., 2018).

## 4. Conclusions

Microbial β-diversity can serve as a reliable estimator of Rs and can play an irreplaceable role due to its biodiversity-insurance effect and assembly indicators (Loreau et al., 2021). Given that numerous biogeographical patterns are frequently influenced by deterministic factors originating from environments, extending β-BEF to diverse ecosystem functions can provide practical significance in the conservation of biodiversity amidst the challenges posed by habitat fragmentation and global climate change. Anthropogenic disturbances often cause biotic homogenization and a loss of β-diversity, while natural disturbances often increase β-diversity since they tend to be patchy, complex, and variable in severity. Consequently, anthropogenic disturbances often hinder ecosystem functioning, while natural disturbances create opportunities for various taxa by preventing competitive exclusion among species. This fosters the preservation of biological diversity and sustains ecosystem functioning that relies on biodiversity.

## CRediT authorship contribution statement

**Yufei Zeng:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Xue Guo:** Data curation. **Jiesi Lei:** Data curation, Writing – review & editing. **Yu Shi:** Data curation. **Xu Liu:** Data curation. **Tianjiao Dai:** Formal analysis. **Qiuting Zhang:** Data curation. **Qun Gao:** Writing – review & editing. **Haiyan Chu:** Conceptualization. **Yongqin Liu:** Conceptualization. **Jizhong Zhou:** Writing – review & editing. **Yunfeng Yang:** Conceptualization, Writing – original draft, Writing – review & editing.

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used chatGPT for the sole purpose of polishing the language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Illumina sequencing data have been deposited in GenBank (National Centre for Biotechnology Information) Short Read Archive, which are publicly available with BioProject numbers PRJNA980427 (bacterial communities) and PRJNA980438 (fungal communities). All original codes are publicly available on GitHub (https://github.com/Yflyer/Microbial-Biogeography-in-Southeastern-Tibet).

## Acknowledgments

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.172263.

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