RESEARCH PAPER

Environmental filtering drives bacterial community structure and function in a subalpine area of northern China

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Microbial community assembly is affected by the trade-off between deterministic and stochastic processes, but the mechanisms underpinning their relative influences remain elusive. This knowledge gap strongly limits our ability to predict the effect of environmental filtering on microbial community structure and function. To improve the understanding of mechanisms underlying community assembly processes, we investigated bacterial community structure and function on a subalpine shady slope and a sunny slope in the Pangquangou National Nature Reserve in North China. By integrating the results of a null model and the RC metric, we inferred that a deterministic process, that is, environmental filtering, drove bacterial community biogeographical patterns. Edaphic factors caused the largest contribution to microbial community structure, followed by vegetation and spatial variables. Among edaphic factors, total carbon (TC) and total nitrogen (TN) were the most important factors as determined by redundancy analysis (RDA). Moreover, network analysis suggested that the status of bacterial community co-occurrence was significantly greater than that of exclusive relationships. Under environmental stress, there was no significant difference in the overall bacterial community structure on the different slopes, while significant differences were observed in relation to community functions. Given this, we inferred that the degrees of response of bacterial community structure and function to varying environments were not consistent. In conclusion, our results contribute to the understanding of deterministic versus stochastic balance in bacterial community assembly and the response mechanisms of community structure and function to environmental heterogeneity.

KEYWORDS
community assembly, environmental filtering, qPCR, soil microbial community, 16S rRNA

1 | INTRODUCTION

The relative importance of deterministic versus stochastic processes that underlie community dynamics has long been a central topic in ecology, albeit under different guises in different times [1,2]. The deterministic process of community assembly emphasizes that niche-based processes (such as environmental filtering and interspecific relationships) determine the presence/absence and relative abundance of species [3–6]. In contrast, the stochastic process asserts that the community assembly pattern is simply governed by neutral stochastic factors such as ecological drift, dispersal and speciation [7,8] and assumes that species are all ecologically equivalent [9]. An increasing number of ecologists appreciate that the two ecological processes are not mutually exclusive, but rather are a continuum [10]. This

continuum can be generally understood as the trade-off between the deterministic and the stochastic processes. Nevertheless, Clark [11], who argued that stochasticity could occur only in mathematical models and not in nature, questioned the universality of this continuum hypothesis [12]. Therefore, to test the hypothesis and to interpret a global map of bacterial diversity patterns, more efforts are necessary to characterize the biogeographic patterns and assembly processes in different environmental contexts and/or under different conditions.

Microbial communities have high taxonomic and metabolic diversity [7,13] and perform important ecological functions [6]. Thus, the assembly processes of soil microbial communities have attracted increasing interest recently [4,5]. However, there are still some controversies relative to the assembly mechanisms of soil microbial communities at different space-time scales [13]. For example, along the well-established glacier forefront chronosequence, a fungal community was found to be initially strongly governed by deterministic processes but less so later [14]. Stochastic processes may dominate microbial community assembly within successional stages while deterministic processes may prevail during transition periods between successional stages [15]. These studies confirmed the importance of the trade-off between the two ecological processes on community assembly processes [16,17], and this trade-off may be dependent on varying environmental conditions or the characteristics of organisms [13]. However, studies focused on the mechanisms of community assembly are limited to specific spatial and temporal scales or sampling scales, and lack of a common standard.

Microbial community composition can display complex variation across spatial or environmental conditions, such as through the ocean water column [18], or an altitudinal gradient in alpine forest soils [19], and this variation can have effects on ecosystem functions [20]. In addition, an increasing number of researches has confirmed that the potential metabolic functions of a microbial community is closely related to environmental conditions [21–23]. However, the mechanisms shaping these conditions remain poorly understood, since the aggregate of multiple mechanisms severely complicates the identification of causal relationships [20]. Therefore, the effect of influential mechanisms of environmental factors on microbial community structure and function needs more attention.

In this study, we investigated the microbial community structure and function on shady and sunny slopes of a subalpine area located in the Pangquangou National Nature Reserve in North China. The environmental stress in a subalpine area, such as altitudinal gradients, slopes, and vegetation types, can provide an ideal platform for insight into microbial community assembly mechanisms. Progress in our general understanding of the trade-off between deterministic versus stochastic processes in community ecology can be facilitated to allow for an improved ability to draw inferences about the ecological processes from various kinds of observations and experiments. The study presented herein was initiated to investigate: (i) the relative roles that deterministic and stochastic processes played in community assembly; (ii) how environmental filtering affected the microbial community structure and community function; and (iii) in terms of the degree that response to environmental filtering, whether the structure and function of the microbial community were consistent.

2 | MATERIALS AND METHODS

2.1 | Site description and soil sampling

Sampling was conducted in the Pangquangou National Nature Reserve (111°32′ E, 37°53′N) in August 2016 (Figure 1). Study sites were initiated on the timberline ecotones between 1950 and 2400 m above mean sea level. Samples were collected across a number of zones, with 18 samples in total. We sampled plots on both slopes of subalpine timberline ecotones with three replicates: in the meadow of a shady slope (SHM), in the timberline of a shady slope (SHT), under the forests of a shady slope (SHU), in the meadow of a sunny slope (SUM), in the timberline of a sunny slope (SUT), and under the forests of a sunny slope (SUU). The distance between each sampling plot was more than 50 m. All samples were collected from the 0–10 cm soil horizon. Soil samples were kept on ice when transported to the laboratory and were sieved through 2 mm meshes to remove roots and stones. Soil samples were preserved at −80 °C for further analysis.

2.2 | Soil biogeochemical and vegetation measurements

Soil temperature and soil respiration were measured in situ by Portable Gas Analysis System (Li-cor, Lincoln, USA) and Soil Respiration Chamber (Li-6400-09, USA). Soil moisture was measured by Portable Moisture Meter (Jia shi Technology, China). In the laboratory, soil total carbon (TC), total nitrogen (TN), and total sulfur (TS) were measured by using Elemental Analyzer (vario EL/MACRO cube, Germany); nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), and nitrite nitrogen (NO_2^--N) were quantified by Automated Discrete Analysis Instrument (CleverChem 380, Germany). Soil pH was measured using a pH Meter (Hl 3221, Italy) after shaking a soil water suspension (1:2.5 mass/volume) for 30 min. An area of 1×1 m was selected in each plot to measure vegetation attributes. Vegetation indices included total coverage, height, and abundance.
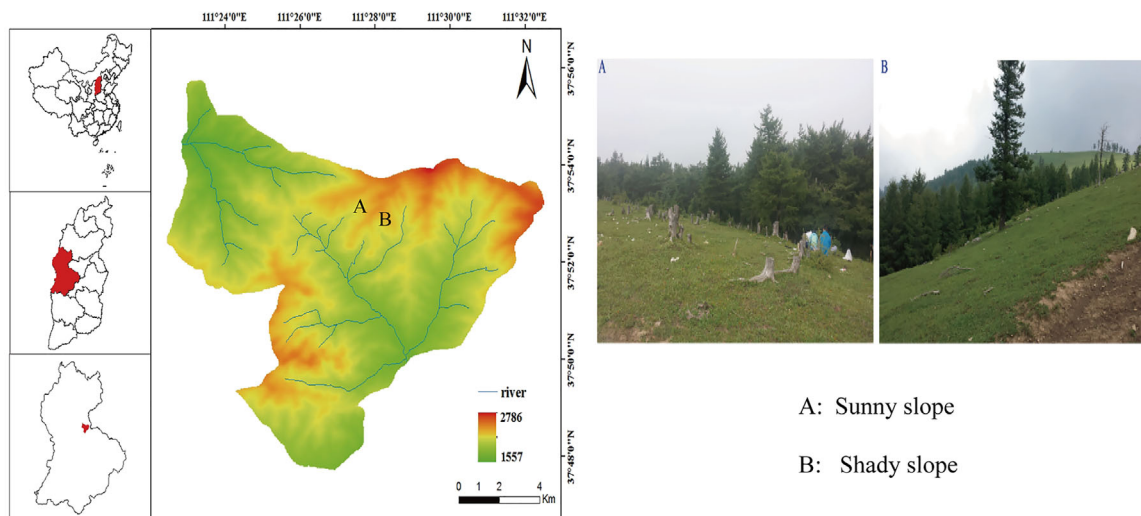
A: Sunny slope

B: Shady slope

FIGURE 1   Sketch map of sample plots. The subalpine timberline ecotone ranged from the canopy forest to the subalpine meadow

2.3 | Soil DNA extraction, purification, and quantitation

Soil DNA was extracted from 1 g soil by using the E.Z.N.A.®Soil DNA Kit (OMEGA, USA) (Supporting Information Figure S1). The quality and quantity of DNA extracts were measured using a Plate Reader Infinite 200 PRO (TECAN, Switzerland). Purity DNA was assessed by determination of A260/A280 ratios. Only DNA extracts with absorbance ratios of 1.8–2.0 were used for bacterial community analyses. The DNA samples from the same vegetation types were mixed into one and sequenced (i.e., a total 6 samples were sequenced).

2.4 | Nucleic acid sequences

High-throughput sequencing was conducted on the bacterial v3-v4 hypervariable region with universal 16S rRNA primers 341F and 806R by Shanghai Personal Biotechnology Co., Ltd using the Illumina MiSeq platform [23]. Sequencing data were analyzed by the QIIME (v1.8.0, http://qiime.org/) pipeline. Filtered sequence alignments were denoised by DeNoiser and then screened for chimeras by UCHIME [24]. Eukaryota, Archaea, and unknown sequences were removed. Sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level by the average neighbor method and taxonomy was assigned using Blast to the SILVA database by a k-mer searching method using MOTHUR. The information of sequencing is listed in the supplementary material (Supporting Information Table S1). Raw sequence data of bacterial 16S rRNA genes has been deposited into the NCBI GenBank under the study accession number SRP135838.

2.5 | Real time qPCR for quantification of abundance of functional genes

Four target genes were quantified, including bacterial 16S rRNA genes and three bacterial functional genes (i.e., *cbb*M [25], *amylase* [26], and *cellulose* [27]). The *cbb*M gene encodes key enzymes of the Calvin-Benson-Bassham (CBB) cycle [25]. The *amylase* gene encodes products within the α-amylase family, which consists of several enzymes that share a number of common characteristics. These include a parallel barrel structure and enzymatic reactions that act on α-glycosidic bonds that are hydrolyzed to yield α-anomeric mono- or oligosaccharides [26]. Cellulose-degrading enzymes (*cellulose*) have been described as members of the superfamily of glycoside hydrolases (GH) in at least ten GH families [27]. Abundances of these genes represent the potential functions of bacterial communities.

All qPCR assays were performed triplicate by using a CFX96 system (BioRad, USA). Template DNA concentrations and plasmid DNA concentrations were quantified by using Infinite M200 PRO (TECAN, Switzerland) and the DNA concentration of each sample was adjusted to a concentration of 10 ng µl^{-1}. Primers selected for qPCR quantification of the 16S rRNA, *cbb*M, *amylase*, and *cellulose* genes are listed in the supplementary material (Supporting Information Table S2), as are the qPCR reaction conditions (Supporting Information Table S3).

Gene copy numbers were calculated from standard curves. Standard curves were constructed from PCR amplicon products of target gene fragments extracted from agarose gel with a Gel Extraction Kit (TIANGEN, China). The purified product was connected to a pMD®18-T plasmid vector (Takara Bio, Dalian), and transformed into competent *Escherichia coli* DH5α cells. Transformant cells were plated

on agar plates supplemented with 1 ml AMP, 5 ml IPTG, and 0.8 ml X-Gal for each liter of Luria-Bertani (LB) and were incubated at 37 °C. Positive clones were sub-cultured into fresh LB. Plasmids were then extracted from the correct insert clones for each target gene and used as standards for quantitative analyses. Ten-fold serial dilutions of known copies of plasmid DNA were then subjected to qPCR in triplicate to generate an external standard curve. A dilution series of 10^8–10^3 gene copies was used as standards in each qPCR run. Efficiencies for the PCR reactions were 81.3% for *cbb*M, 79% for *amylase*, 86.8% for *cellulose*, and 101.1% for 16S rDNA.

2.6 | Statistical analyses

The β-diversity null deviation approach takes advantage of a null model to create stochastically assembled communities from the regional species pool in order to determine the degree to which the observed β-diversity patterns deviate from stochastic assembly [28,29]. Null deviation is the deviation between the observed value and the null expectation. Large deviations from the null expectation suggest a strong role for deterministic processes, whereas smaller deviations indicate that stochastic processes prevail [14,29]. Detailed process descriptions provided in Tucker et al. [28].

To identify more details in the roles and the weight of the different community assembly mechanisms, a slight modification of the Raup-Crick (RC) index was also used to disentangle the underlying assembly mechanisms [30]. The R script of the model used can be found at https://github.com/stegen/Stegen_etal_ISME_2013. The RC probability metric indicates whether local communities are more dissimilar (i.e., values closer to 1), dissimilar (i.e., values near 0), or less dissimilar (i.e., values closer to −1), than expected by random chance.

By analyzing and integrating the above two methods of community assembly processes, a new perspective can be proposed for understanding community assembly mechanisms.

Ecological analyses (e.g., species diversity analysis) were performed using the Vegan package (v2.4-1) in R. Principal coordinates analysis (PCoA) was used to visualize ordination of the microbial community structure. Redundancy analysis (RDA) was used to evaluate the link between microbial community composition and environmental attributes. The principal coordinates of neighbor matrix (PCNM) eigenfunctions, which represent the spectral decomposition of the spatial relationship across sampling locations, can be considered as the spatial variables in the ordination-based analysis. The PCNM was completed by the pcnm package in R. The partial least-square (PLS) method path modeling is typically referred to as the partial least-square method approach to structural equation modeling (SEM). This analysis revealed direct and indirect effects of different latent variables, and was completed by the plspm package in R. Network analysis was used to investigate the patterns of microbial communities and the interaction of species [31]. The package igraph was used to visualize the network relationships of dominant species. LDA Effect Size (LEfSe) is an algorithm for high-dimensional biomarker discovery and explanation that identifies taxa characterizing the differences between different slopes (http://huttenhower.sph.harvard.edu/galaxy/). The Venn diagram can be used to visualize the shared OTUs, and was completed by VennDiagram package in R. Analysis of similarities (ANOSIM) was used to determine whether there was a significant difference in the bacterial community structure or functional attributes on different slopes.

3 | RESULTS

3.1 | Soil physicochemical factors and above-ground vegetation

TC, TN, TS, the ratio of carbon and nitrogen (C/N), NH_4^+-N, NO_3^--N, and NO_2^--N significantly varied among sample plots ($p < 0.05$), indicating that soil fertility varied significantly in this area. Although the divergence in pH was significantly associated with sampling sites ($P < 0.05$) (Table 1), soil respiration was not. In addition, the diversity indexes of above-ground vegetation varied among different plots (Figure 2). The Shannon index was higher in SUU site (1.66) and in SHU site (1.64) than other plots, indicating that species richness of forest vegetation on both shady and sunny slopes were higher than any others. Variance of environmental factors formed an ecological gradient across different slopes. Based on this ecological gradient, this study aimed to explore the driving factors that governed the structure of the soil bacterial communities and community function.

3.2 | Distribution pattern of soil bacterial communities

A total of 733 OTUs were identified by 105,655 high-quality sequences recovered from six soil samples. Good's coverage ranged from 98.91% to 99.59%, indicating that sequences identified represent the majority of bacterial sequences in the collected soil samples. The OTU rarefaction curves are shown in Supporting Information Figure S2. In the rarefaction curves, when the curve tends to be flat, the number of samples can be considered sufficient and indicates that further samples would detect only a small amount of additional OTUs.

Both the Chao1 index and ACE index were higher in the SHM site (2705 and 3221.63, respectively), and lower in the SUM site (2127 and 3015.25, respectively) (Table 2). The

TABLE 1 Soil physicochemical characteristics among the different plots

Plots	SHM (shady slope meadow)	SHT (shady slope timberline)	SHU (shady slope forest)	SUM (sunny slope meadow)	SUT (sunny slope timberline)	SUU (sunny slope forest)
Elevation (m)	2552	2496	2473	2589	2566	2531
pH	$6.21 \pm 0.03ab$	$6.39 \pm 0.02a$	$6.15 \pm 0.06b$	$6.26 \pm 0.12ab$	$6.41 \pm 0.02a$	$6.04 \pm 0.15b$
Soil moisture (m^3/m^3)	$0.42 \pm 0.01ab$	$0.45 \pm 0.01a$	$0.43 \pm 0.02a$	$0.42 \pm 0.02ab$	$0.43 \pm 0.01a$	$0.38 \pm 0.01b$
N (%)	$0.4 \pm 0.01bc$	$0.44 \pm 0.03b$	$0.52 \pm 0.03a$	$0.45 \pm 0.01b$	$0.38 \pm 0.02c$	$0.42 \pm 0.001bc$
C (%)	5.17 ± 0.27 cd	$6.03 \pm 0.35bc$	$8.68 \pm 0.58a$	$5.43 \pm 0.15bcd$	$4.951 \pm 0.18d$	$6.094 \pm 0.1b$
S (%)	$0.21 \pm 0.1a$	$0.12 \pm 0.01ab$	$0.11 \pm 0.003ab$	$0.09 \pm 0.004ab$	$0.08 \pm 0.004b$	$0.08 \pm 0.01ab$
C/N	$12.91 \pm 0.22d$	$13.58 \pm 0.01c$	$16.68 \pm 0.42a$	$12.15 \pm 0.21e$	12.97 ± 0.13 cd	$14.49 \pm 0.31b$
Soil temperature (°C)	$15.28 \pm 0.59a$	$14.31 \pm 0.81b$	$11.98 \pm 0.58c$	$17.31 \pm 1.08a$	$15.19 \pm 1.63b$	$12.9 \pm 0.37c$
Soil respiration	$10.12 \pm 1.07a$	$9.86 \pm 1.29a$	$8.51 \pm 0.99a$	$10.22 \pm 1.08a$	$8.60 \pm 1.29a$	$7.44 \pm 0.99a$
NH_4^+-N ($mg \cdot kg^{-1}$)	$1.13 \pm 0.83b$	$2.84 \pm 0.76ab$	$3.68 \pm 1.62ab$	$1.57 \pm 1.1b$	$5.87 \pm 1.67a$	$4.02 \pm 1.14ab$
NO_3^--N ($mg \cdot kg^{-1}$)	$1.15 \pm 0.37c$	$1.95 \pm 0.67ab$	$1.18 \pm 0.39c$	$1.28 \pm 0.11c$	$1.32 \pm 0.56bc$	$2.38 \pm 0.55a$
NO_2^--N ($mg \cdot kg^{-1}$)	$0.19 \pm 0.07b$	$0.29 \pm 0.06a$	$0.2 \pm 0.09b$	$0.21 \pm 0.05b$	$0.16 \pm 0.03b$	$0.25 \pm 0.06ab$

Values with different letters in a row means significant difference at $p = 0.05$. Values are means of three replicates $\pm$ SE.
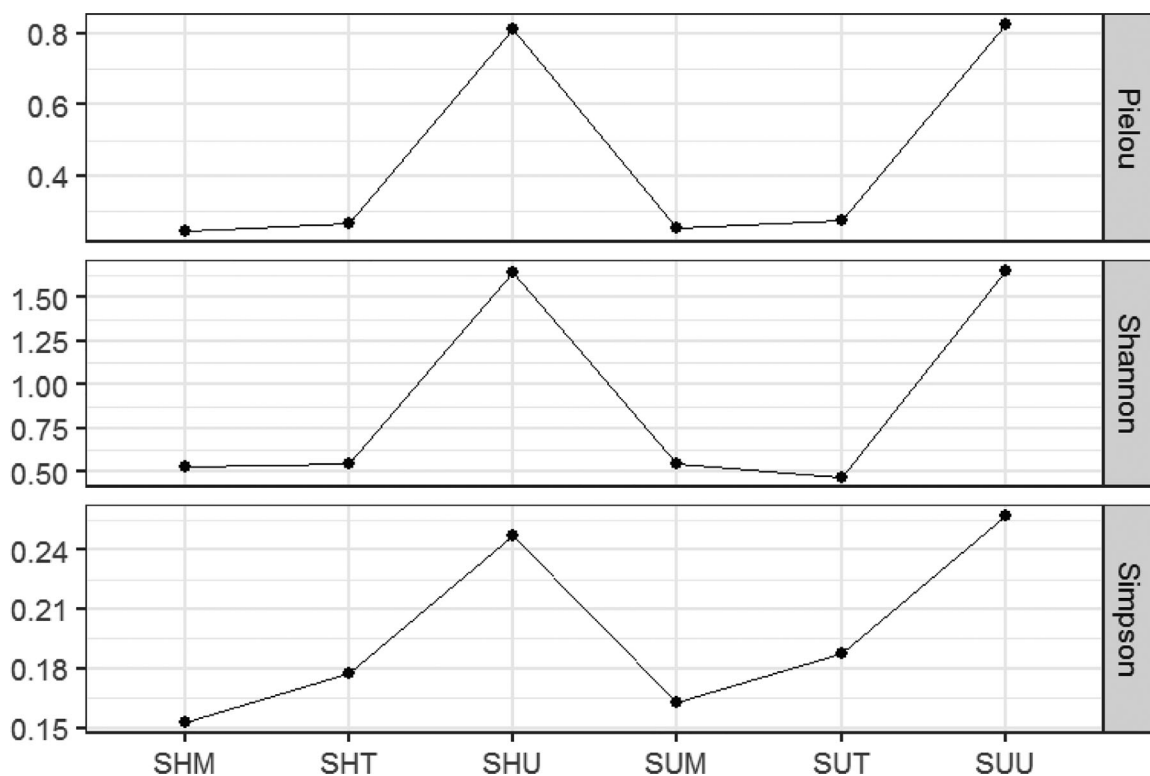

FIGURE 2 The diversity index of above-ground vegetation. Samples plots include SHM, sampling plot in a meadow of a shady slope; SHT, sampling plot in the timberline of a shady slope; SHU, sampling plot under the forests of a shady slope; SUM, sampling plot in a meadow of a sunny slope; SUT, sampling plot in the timberline of a sunny slope; and SUU, sampling plot under the forests of a sunny slope

TABLE 2 Diversity of soil bacterial communities in different plots

	Chao1	ACE	Simpson	Shannon
SUM	2127	3015.25	0.9944	9.32
SUT	2633	3194.01	0.9956	9.60
SUU	2536	3192.71	0.9963	9.76
SHM	2705	3221.63	0.9958	9.61
SHT	2551	3106.75	0.9959	9.64
SHU	2552	3054.41	0.9932	9.38

FIGURE 3 Principal coordinate analysis based on Bray-Curtis similarities of bacterial communities

Simpson index were higher in the SUU site (0.9963), and lower in the SUM site (0.9932). The Shannon index was the highest in the SUU site (9.76) and the lowest in the SUM site (9.32). Overall, the species richness and diversity indices of bacterial communities on the shady slope were higher than those on the sunny slope. In addition, the PCoA (Figure 3) illustrated the biogeographic pattern of bacterial communities on a two-dimensional ordination, indicating a lower similarity of community composition between the sampling plots.

A total of 43 bacterial phyla were identified among all sampling plots. As shown in the Venn diagram, there were 644 shared bacterial OTUs (Supporting Information Figure S3). There were nine bacterial phyla with a relative abundance of 1% or more. The relative abundance of *Proteobacteria* in all plots was the highest (mean relative abundance = 33.64%) (Figure 4). The relative abundance of *Actinobacteria* was the highest in the SUM site (24.27%), whereas *Acidobacteria* was the highest in the SHM site (23.43%). There were 26 bacterial genera with relative abundances higher than 0.1%. Based on LEfSe analysis, the most significant difference in order level between the different slopes was detected in *Acidobacteria* and *Rhizobiales* (Supporting Information Figure S4). However, for the overall microbial community structure, there were no significant differences between the two slopes (ANOSIM, $R = 0.04$, $p = 0.42$).

Network analysis indicated the pattern of bacterial community interactions between different slopes (Figure 5). There were 8 main modules, 1224 edges, and 252 vertices that emerged in the network. The connectability was 0.0387 and the clustering coefficient was 0.483. Community co-occurrence was significantly higher compared to the exclusion relationships, inferred from the numbers of positive correlations (1189) that were far higher than those of negative correlations (35).

3.3 | Bacterial community assembly processes

The relative contribution of deterministic processes and stochastic processes changed along different plots, since the null deviation varied ranging from −0.37 to −0.59 (mean value = −0.48) (Figure 6). The null deviation on the shady
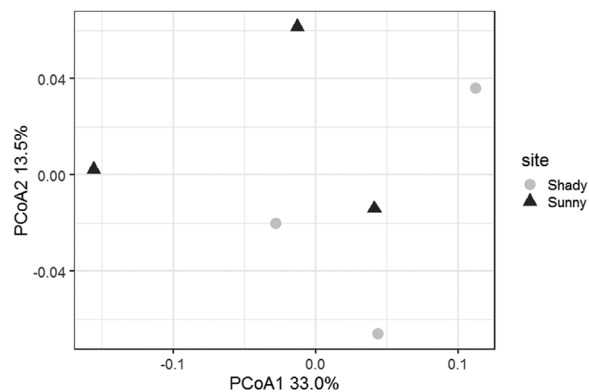
slope was −0.512 and was significantly different from that on the sunny slope (−0.429, $p < 0.05$). Compared with the sunny slope, deterministic processes played more important roles on the microbial community in shady slope, because of the larger deviations from random expectation.

Based on the results of the Raup-Crick metric (Figure 7), the RC values for different slopes were negative (ranging from −0.53 to −0.79, mean value = −0.63), indicating less dissimilar than expected by random chance.

3.4 | Environmental filtering affects soil bacterial community composition

The results of the PLS path modeling analysis indicated that the direct effect of edaphic factors on microbial community structures was 0.9736 (Figure 8), followed by vegetation and spatial variables. Redundancy analysis (RDA) showed that environmental factors influenced on the microbial community structure. Results demonstrated *Elusimicrobia* and *Cyanobacteria* were mainly shaped by TC and TN, while NH_4^+-N was the main abiotic driver of *Bacteroidetes* and *Proteobacteria*.

3.5 | Environmental filtering affect soil bacterial community metabolism

Copy numbers of 16S rRNA genes for the shady slopes (9.42) were higher than those for the sunny slopes (8.54) (Figure 9) ($p < 0.05$). Copy numbers of *cbb*M genes, which encode the key enzymes of the Calvin-Benson-Bassham (CBB) cycle, for the shady slopes (7.02) were significantly higher than those for the sunny slopes (6.59) ($p < 0.05$). However, there were no significant difference in the copy numbers of *amylase* and *cellulose* genes on different slopes ($p > 0.05$). In terms of the functions of the overall microbial community, there were significant differences between the different slopes (ANOSIM, $R = 0.64$, $p = 0.001$).
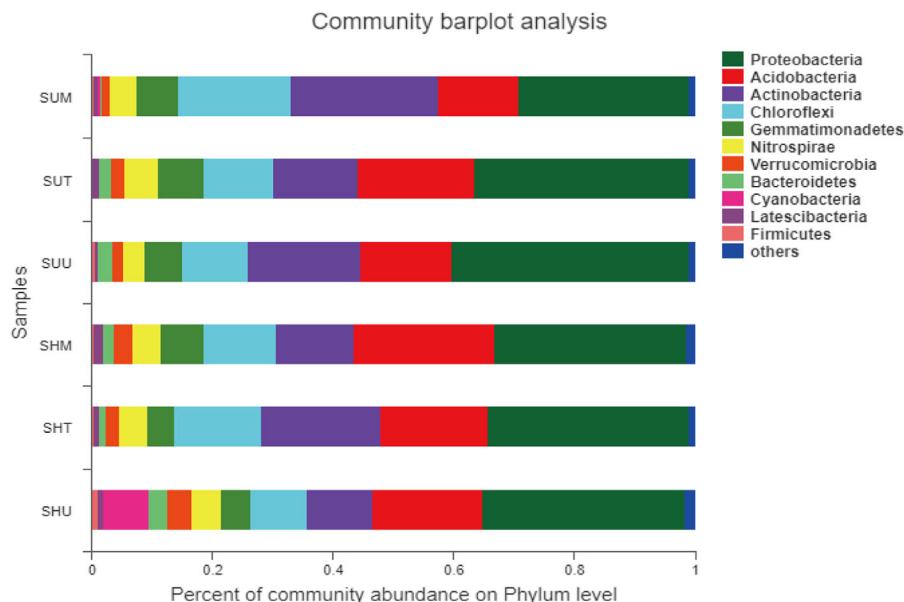
FIGURE 4　Relative abundance of different phyla in different plots

4 | DISCUSSION

Ecological processes that underlie community dynamics govern the complicated biogeographical patterns of microbes, and these processes have long been a hotspot of discussion in community ecology. However, the mechanisms shaping these patterns remain poorly understood, given that the aggregate of multiple ecological pro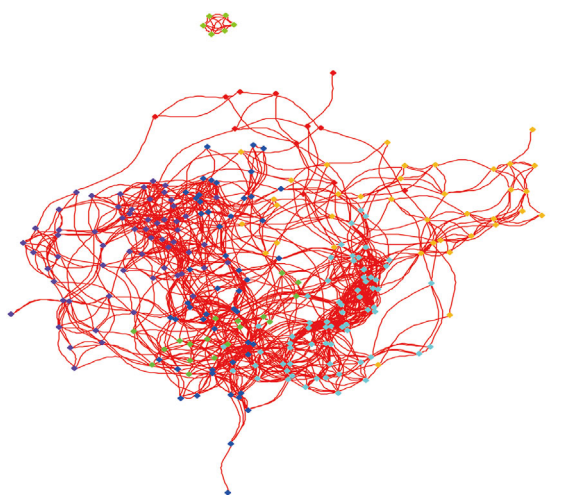cesses severely complicates the identification of causal relationships. It is necessary to investigate the community assembly mechanism under different environmental contexts.

Our results from the null model analysis demonstrated that the deviations were negative (mean value $= -0.48$), which were interpreted as showing more deterministic processes (especially environment filtering) of the community assembly, since communities were more similar than expected by chance. This is consistent with the results of the RC metric. The degree of deviation reported herein was lower than that of a previous study focused on a soil fungal community along a retreating glacier [14]. This could be because bacteria may possess greater metabolic versatility [32], and are thus less affected by environmental filtering

FIGURE 5　Network analysis based on Spearman rank correlation showing potential interactions of the bacterial communities. The node size is proportional to a taxon's average relative abundance (log transformation) across all samples. Lines connecting nodes (edges) represent positive (red) or negative (blue) co-occurrence relationships. The nodes are colored by modularity class
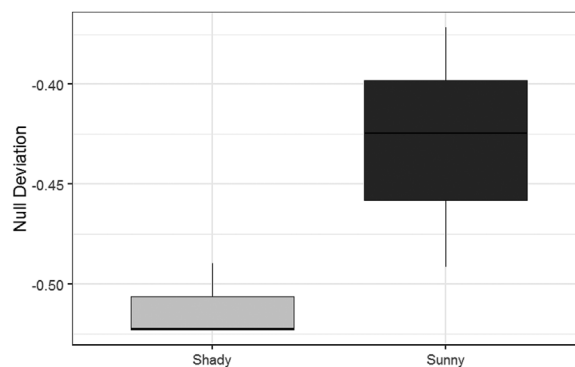

FIGURE 6　The null model analysis showing the null deviation of bacterial communities on different slopes. A null deviation close to zero suggests that stochastic processes are more important in structuring the community, whereas larger positive or negative null deviations suggest that deterministic processes play more important roles
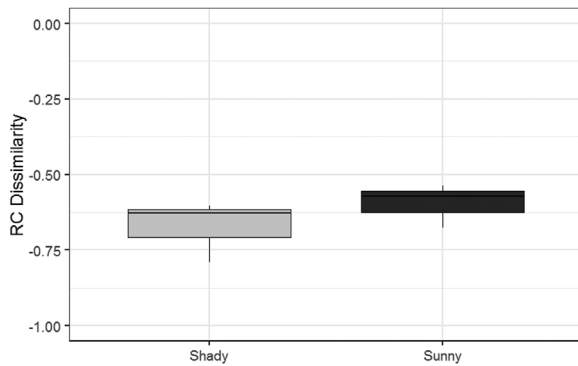
FIGURE 7 Mean dissimilarity according to the Raup_Crick metric (RC) of sampling plots. The RC metric ranges from −1 to 1 and indicates whether a pair of plots is less dissimilar (approaching −1), as similar (approaching 0), or more dissimilar (approaching 1)

(compared to a fungal community). The combination of both the null model and RC metric [28,30] confirmed the importance of environmental filtering in shaping the soil microbial community structure in the subalpine timberline area.

Our results demonstrated that the edaphic factors were the main driving force of bacterial community assembly, followed by the vegetation and spatial variables. The effect of environmental filtering on the assembly of bacterial community suggests that everything is everywhere, but the environment selects [33]. A previous study have confirmed that high environmental stress can potentially increase environmental selection [34].

The environmental conditions in subalpine timberline ecotones consist of pronounced climatic gradients [35] and climosequences within short distances, with a high level of environmental heterogeneity [36]. The harsh but homogeneous environmental factors could have a strongly selective effect on the local species pools, thus reducing the dissimilarity of the community. As nested sieves, the environmental factors could eliminate some unsuitable species, which keep the whole community in a state of co-occurrence. Thus, only species that are suitable for the environmental selection survive. Reducing the dissimilarity of the community may be relative to the decreasing difference in microbial community structures on different slopes.

On the other hand, the results of this study indicate that the biotic factors, such as interspecies relationships, had also influenced the bacterial community structure to some extent. Network analysis can be used to investigate the potential interaction between microbes and the symbiosis patterns of the dominant groups [37,38]. Our results demonstrated that community co-occurrence was more obvious, since the positive correlations in network indicate that the abundance of OTUs varied along the same trend (co-occurrence) [35]. This could be relative to the competition being more important under high resource availability, whereas environmental filtering prevailed during periods of high environmental stress [39].

Previous studies have shown that microbial communities displayed complex variation in composition across different habitats, and this variation can have profound effects on ecosystem functions [20,40]. This study found that the copy numbers of *cbbM* genes on the shady slope were significantly higher than those on the sunny slope, indicating a greater carbon sequestration capability on the shady slope. In addition, the abundance of bacteria (i.e., copy numbers of 16S rRNA genes) on the shady slope was higher than that on the sunny slope. The intermediate disturbance hypothesis (IDH) suggests that local species diversity is maximized, when ecological disturbance is neither too rare nor too frequent [41,42]. Diversity of the bacterial community on the shady slope was higher than that on the sunny slope. Therefore, we inferred that environmental factors on a shady slope may cause intermediate disturbances to the microbial community, but may increase the overall diversity of the
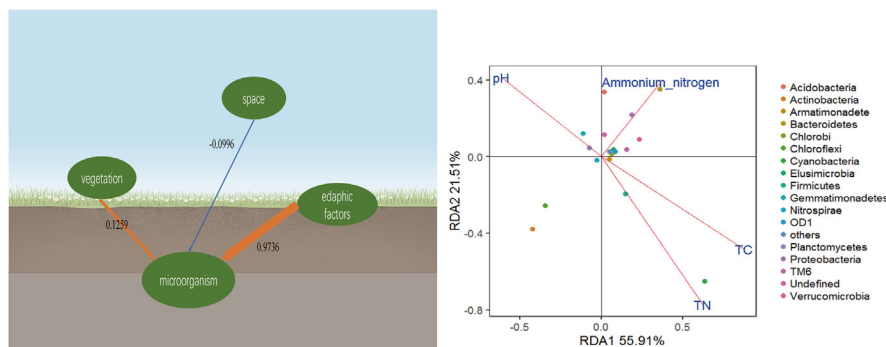

FIGURE 8 Redundancy analysis (RDA) (right) and PLS path model analysis (left). In the path model, the spatial variables include PCNM1, PCNM2, and PCNM3, which were operated by principal coordinates of neighbour matrices. Environmental variables include TC, TN, and pH, which indicated greater contribution to microbial community structure as demonstrated RDA
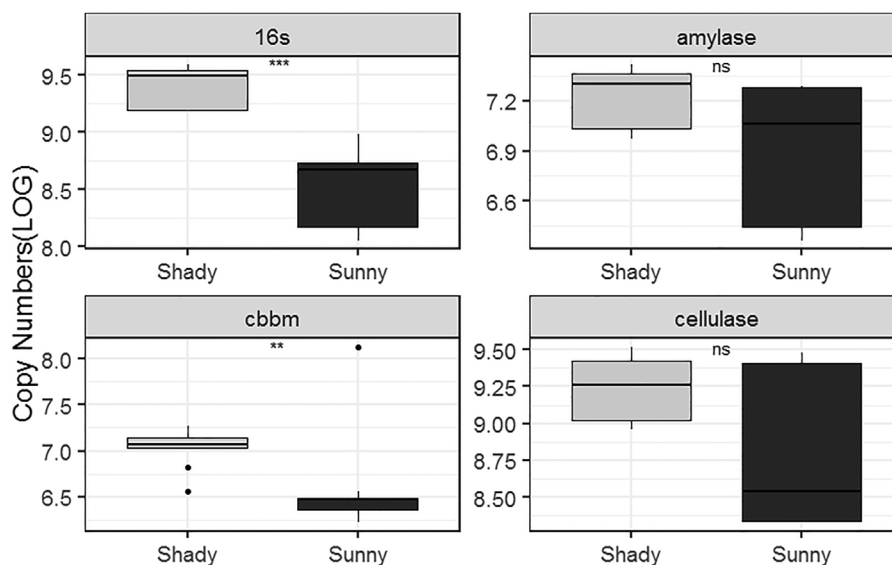
FIGURE 9　The copy numbers of 16S rRNA, *cbb*M, cellulose, and amylase genes

microbial community. This suggests that the higher the diversity of the community, the more complementary taxa are likely to be included to adapt to certain stresses on a shady slope [43], so as to ensure that the community has a faster ability to recover after stress [44]. Thus, bacterial communities on shady slopes could be more resilient and resistant to varying environmental conditions. Stable communities are conducive to species colonization and reproduction. In addition, species richness and abundance are positively correlated with ecosystem function [45]. For the reasons discussed above, the microbial community on shady slope had the highest abundance and strongest carbon sequestration ability.

Copy numbers of *amylase* and *cellulose* genes did not differ significantly between the different slopes, indicating that there was no significant difference in carbon metabolism. This may be due to many microbial taxa exhibiting carbon-use plasticity, as taxa have been reported to be able to alter their usage of glucose and soil organic matter depending upon environmental conditions [46,47].

Overall, our results demonstrated that with respect to the degree of the response to environmental filtering, the bacterial community structure and function were not consistent. This may be attributed to the significant difference between the functional and taxonomic community structure, which arises because mechanisms that lead to a convergence of metabolic function do not necessarily lead to a convergence of taxonomic composition [36].

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.