Multiple ecological processes jointly drive the soil microbial community assembly in subalpine coniferous forests (#30949)

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Multiple ecological processes jointly drive the soil microbial community assembly in subalpine coniferous forests

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The mechanisms underlying community dynamics, which govern the complicated biogeographical patterns of microbes, have long been a research hotspot in community ecology. However, the mixing of multiple ecological processes and the one-sidedness of analytical methods make it difficult to draw inferences about the community assembly mechanisms. In this study, we investigated the driving forces of the soil microbial community in subalpine coniferous forests of the Loess Plateau in Shanxi, China, by integrating multiple analytical methods. The results of the null model demonstrated that deterministic processes (especially interspecific relationships) were the main driving force of the soil microbial community assembly in this study area, relative to stochastic processes. Based on the results of the net relatedness index (NRI) and nearest taxon index (NTI), we inferred that historical and evolutionary factors, such as climate change and local diversification, may have similar effects on microbial community structure based on the climatic niche conservatism. Based on the results of a functional traits analysis, we found that the effects of ongoing ecological processes on the microbial community assembly varied among sites. Therefore, the functional structures seemed to be more related to ongoing ecological processes, whereas the phylogenetic structures seemed to be more related to historical and evolutionary factors, as well as the tradeoff between deterministic and stochastic processes. The functional and phylogenetic structures were mainly shaped by different ecological processes. By integrating multiple ecological processes, our results provide more details of the mechanisms driving the community assembly

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Abstract The mechanisms underlying community dynamics, which govern the complicated biogeographical patterns of microbes, have long been a research hotspot in community ecology. However, the mixing of multiple ecological processes and the one-sidedness of analytical methods make it difficult to draw inferences about the community assembly mechanisms. In this study, we investigated the driving forces of the soil microbial community in subalpine coniferous forests of the Loess Plateau in Shanxi, China, by integrating multiple analytical methods. The results of the null model demonstrated that deterministic processes (especially interspecific relationships) were the main driving force of the soil microbial community assembly in this study area, relative to stochastic processes. Based on the results of the net relatedness index (NRI) and nearest taxon index (NTI), we inferred that historical and evolutionary factors, such as climate change and local diversification, may have similar effects on microbial community structure based on the climatic niche conservatism. Based on the results of a functional traits analysis, we found that the effects of ongoing ecological processes on the microbial community assembly varied among sites. Therefore, the functional structures seemed to be more related to ongoing ecological processes, whereas the phylogenetic structures seemed to be more related to historical and evolutionary factors, as well as the tradeoff between deterministic and stochastic processes. The functional and phylogenetic structures were mainly shaped by different ecological processes. By integrating multiple ecological processes, our results provide more details of the mechanisms driving the community assembly.

Key words: ecological process; community assembly; phylogenetic structure; functional traits; soil microbial community

Introduction

Understanding the fundamental ecological mechanisms that drive the assembly process of microbial

communities is a major challenge in community ecology [1]. The assembly process of the microbial community in a local community is generally influenced by two types of ecological processes, namely deterministic and stochastic processes. The deterministic process hypothesizes that deterministic factors such as organism traits, interspecies relationships (e.g., competition, predation, mutualisms), and environmental stresses (e.g., pH, temperature, salt, and moisture) govern community succession [2-4]. For example, ecologists have traditionally tended to consider that the environmental context determines the assembly process of microbial communities: "Everything is everywhere, but the environment selects" [5]. For example, environmental factors such as pH [6], temperature [7], and nitrogen levels [8] may be major determinants of microbial community structure. However, there is no doubt that interspecies relationships may also be an important force that influences community structure and dynamics [9]. Although ecologists accept that competition and environmental processes act simultaneously [10], biogeographic patterns have usually been ascribed to environmental filtering alone [11]. Little attention has been paid to the relative contributions of competition and environmental stress.

For the other types of community assembly processes (i.e., stochastic processes), it is assumed that community structures are independent of organism traits and are governed by birth, death, colonization, extinction, drift, and speciation [12], and it is hypothesized that species are all ecologically equivalent [13]. It has recently been accepted that the two ecological processes are not mutually exclusive, but rather form a continuum [14]. However, Clark [11] argued that stochasticity could occur only in mathematical models and not in nature, and therefore questioned the universality of the continuum hypothesis [12]. Therefore, to interpret a global map of bacterial diversity patterns, more studies are necessary to characterize the biogeographic patterns and assembly processes in different environmental contexts or conditions.

Communities at different stages of succession [15] or in different sub-communities[16] are driven by

different assembly processes. Most studies of ecological mechanisms have been limited to specific spatial or temporal scales [6, 17-19]. For example, stochastic processes may dominate microbial community assembly within successional stages, while deterministic processes may prevail during the transition periods between successional stages. Moreover, some of the conclusions reached in typical examples do not apply to all environmental contexts [20]. This may be related to the mixing of multiple ecological processes and the one-sidedness of analytical methods.

The biogeographic patterns of the community are the aggregate of multiple ecological processes (e.g., the evolutionary process [21, 22] and ongoing ecological processes [23]) operating on multiple axes. This aggregation severely complicates the identification of causal relationships in a local community [20]. Therefore, the aggregation of multiple ecological processes makes it difficult to draw inferences about the community assembly [20].

On the other hand, the one-sidedness of analytical methods also makes it difficult to draw inferences about the ecological processes. Ecologists investigating the community assembly process mainly rely on community β-diversity [2, 24-26], phylogenetic structure [6, 27], and functional traits [28]. First, the β-null deviation measure can be used to create stochastically assembled communities from the regional species pool and investigate the degree to which the observed β-diversity patterns deviate from the stochastic assembly [2, 25, 26]. However, it is difficult to precisely and robustly disentangle the different multiple ecological processes structuring communities [26]. Second, the net relatedness index (NRI) [29] and nearest taxon index (NTI) are two important indexes that can be used to characterize phylogenetic structure. Both the NRI and NTI increase with increasing clustering and become negative with over-dispersion [29]. However, little attention has been given to the differences or relationships between NRI and NTI. In most cases, this approach completely relies

on phylogenetic relatedness being a strong proxy of ecological similarity. The result of this is the aggregation of multiple processes. Thus, it is difficult to draw inferences of the complete community assembly process. Finally, the functional traits should be closely linked to ongoing ecological processes [30]. Functional trait analyses have followed an almost identical trajectory to the phylogenetically-based analyses [28, 30]. However, the measured traits cannot represent the whole functioning of an organism. It would be incorrect to describe the process of community assembly only according to functional traits. In essence, the different analytic methods infer the process of community assembly based on different perspectives. Therefore, by integrating these three analytical methods, more information is provided regarding the biogeographic distribution patterns of the community.

In this study, soil was sampled from 23 soil plots in subalpine coniferous forests located on the Loess Plateau in Shanxi Province, China. Microbial communities have a high taxonomic and metabolic diversity [31, 32], and perform important ecological functions [33]. Thus, microorganisms are ideal research objects for the study of community assembly mechanisms. The 16S ribosomal RNA genes of bacteria were analyzed using high-throughput sequencing. Linking data on soil microbial communities to data on the community turnover rate, historical or evolutionary factors, and ongoing ecological processes to investigate the community assembly process may provide more evidence of the biogeographic distribution patterns of a community. Specifically, we aimed to (i) quantify the relative roles of deterministic and stochastic processes in bacterial community dynamics; (ii) disentangle the relative importance of environmental filtering and interspecific relationships on the community assembly process; and (iii) determine the effects of historical or evolutionary factors and the ongoing ecological processes on the assembly of microbial communities.

1. Materials and methods

2.1 Study site and sampling

A total of 23 soil plots were sampled (Figure 1) in August 2016 and 2017. The plots were located in subalpine coniferous forests at an altitude between 1900 and 3055 m above mean sea level. In addition, the distance between the samples of each plot was more than 50 m. All samples were collected from the 0–10 cm soil horizon. Soil samples were sealed in plastic bags and refrigerated, immediately transported to the laboratory, and sieved through a 2-mm mesh. The soil samples were stored at -80°C prior to analysis.

The soil samples were subsampled for a molecular analysis, with the extraction of 1 g of soil using an E.Z.N.A. ® Soil DNA Kit (Omega Bio-tek, Inc., Norcross, GA USA). The quality and quantity of DNA extracts were then measured by an Infinite 200 PRO plate reader (Tecan, Männedorf, Switzerland). The DNA purity was assessed by a determination of the A260/A280 absorbance ratios, and only DNA extracts with absorbance ratios of 1.8–2.0 were used in further analyses. Three DNA samples were extracted from each soil sample and were then mixed and sequenced on the Illumina MiSeq sequencing platform (Majorbio Biotechnology Co., Ltd.,Shanghai, China) in the bacterial v3-v4 hypervariable region using the bacterial 16S universal primers (341F 5′-ACTCCTACGAGGAGCA-3′ and 805R 5′- TTACCGCGGCTGCTGGCAC -3′) [34].

2.2 Bioinformatics analysis

The sequencing data were analyzed by the QIIME (v1.8.0, http://qiime.org/) pipeline [35]. The filtered sequence alignments were denoised by DeNoiser [36] and then screened for chimeras by UCHIME [37]. The Eukaryota, Archaea, and unknown sequences were removed. The sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level by the average neighbor method and taxonomy was blasted to the SILVA database by the k-mer searching method using MOTHUR [38]. The OTU table was rarefied to 4020 sequences per sample. Ten independent maximum-likelihood phylogenetic trees, with the Jukes-Cantor distance, were then constructed using FastTree2 [39] after removing gaps and hypervariable regions using a Lane mask

127 wrapped within QIIME to support the phylogenetic diversity (pd) calculations.

128 **2.3 Environmental variables**

129 In the laboratory, soil total carbon (TC), total nitrogen (TN) and total sulfur (TS) were measured using an
130 elemental analyzer (Vario EL/ MACRO cube, Elementar, Hanau, Germany); nitrate nitrogen (NO_3^-_N),
131 ammonium nitrogen (NH_4^+_N), and nitrite nitrogen (NO_2^-_N) were measured using an automated discrete
132 analysis instrument (CleverChem 380, DeChem-Tech. GmbH, Hamburg, Germany). After shaking the soil :
133 water (1:2.5 mass/volume) suspension for 30 min, the soil pH was measured by a pH meter (Hl 3221, HANNA
134 Instruments Inc., Woonsocket, RI, USA). The soil organic carbon in each soil sample was measured by the
135 potassium dichromate volumetric method [40].

136 **2.4 Null model analysis**

137 The null model accounted for such changes in β-diversity, while controlling for stochastic variation and
138 associated changes in α-diversity (i.e., local species richness) [25]. We considered the null deviation to be the
139 relative difference between the observed β-diversity and the null-model β-diversity, $\beta_{obs} - E(\beta_{null})$, and the β-
140 diversity was measured as the Sorenson-Czekanowski dissimilarity [26]. As such, null deviation values may
141 represent communities that are more similar than expected by chance (a negative null deviation value), less
142 similar than expected by chance (a positive null deviation value), or close to the chance expectation (values near
143 zero). The detailed calculation process is provided in previous studies [15, 26, 41, 42].

144 **2.5 Phylogenetic analysis**

145 The pd was determined with the method of Stegen et al. using the picante library for R [43]. The NRI and
146 the NTI were used to quantify the phylogenetic structure. The NRI measures the mean pairwise phylogenetic
147 distance between all species or individuals in a sample (MPD), while the NTI measures the mean phylogenetic

distance between a species or individual and its closest relative mean nearest taxon distance (MNTD), in both cases adjusting for the null-model expectation by random sampling from a species pool. They are calculated as follows:

$$-1 \times \frac{r_{obs} - mean(r_{rand})}{sd(r_{rand})}$$

where r_{obs} is the observed NRI/NTI and r_{rand} is the MPD/MNTD from a null model, which is built by permuting the species labels across a phylogeny covering all species in a given species pool and using the "taxa labels in phylum level" null model in picante, to preserve the community structure and achieve a reliable randomization [44]. Positive values represent phylogenetic clustering, whereas species in the community are more closed related than expected. The negative values indicate phylogenetic over-dispersion, where species in the community are more distantly related than expected.

2.6 Functional attributes

Functional community structure was calculated based on a single functional trait of key importance in microbial communities, e.g., niche breadth [45]. This was because the niche breadth was the only trait broadly available for the species studied. The formula below was used:

$$B_j = \frac{1}{\sum_{i=1}^{N} P_{ij}^2}$$

where B_j indicates niche breadth and P_{ij} is the relative abundance of species j present in habitat i [46, 47]. Niche breadth is the sum of all the resources that can be used by organisms. The abundance of a given bacterial species is the result of the balance between its growth rate and loss factors [48]. Thus, the niche breadth can reflect important functional information of the community.

A functional traits analysis was conducted in the same way that the NRI was calculated [49], but using the

functional niche breadth-based dendrogram. A similarity distance matrix for the niche breadth of all species was then constructed, comparing the niche breadth values of all pairs of species using the Euclidian distance. Subsequently, we conducted a cluster analysis (i.e., a complete linkage method that identified similar clusters) of this distance matrix and constructed a dendrogram based on the results of the cluster analysis. Finally, functional trees were calculated.

2.7 Statistical analysis

All statistical analyses were performed in the R environment using the vegan, gplots, ggpubr. and corrplot packages. A Venn diagram was used to show the shared OTUs among the sites. A correlation matrix graph was used to demonstrate the correlation between soil physicochemical factors. A multivariate regression tree (MRT) analysis was used to explain the relationship between bacterial α-diversity estimates and environmental variables in a visualized tree, and the diversity indices were normalized to the same mean before performing the MRT analysis [50]. To test the effects of soil physical and chemical factors on NRI across all datasets, we used a generalized additive mixed model (GAMM). The GAMM was fitted using the "gamm" function in the "mgcv" R package. A combination of soil physicochemical data and community matrices were used in a redundancy analysis (RDA) to visualize the effect soil physicochemical properties on the structure of soil microbial communities (Hellinger-transformed data) using the vegan package in R. The forward selection of the principal coordinates of neighbor matrices (PCNM) variables based on permutation tests was chosen to identify two of the 23 extracted PCNM variables, which could significantly (P <0.05) explain the spatial structure. The PCNM eigenfunctions, which represent the 'spectral decomposition of the spatial relationship across sampling locations', were considered to be the spatial variables in the ordination-based analysis. The contributions of environmental filtering and the space variable (PCNM) to the variation of the bacterial community composition

were calculated using the variance partitioning analysis (VPA) (CANOCO for Windows Version 5.0).

Results

Physicochemical properties of the soils at different sites

The soil physicochemical properties varied across the different sampling sites (Figure 2). Briefly, the ammonium nitrogen and nitrite nitrogen concentrations were highest in LY sites and lowest in WT sites (P <0.05). The nitrate nitrogen, SOC, TC, and TN concentrations were highest in WT sites and lowest in LY sites (P <0.05). TN was significantly positively correlated with TC and SOC (P <0.05) and significantly negatively correlated with pH (P <0.05). TC and pH were significantly negatively correlated (P <0.05). SOC was significantly positively correlated with nitrate nitrogen (P <0.0.5), and was significantly negatively correlated with nitrite nitrogen (P <0.05). Ammonium nitrogen was significantly negatively correlated with ammonium nitrogen, nitrate nitrogen, and nitrite nitrogen (P <0.05). The difference in the environmental factors formed an ecological gradient along the different sites. Based on this ecological gradient, the study aimed to investigate the assembly process of the bacterial community by integrating multiple analytical methods.

Dynamics of the bacterial community composition and diversity

A total of 4258 OTUs were identified by 1,062,241 high-quality sequences recovered from 23 soil samples. Good's coverage index ranged from 95.19 to 99.75%, indicating that the sequences identified represented the majority of the bacterial sequences in the soil samples.

As shown in the Venn diagram, 869 bacterial shared OTUs were observed from all sampling sites. There were 46 bacterial phylum identified (Figure 3). There were 15 bacterial phylum with a relative abundance of more than 0.01%. The highest abundance at all sites was recorded for *Proteobacteria* (mean relative abundance = 30.59%), followed by *Acidobacteria* (19.63%), *Actinobacteria* (16.51%), and *Chloroflexi* (13.22%). The mean

relative abundance of *Proteobacteria* was highest in PQG sites (34.39%), while the abundance of *Actinobacteria* was highest in LY sites (26.29%). The mean relative abundances of *Acidobacteria* (28.68%) and *Chloroflexi* (16.09%) were highest in WT sites. There were 20 bacterial classes with a relative abundance of more than 0.01% in the study area. The sample plots for each site could be roughly clustered together (Figure 3d).

The community α-diversity indices varied at the different sites (Figure 4). Briefly, the pd and the number of observed species (sobs) were greater in WT sites (P <0.05). There was no significant differences in the Ace, Chao, Shannon, and Simpson indexes among the different sites (P >0.05).

Effects of environmental factors on microbiome dynamics

Redundancy analyses were used to identify the abiotic environmental drivers that influenced bacterial community composition. The results demonstrated that *Proteobacteria* and *Cyanobacteria* were mainly shaped by pH, while SOC, TC, and TN were the main abiotic drivers of *Parcubacteria* and *Planctomycetes* (Figure 5). In addition, SOC made the largest contribution to the microbial community structure (i.e., the arrow had the longest length). From the MRT analysis (Figure 6), we found that normalized diversity estimates were mainly split by SOC, which explained 36.75% (in the first spilt), followed by pH (6.68%).

The variation partitioning analysis showed that environmental factors (20.3%) and the spatial variables (1.9%) were minor contributors to the bacterial biogeographic distribution pattern, because there was a 78.6% contribution from an unexplained variable (Figure 7).

Because the NRI is a standardized measure of the mean pairwise phylogenetic distance of taxa in a given sample [29] and its calculation relies on both phylogenetic and species abundance information. The NRI can effectively reflect the process of community assembly. To investigate the effect of environmental factors on the NRI across all datasets, we used a GAMM (Table 1). Our results showed that SOC had a slight (the estimate

231 was 0.004) but significant (P <0.05) effect on the NRI.

232 **Nonrandom co-occurrence patterns of the microbial community**

233 Network analysis was applied to explore interspecific relationship patterns in the complex microbial
234 communities. The results demonstrated that the number of edges (595675), vertices (4014), and the average
235 degree (296.799) were greater in the WT sites (Figure 8). The diameter (5) and modularity (0.975) were greater
236 in the LY site. The significant and strongly correlated OTUs were mainly distributed in the different modules in
237 the network. The modules were more frequently observed in PQG sites (9), followed by WT sites (7) and LY
238 sites (6). A module is a group of OTUs that are highly connected within the group, but with very few connections
239 outside the group [51]. Thus, we considered that the changes of modules represented changes in interspecific
240 relationships.

241 **The bacterial community assembly process**

242 According to the null model analysis, our results demonstrated that the null deviation values varied at
243 different sites (ranging from 0.29 to 0.57) (Figure 9). Positive null deviation values can represent communities
244 that are more dissimilar than the null expectation [2, 25]. The bacterial communities in WT sites deviated
245 significantly from the null expected value (relative null deviation = 0.45) and there were more communities in
246 WT sites than in the LY and PQG sites (relative null deviation = 0.32 and 0.34) (P <0.05).

247 The functional community structure ($NRI_{(FUN)}$) varied among the different sites (ranging from -1.02 to -
248 0.31,) (P >0.05). The $NRI_{(FUN)}$ was negative, indicating a traits divergence in communities. The ($NRI_{(FUN)}$) was
249 lowest in WT sites (-0.75).

250 The NRI (ranging from -0.94 to -0.38) and NTI (ranging from -1.04 to -0.46) varied among the different
251 sites (P >0.05). Both the NRI and NTI were negative, indicating over-disperse phylogenetic patterns. There were

no significant differences between the NRI and NTI (P >0.05).

Disscussion

Microorganisms typically form diverse communities of interacting species, whose activities have a tremendous impact on the plants, animals, and humans they associate with [52]. The mechanism or ecological processes that drives the structure of these complex communities is crucial to understanding and managing them. The integration of multiple ecological processes can provide more clues for drawing the biogeographic patterns of communities. The results of this study indicate that the ongoing ecological processes and historical or evolutionary factors, as well as the trade-off between deterministic and stochastic processes, jointly drive the assembly processes of the soil microbial community in subalpine coniferous forests on the Loess Plateau, China. By integrating multiple analytical methods, the one-sidedness of a single method can be avoided and a more scientific and accurate conclusion can be drawn. There are some inconsistencies in the results obtained for the same microbial community data when using different analytical methods, and this may be the main reason why the universality of ecological mechanisms is often challenged. What these conclusions have in common is that interspecific relationships are driving factors in the process of community assembly.

Trade-off between deterministic and stochastic process in driving the community assembly process

In the null model analysis, the degree of deviation from the random expectation is understood to reflect community assemble processes through environmental filtering (negative values; communities less dissimilar than expected by chance) or competitive interactions (positive values; communities more dissimilar than expected by chance) [2, 26]. The large deviations from the random expectation could be interpreted as reflecting communities structured by deterministic assembly mechanisms [26]. The results demonstrated that null deviation values varied among sites (P <0.05); thus, we inferred that the trade-offs between deterministic and stochastic

processes drove the composition of microbial communities in the study area [25, 26]. Previous studies have confirmed that the trade-off could be dependent on varying environmental conditions or the characteristics of organisms [53]. The null deviation in WT sites significantly deviated from the stochastic assembly model to a greater extent than for the other two sites, indicating a stronger deterministic process. From the VPA, the spatial variables (1.9%) were found to be the minimal contributor to the bacterial biogeographic distribution pattern, indicating the minimal role of stochastic processes. Therefore, we inferred that a deterministic process was predominant for governing the biogeographic distribution patterns of the microbial community in current study.

Relative to environmental filtering, interspecific relationships dominate the biogeographic patterns of microbial communities

Environmental factors, such as salinity [54], pH [55, 56], C/N ratio [57], soil C [58], soil N [59], and the structure of the plant community [60] may be major determinants of microbial community structure. Our results demonstrated that pH, SOC, TC, and TN were the main abiotic drivers of microbial community composition. More importantly, it was SOC that had the most significant effects on community diversity (MRT analysis), structures (RDA), and NRI (GAMM). We observed that SOC was significantly different at different sites, and was significantly correlated with nitrate nitrogen, nitrite nitrogen, and TN (P <0.05). Thus, SOC was found to be closely related to many soil environmental factors and had the highest weighting. Along the northern slope of the Changbai Mountains, the SOM decomposition rate had a significant positive relationship with the total microbial, bacterial, and *Actinomycetes* PLFAs and soil enzyme activity [61]. Thus, SOC was closely related to microbial community structure, composition, and diversity [62], and was therefore related to the community assembly process.

The VPA showed that environmental factors and spatial variables were minor contributors to the bacterial

biogeographic distribution pattern, explaining only 21.4% of the total variation. The sample plots used in this study were established under subalpine coniferous forest. The environmental context of subalpine regions includes pronounced climatic gradients and climosequences within short distances, with a high level of environmental heterogeneity [17]. However, crowded coniferous forests can block most of the sunlight and reduce wind, reducing environmental heterogeneity. In addition, the current study was initiated in a subalpine coniferous forest soil, where the composition of litter was relatively simple. Although the sites were different, there was little variation in the aboveground vegetation (dominant species: C. *breviculmis*, *Stipa capillata* Linn.). This could be the reason why environmental filtering made only a minor contribution to the bacterial biogeographic distribution pattern.

The unexplained variation in VPA (78.6%) could also be due to unmeasured environmental variables and unincorporated neutral factors. We inferred that this was more related to the interspecific relationship.

We observed that the null deviations were positive in the null model, which were interpreted as showing competitive interactions within the community, because communities were more dissimilar than expected by chance [2, 25]. In addition, we also observed an over-dispersion of phylogenetic patterns (NRI and NTI) and trait divergence in communities ($NRI_{(fun)}$) [10]. By integrating the results of the three analytical methods, we inferred a consistent conclusion that interspecific relationships were the driving factor of community assembly processes rather than environmental filtering. The driving effect of interspecific relationships in the process of community assembly can be represented by the changes of modules in the network analysis (Figure 8).

Many analytical methods can be used to separate the relative roles of competition and abiotic filtering, but their distinction is frequently fuzzy. This may be because competitive interaction and environmental stress act synchronously, as suggested by the existence of a balance between stress tolerance and nutrient access [11].

315 Many ecologists tend to appreciate that environmental filtering is the dominant process in community assembly,
316 because such conditions have traditionally been ascribed to environmental filtering alone in most cases [9].
317 However, it is not correct to ignore the roles of interspecific relationships to infer the process of community
318 assembly. Many studies have confirmed the importance of interspecific relationships. For microorganisms,
319 competition is most important under conditions of high resource availability while abiotic filtering prevails
320 during periods of high environmental stress [63]. For macroorganisms, the interactions due to competition were
321 more important than the regional climate in governing long-term changes in tree mortality [64]. In the current
322 study, the dense coverage of coniferous forest litter on the surface of the soil formed an unventilated
323 environment, which was conducive to the accumulation of soil nutrients. This nutrient accumulation promoted
324 substrate availability. The high resource availability then accelerated interspecies competition [63].
325 **Effects of historical factors and ongoing ecological processes on community assembly**
326 The phylogenetic patterns could reflect the imprints of evolutionary and biogeographic history on
327 community structure [65]. The NRI primarily reflects the structure in deeper parts of the phylogeny, while NTI
328 mainly reflects the shallow parts of the phylogeny [29, 49]. For example, previous studies found that broad scale
329 deep-time intercontinental migration (inferred by the NRI index), together with climatic niche conservatism,
330 appear to influence the tree community phylogenetic structure in East Asian forests, with a shallow phylogenetic
331 imprint of local diversification (inferred by the NTI index) [27]. This may be because patterns of relatedness,
332 where related taxa have disjunctive occurrences, often occur at the genus or higher taxonomic levels and could
333 reflect historical factors (migrations that occurred millions of years ago) [66]. Climatic conservatism is a
334 necessary component in maintaining such disjunctions [27]. The results of the current study demonstrated that
335 there were no significant differences between the NRI and NTI, indicating little difference between the deeper

and the shallow parts of the phylogeny. We therefore inferred that historical and evolutionary factors, such as the paleoclimate, current climate, and local diversification, have similar effects on microbial community structure based on climatic niche conservatism [67, 68]. This may be because there is no significant difference between the paleoclimate and current climate, or that the difference between the two is not the driving factor in the process of microbial community assembly.

The functional traits should be directly linked to ongoing ecological processes [30]. Because niche breadth is the sum of all the resources that can be used by organisms, it is an ideal proxy of the functional traits. The functional community structure (NRI_{fun}) also showed functional dispersal patterns (traits divergence) [69]. The effects of ongoing ecological processes on microbial community assembly were greater in WT sites. Both the null deviation and the NRI_{fun} were greater in WT sites, which is probably related to the larger elevation gradient in WT sites (Table S1). The ongoing ecological processes may be related to the community turnover. The functional and phylogenetic structure were shaped by divergent processes, which is consist with the results of previous studies [27, 28].

Conclusion

The most important finding in this study was that deterministic processes (especially interspecific relationships) drove the bacterial community assembly in subalpine coniferous forests on the Loess Plateau, China. Historical and evolutionary factors, such as the paleoclimate and current climate, had similar effects on microbial community structure based on climatic niche conservatism. The effects of ongoing ecological processes on microbial community assembly were largest in WT sites. The functional and phylogenetic structures were shaped by divergent processes. The results of this study will improve our understanding of the trade-off between deterministic versus stochastic process in bacterial community assemblages and the shaping

of bacterial biogeography from multiple dimensions.

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The bacterial sequences have been deposited in the SRA of the NCBI database under Accession No. SRP135838.

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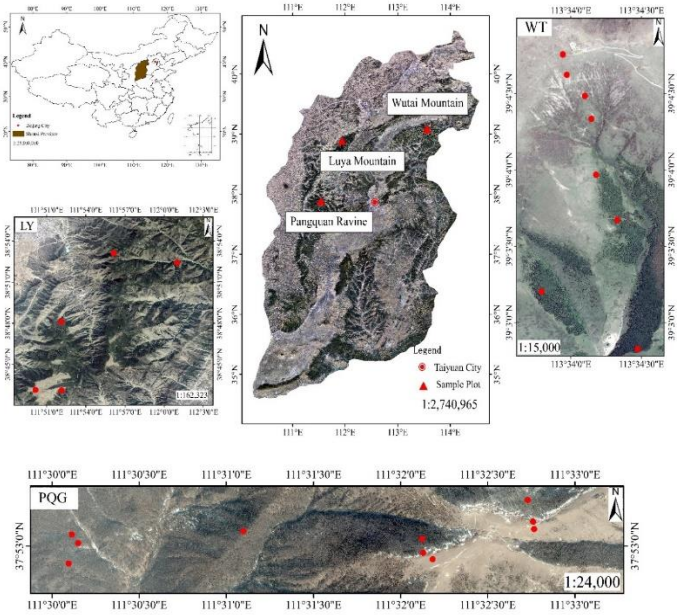
521

Figure 1(on next page)

Figure 1 Geographic distribution of the 23 sampling plots located on Loess Plateau, China

WT: Wutai mountain; LY: Luya mountain; PQG: Yunding mountain located on Pang Quangou National Nature Reserve


Wutai Mountain
Luya Mountain
Pangquan Ravine
Legend
Taiyuan City
Sample Plot
1:2,740,965
WT
1:15,000
LY
1:62,223

PQG
1:24,000

Figure 2(on next page)

Figure 2 Barplots (a) show the soil physicochemical factors of each sites. Correlation matrix graph (b) shows the correlation between soil physicochemical factors

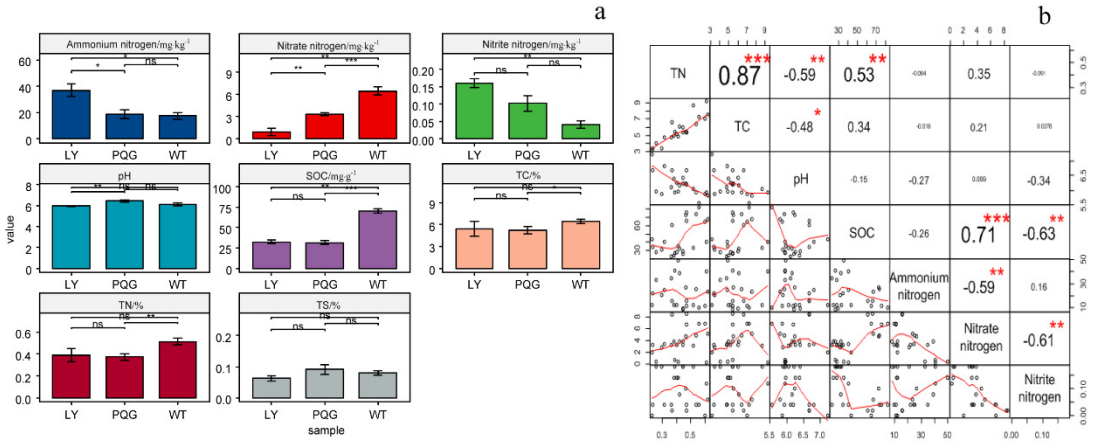

a
Ammonium nitrogen/mg·kg-1
Nitrate nitrogen/mg·kg-1
Nitrite nitrogen/mg·kg-1
pH
SOC/mg·g-1
TC/%
TN/%
TS/%
value
LY
PQG
WT
sample
b
TN
0.87
-0.59
0.53
0.35
TC
-0.48
0.34
0.21
pH
-0.15
-0.27
-0.34
SOC
-0.26
0.71
-0.63
Ammonium nitrogen
-0.59
0.16
Nitrate nitrogen
-0.61
Nitrite nitrogen

Figure 3(on next page)

Figure 3 Relative abundance of the dominant bacterial phylum (a) and class (c) across the sites. Venn Diagram (b) showed the shared OTUs in all plots. The heat map (d) shows clustering patterns in different plots

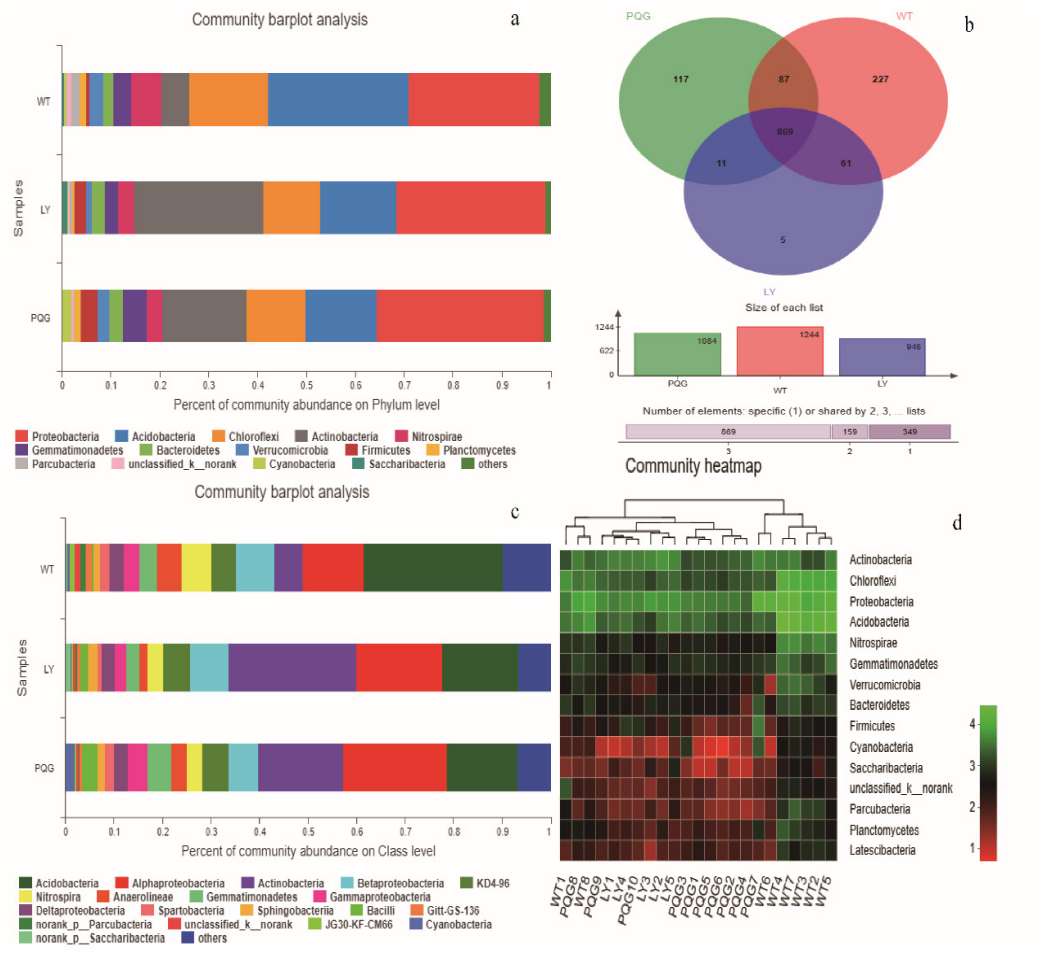

Community barplot analysis
a
WT
LY
PQG
Samples
Percent of community abundance on Phylum level
Proteobacteria
Acidobacteria
Chloroflexi
Actinobacteria
Nitrospirae
Gemmatimonadetes
Bacteroidetes
Verrucomicrobia
Firmicutes
Planctomycetes
Parcubacteria
unclassified_k__norank
Cyanobacteria
Saccharibacteria
others
PQG
WT
LY
117
87
227
869
11
61
5
b
Size of each list
1244
622
0
1084
1244
946
PQG
WT
LY
Number of elements: specific (1) or shared by 2, 3, ... lists
869
159
349
3
2
1
Community barplot analysis
c
WT
LY
PQG
Samples
Percent of community abundance on Class level
Acidobacteria
Alphaproteobacteria
Actinobacteria
Betaproteobacteria
KD4-96
Nitrospira
Anaerolineae
Gemmatimonadetes
Gammaproteobacteria
Deltaproteobacteria
Spartobacteria
Sphingobacteriia
Bacilli
Gitt-GS-136
norank_p__Parcubacteria
unclassified_k__norank
JG30-KF-CM66
Cyanobacteria
norank_p__Saccharibacteria
others
Community heatmap
d
Actinobacteria
Chloroflexi
Proteobacteria
Acidobacteria
Nitrospirae
Gemmatimonadetes
Verrucomicrobia
Bacteroidetes
Firmicutes
Cyanobacteria
Saccharibacteria
unclassified_k__norank
Parcubacteria
Planctomycetes
Latescibacteria
4
3
2
1
WT1
PQG8
WT8
PQG9
LY7
LY4
PQG10
LY3
LY2
LY5
PQG3
PQG1
PQG5
PQG2
PQG4
PQG7
WT6
WT4
WT3
WT2
WT5

Figure 4(on next page)

Figure 4 Bacterial community diversity on different sites

ace: ACE index; chao: Chao index; shannon: Shannon index; simpson: Simpson index

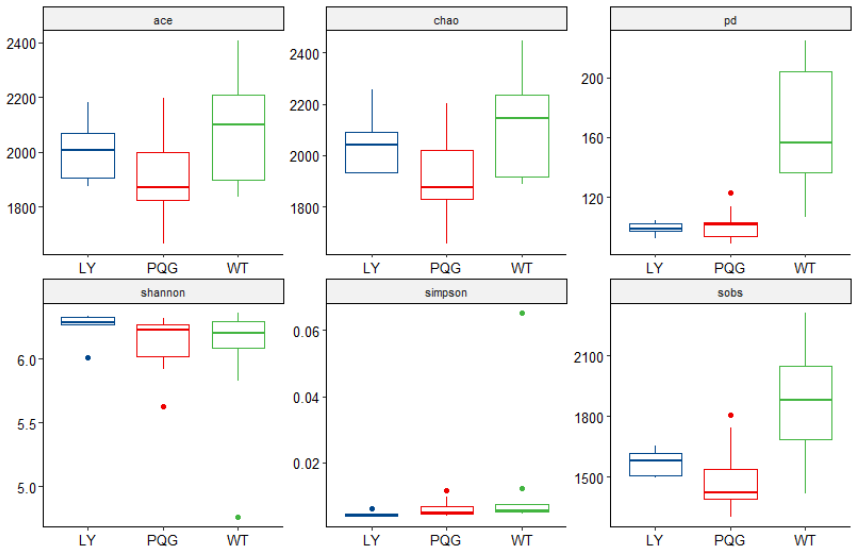

ace
chao
pd
shannon
simpson
sobs
LY
PQG
WT
2400
2200
2000
1800
200
160
120
6.0
5.5
5.0
0.06
0.04
0.02
2100
1800
1500

Figure 5(on next page)

Figure 5 Redundancy analysis (RDA) plots of bacterial communities and the response of these communities to significant soil physicochemical properties

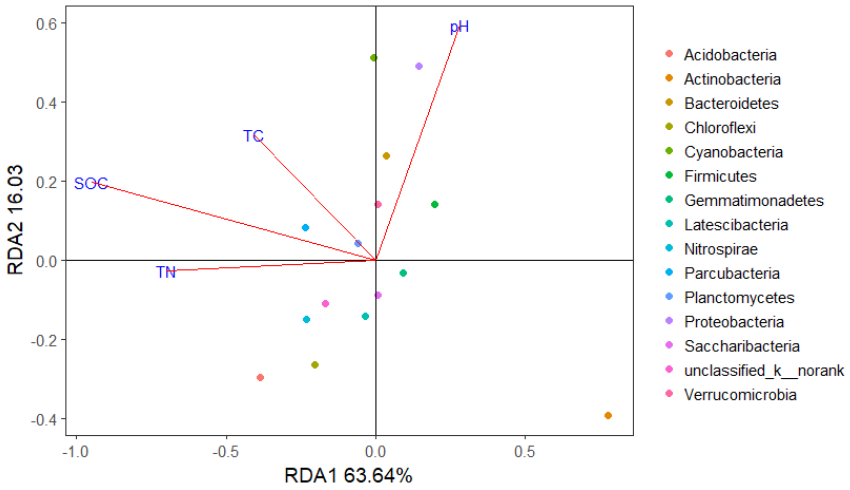

pH
TC
SOC
TN
RDA2 16.03
RDA1 63.64%
0.6
0.4
0.2
0.0
-0.2
-0.4
-1.0
-0.5
0.0
0.5
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Cyanobacteria
Firmicutes
Gemmatimonadetes
Latescibacteria
Nitrospirae
Parcubacteria
Planctomycetes
Proteobacteria
Saccharibacteria
unclassified_k__norank
Verrucomicrobia

Figure 6(on next page)

Figure 6 Multivariate regression tree (MRT) of bacterial α-diversity data associated with key environmental factors

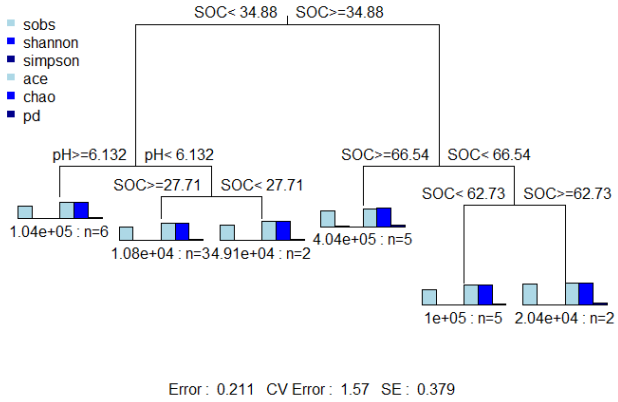
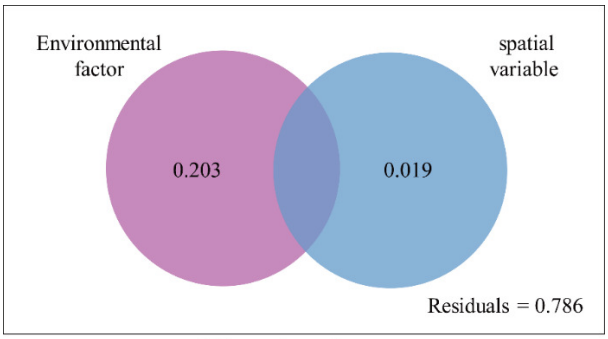

sobs
shannon
simpson
ace
chao
pd
SOC< 34.88
SOC>=34.88
pH>=6.132
pH< 6.132
SOC>=66.54
SOC< 66.54
SOC>=27.71
SOC< 27.71
SOC< 62.73
SOC>=62.73
1.04e+05 : n=6
1.08e+04 : n=3
4.91e+04 : n=2
4.04e+05 : n=5
1e+05 : n=5
2.04e+04 : n=2
Error : 0.211 CV Error : 1.57 SE : 0.379

Figure 7 (on next page)

Figure 7 Variation partitioning analysis showing the percentages of variance in bacterial communities explained by environment factor, spatial variable (PCNM)



Values <0 not shown

Figure 8(on next page)

Figure 8 Network of co-occurring OTUs colored by modularity class

A connection stands for a strong (Spearman's ρ > 0.6) and significant (p-value < 0.01) correlation network. For each panel, the size of each node is proportional to the betweenness centrality; the thickness of each connection between two nodes (edge) is proportional to the value of Spearman's correlation coefficients (> 0.6)

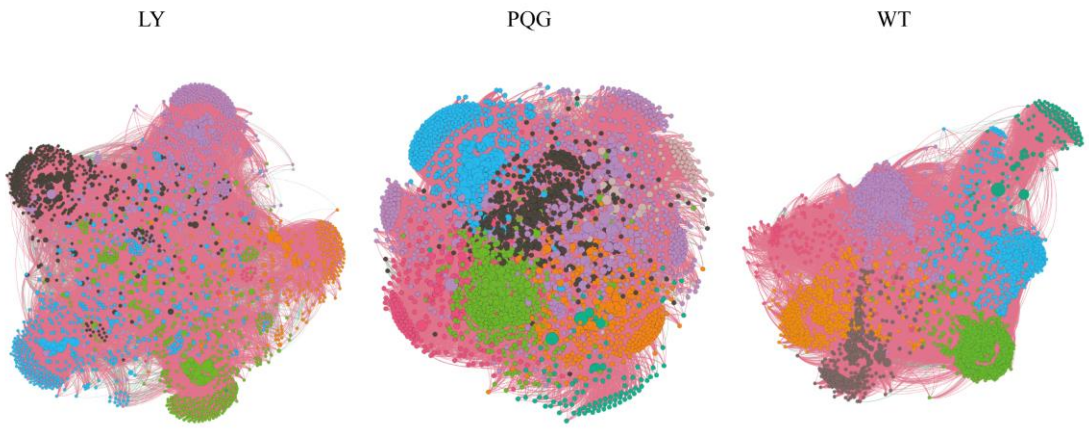

LY
PQG
WT

Table 1(on next page)

Table 1 Generalized Additive Mixed Model fitted to the NRI data across all sites

Table 1 Generalized Additive Mixed Model (GAMM) fitted to the NRI data across all sites

□	Estimate	SE	T	Pr(>|t|)
(Intercept)	-1.044	0.676	-1.546	0.141
TN	-0.129	0.578	-0.223	0.826
TC	0.014	0.034	0.411	0.686
pH	0.023	0.096	0.24	0.813
SOC	**0.004**	**0.002**	**2.472**	**0.024 ***
PCNM	0.0001	0.0001	1.158	0.263

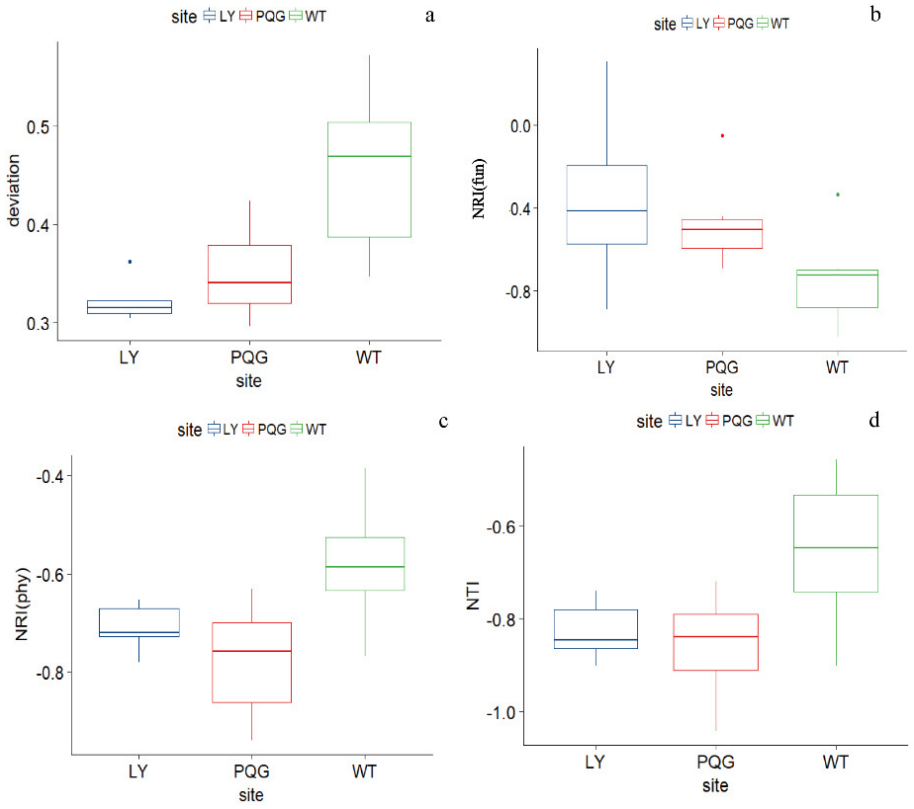
SE: standard error

1

Figure 9(on next page)

Figure 9 The community assembly preocesses in different sites

a: null deviation values; b:NRI(fun); c: NRI(phy); d: NTI


site LY PQG WT
a
deviation
0.5
0.4
0.3
LY
PQG
WT
site
b
NRI(fun)
0.0
0.4
-0.8
c
NRI(phy)
-0.4
-0.6
-0.8
d
NTI
-0.6
-0.8
-1.0