



RESEARCH ARTICLE

Soil pH dominates elevational diversity pattern for bacteria in high elevation alkaline soils on the Tibetan Plateau

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One sentence summary: Soil pH dominates elevational diversity pattern for bacteria in high elevation alkaline soils on the Tibetan Plateau.

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ABSTRACT

Although studies of elevational diversity gradient for microbes have attracted considerable attention, the generality of these patterns and their underlying drivers are still poorly understood. Here, we investigated bacterial distribution across a high elevational gradient (4328–5228 m a.s.l.) along the Nyainqêntanglha mountains on the Southwestern Tibetan Plateau. We found a decreasing diversity trend with increasing elevation, with pH contributing most to the diversity variation, followed by elevation and mean annual temperature (MAT). Bacterial community composition differed taxonomically and phylogenetically with elevation. Furthermore, partial Mantel analyzes showed that bacterial community composition was significantly influenced by elevation, pH and MAT. Phylogenetic structure analysis indicated that deterministic processes (related to pH) determined bacterial community assembly along this elevation gradient. Our results reinforce the observed rule that pH is of foremost importance in driving bacterial elevational diversity patterns. We also suggest that absence of tree cover and of human disturbance might be important in allowing an underlying elevational trend to reveal itself. Review of other studies suggests that the trend of decreasing diversity at higher elevations might be generally true above the tree line.

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INTRODUCTION

Studies of elevational gradients in species richness are a fundamental aspect of ecology. Elevational diversity gradients on plant and animal taxa have been well studied, with decreasing diversity with increasing elevation being frequently reported (Rahbek 2005; Gaston 2000; McCain 2005). In contrast to macroorganisms, microbial elevational diversity patterns were not investigated until Bryant et al. (2008) first documented a decreasing trend for soil acidobacterial diversity with elevation. Some researchers concluded that the elevational diversity patterns for microbes are fundamentally different from those of macroorganisms, based on the findings that soil bacteria exhibited no evident trends in diversity along elevation gradient (Fierer et al. 2011; Shen et al. 2014). However, many others did observe a decreasing elevational diversity pattern for soil microbes including bacteria, fungi and even specific functional taxa (e.g. arbuscular mycorrhizal fungi and ammonia-oxidizing microorganisms) (Lugo et al. 2008; Zhang et al. 2009; Liu et al. 2015; Shen et al. 2015; Wang et al. 2015). There is a need for further investigation of mountain ecosystems in order to reach general conclusions about the prevalence of elevational diversity trends in soil microbes, as part of the search for generalized elevational patterns of biodiversity (Lomolino 2001).

Climate-related factors that cause physiological stress and reduce resource availability have long been recognized as principal drivers in causing the decreasing elevational diversity patterns that are often seen in plants and animals (McCain 2005). For soil microbes, aspects of the soil itself—rather than climate—appear to be more important in affecting the diversity patterns (Fierer and Jackson 2006; Chu et al. 2016). Evidence from our previous work on Changbai Mountain suggested that soil pH is a major factor influencing bacterial elevational distributions (Shen et al. 2013). Previous studies on Segrila Mountain indicated that pH contributed most to the decreasing elevational diversity pattern shown by soil bacteria and fungi (Wang et al. 2015). Temperature may also have an important role in explaining microbial diversity patterns (Hendershot et al. 2017). In ecology, temperature is often considered as closely linked with the metabolic theory of ecology (van der Meer 2006), which predicts that individuals' metabolic rates, the population growth and species richness increase exponentially with the environmental temperature. For instance, an Andean transect suggested that temperature was the dominant driver of decreasing soil microbial diversity pattern (Nottingham et al. 2018). Therefore, it is unclear whether microbial diversity can sometimes be driven by the same factors as for plants and animals.

The Tibetan Plateau, which has been dubbed 'the third pole', is the Earth's largest (2.5×10^6 km²) and highest (mean elevation 4500 m) plateau with harsh environment and high sensitivity to climate change (Yao et al. 2000). In this study, we collected soil samples from 4328 m to 5228 m along six elevation gradients on Mt. Nyainqêntanglha at Shigatse on the southwestern part of the Tibetan Plateau. Soil bacterial communities were analyzed based on 16S rRNA gene sequencing. As two major processes including deterministic (niche theory or environmental filters) and stochastic processes (neutral effects) were recently used to explain microbial elevational diversity patterns, a phylogenetic approach was further used to infer the bacterial assembly processes (Hanson et al. 2012). We also summarized the findings of 20 published studies of elevational microbial studies from

2008 to 2018 to better understand the prevalence of elevational diversity patterns, their correlates and their possible underlying mechanisms. Our study aimed to answer the following questions: (i) Do soil bacteria show a decrease in diversity with elevation in this high-elevation meadow ecosystem? (ii) Between soil pH and temperature, which is more important in affecting bacterial diversity and community composition along this elevation gradient? (iii) Does the relative role of stochasticity vs determinism in microbial community assembly vary with elevation?

MATERIALS AND METHODS

Site selection and soil sampling

We sampled soil samples at six sites along an elevational transect on Mt. Nyainqêntanglha located at Shigatse (28°38'~28°56'N, 87°09'~87°26'E) in Southwest Tibet, China. The sites were located between 4328 and 5228 m above sea level and geographic distance across all sites was up to 46.5 km. The vegetation type was identified as alpine meadow across all sites. We collected soil samples in summer of July 2011. At each site, three separated 10 × 10 m² plots were chosen and eight upper 10-cm soil cores were pooled randomly from every plot. For each sample, soil was divided into two part after sieving through 2 mm sieve, with one for physicochemical analyzes and another for DNA extraction. Details of the general site characteristics are in Table S1 (Supporting Information).

Soil physicochemical analyzes

Detailed soil physicochemical analyzes were described in Shen et al. (2015). Briefly, we measured four representative soil features including pH, total carbon (TC), total nitrogen (TN) and dissolved organic carbon (DOC).

DNA extraction and MiSeq sequencing

MoBio PowerSoil DNA Extraction Kit (MO BIO, Carlsbad, CA, USA) was used to extract soil DNA. The primer set 515F/806R (cited by Earth Microbiome Project) were used to amplify the V4 region of the 16S rRNA gene (triplicate reactions for each sample). PCR reaction systems and conditions were described in Fan et al. (2017). The amplicons were quantified using Qubit Fluorometer (Thermo Fisher Scientific Inc., USA) and then were sequenced by Illumina MiSeq PE250. Sequence data have been uploaded to the MG-RAST database under the accession numbers from 4707284.3 to 4707301.3.

Bioinformatics

FLASH V1.2.7 (<http://ccb.jhu.edu/software/FLASH>) was used to assembly paired-end sequences from MiSeq platform (Magoc and Salzberg 2011). QIIME v. 1.9.0 (<http://qiime.org/index.html>) were used to conduct quality filtering, length trimming, chimera checking, OTU clustering and taxonomy assignment (Caporaso et al. 2010). USEARCH algorithm was utilized to conduct chimeric sequences detection and OTUs clustering (97% similarity) (Edgar et al. 2011). Bacterial OTUs were assigned taxonomy using the Ribosomal Database Project (RDP) classifier based on GreenGenes database from the latest QIIME releases version (gg.13.8).

To avoid sequencing and sampling bias, 29 000 sequences per sample were randomly selected.

Statistical analyzes

Taxonomic diversity (OTU richness, Chao1, Shannon index, Pielou's evenness) and phylogenetic diversity (Faith 1992) were estimated in QIIME with 'alpha_diversity.py' script. For the relationship between the diversity and environmental variables, between the relative abundances of phyla/OTUs and environmental variables, correlation analyzes (Pearson) and Linear models were applied. The relative importance of environmental variables that explained variations of alpha diversity was analyzed by Aggregated boosted tree (ABT) models (De'ath 2007; Ge et al. 2008). Bray-Curtis distance and unweighted UniFrac distance were used to estimate community dissimilarity (Lozupone, Hamady and Knight 2006). For the relationship between community dissimilarity and elevation, non-metric multidimensional scaling analyzes (NMDS), canonical correspondence analysis (CCA), analysis of similarities (ANOSIM) and multi response permutation procedure (MRPP) were performed. For the relationship between community composition and environmental variables, Mantel and partial Mantel tests and distance-based multivariate analysis for a linear model were performed (DistLM; McArdle and Anderson 2001). All statistical analyzes were conducted in R software or DISTLM.forward3 software (Anderson 2003; R Development Core Team 2006).

Phylogenetic structure analyzes

Before mean nearest taxon distance (MNTD) was used, the phylogenetic signal was tested to ensure the niche conservatism across short phylogenetic distance (Webb et al. 2002). The standardized-effect size of the MNTD (ses.MNTD) and beta nearest taxon index (betaNTI) were calculated in R and phylocom software, respectively (Webb et al. 2002; Hardy 2008). The ses.MNTD below 0 and betaNTI below -2 or above 2 indicates deterministic processes play a dominant role (Stegen et al. 2012).

Network analyzes

To elucidate the interactions between bacterial phylotypes and environmental variables, non-random co-occurrence network analysis was conducted. In brief, analyzes were performed using the approach SparCC which estimating correlation values from OTUs and environmental variables data (Friedman and Alm 2012). The 1000 most dominant OTUs were retained for network analysis. Correlation coefficients (ρ) with statistically significant ($P < 0.05$) and an absolute value over 0.75 were retained in network analyzes (false discovery rate correction was applied). The nodes represented the dominant OTUs and environmental variables, whereas the edges corresponded to correlations between nodes. Gephi platform was used to make the networks visualized (Bastian, Heymann and Jacomy 2009). The size of each node represents the number of connections (i.e. degree). The color of each node is based on dominant phylum and environmental variables.

RESULTS

Bacterial taxonomic features

Across all soil samples, we obtained 647 547 sequences from the lowest 29 849 to the highest 39 785 sequences per sample. Of

the 5966 OTUs observed, 3311 OTUs occurred in at least two elevations and 2464 OTUs shared by all elevations accounting for 41.30% of the total number of OTUs (Table S2, Supporting Information). As Fig. 1 showed, Actinobacteria (31.71%) was the most dominant phyla across all soil samples, followed by Proteobacteria (25.68%), Acidobacteria (11.88%), Chloroflexi (6.84%), Gemmatimonadetes (4.31%), Bacteroidetes (3.88%), Planctomycetes (3.52%) and Verrucomicrobia (1.43%). These phyla covered about 90% of the bacterial sequences. Pearson correlation analysis revealed that elevation, mean annual temperature (MAT) and pH was main factors correlated with the relative abundance of dominant phyla/OTUs (Fig. 1).

Bacterial diversity

The alpha diversity index estimated by OTU richness, Faith's PD, Chao1, Shannon index and evenness decreased monotonically with increasing elevation from 4328 to 5228 m (Fig. 2; Table S4, Supporting Information). We also tested the elevational diversity patterns of specific dominant phyla, and found the diversity of 9 phyla also decreased with increasing elevation ($P < 0.05$; Fig. S2 and Table S5, Supporting Information). Factors including elevation, MAT, pH and C:N ratio are significant factors that correlated ($P < 0.05$) with all diversity index (Table S4, Supporting Information). ABT model showed that pH was the largest contributor to the variation of bacterial OTU richness and PD (Fig. 3).

Bacterial community dissimilarity

NMDS analysis showed samples clustered together at the same elevations and separated among elevations (Fig. 4). ANOSIM and MRPP further demonstrated community dissimilarities among different elevations are significant ($P < 0.05$) (Table S6, Supporting Information). As shown in Fig. 5, significant distance-decay relationship between elevation and bacterial community similarity were observed ($P < 0.001$). The taxonomy based dissimilarities were significantly correlated with the obtained environmental variables except DOC from Mantel tests (Table S7, Supporting Information). In the partial Mantel tests, however, only Elevation, pH and MAT showed significant correlations with bacterial community composition when other factors are controlled. DistLM analysis showed that Elevation explained 25.14% of the total variation in bacterial community composition, providing the greatest explanatory power, followed by pH (24.52%) and MAT (24.10%) (Table S8, Supporting Information). Network analysis showed that elevation, pH and MAT closely interacted with bacterial communities (Fig. 6). These results indicate that elevation, pH and MAT are the predominant factors shaping the bacterial community composition.

Bacterial community phylogenetic structure

Significantly phylogenetic signals (i.e. relationships between ecological-niche distances and phylogenetic distances across relatively short phylogenetic distances are significant) were observed (Fig. S4, Supporting Information), indicating that niche conservatism occurs among OTUs. The MNTD value was minimum at the lowest elevation and significantly increase with increasing elevation, indicating phylogenetic dispersion towards higher elevation. Both ses.MNTD and betaNTI were significantly below -2, indicating that bacterial community assembly processes along elevation are dominated by deterministic processes (environmental filtering) (Fig. 7). Linear regression analysis showed that the betaNTI was significantly correlated with pH euclidean distance (Δ pH).

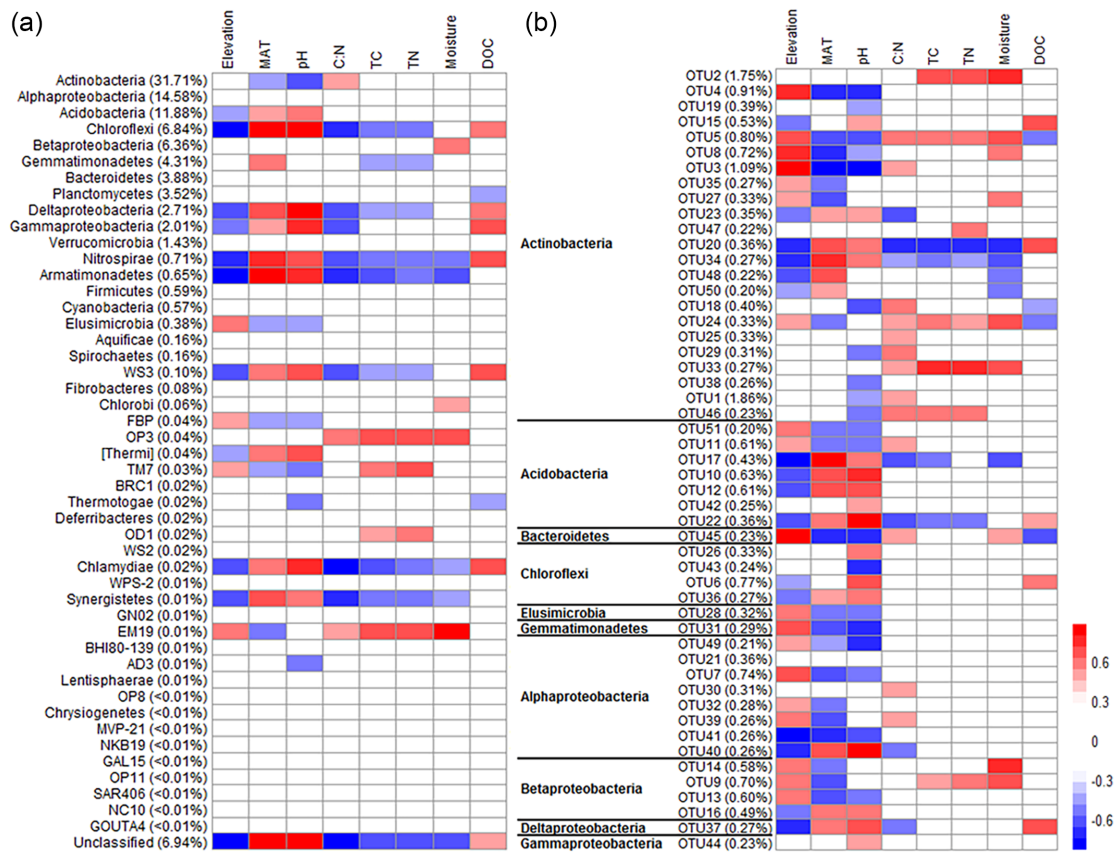


Figure 1. Pearson correlations between the environmental factors and the relative abundance of (a) bacterial phyla and (b) dominant OTUs (top 100). Only the significant correlations ($P < 0.05$) are shown and insignificant ones ($P > 0.05$) are left blank. Numbers in the parentheses are values of average relative abundance. MAT, mean annual temperature. TC, total carbon. TN, total nitrogen. C:N, carbon:nitrogen ratio. DOC, dissolved organic carbon.

Summary of literature of elevational microbial studies in soil environments

We summarized the findings of 21 published studies of elevational microbial studies from 2008 to 2018 (Table S9, Supporting Information). Seven characteristics were listed, including location, targeted microbes, elevational diversity pattern, key factor, pH range, elevation range, treeline or not. Of the 20 published studies, 15 studies focused on bacteria, 11 studies found decreasing diversity patterns, 10 studies identified pH as a key factor and 7 studies identified temperature as a key factor. Among these studies, pH was alkaline in only 2 studies, and treeline was absent in 8 studies with a decreasing diversity pattern.

DISCUSSION

The generality of the trend of decreasing microbial diversity with elevation

In this high-elevation meadow ecosystem, along with a 900 m elevation gradient (from 4328 to 5228 m), there was a monotonically decreasing diversity pattern with increasing elevation.

A decrease in diversity of soil microbes with increasing elevation has been reported by many recent studies (Shen et al. 2015; Li et al. 2016; Nottingham et al. 2018). We reviewed 20 elevational soil microbial studies published since 2008, and found that almost half of these studies showed a decreasing pattern,

whereas other studies found no significant trend (Table S9, Supporting Information). This led us to ask why a decreasing elevational diversity patterns for soil microbes may occur in some cases rather than others. Here, based on considering the range of previous studies, we propose three primary factors including wide pH range, the absence of treeline and very high-elevations, which could largely lead to the generalized decreasing elevational diversity patterns for soil microbes.

What factors affect bacterial diversity and community composition?

Along latitudinal gradients, extensive studies have reported soil pH as having the strongest predictive power in bacterial diversity (Griffiths et al. 2011; Bahram et al. 2018). Along elevational gradients, several studies have identified an important role of pH resulting in the decreasing elevational diversity pattern, despite the effect of temperature and other soil characteristics (e.g. C:N ratio) was often detected (Wang et al. 2015; Nottingham et al. 2018). In this study, pH, MAT and C:N ratio were significant ($P < 0.05$) factors correlated with bacterial diversity (Table S4, Supporting Information). To discern their relative influence on bacterial diversity, we conducted ABT model analysis and the result suggested that pH contributed most to variation in diversity (Fig. 3). This finding parallels with our previous work that found that pH made the largest contribution to the variation of bacterial diversity on Changbai Mountain (Shen et al. 2013). However, the difference in the case of Changbai is that the soil pH is acidic (3.89–6.31), rather than alkaline (7.31–9.37) as it is in our present

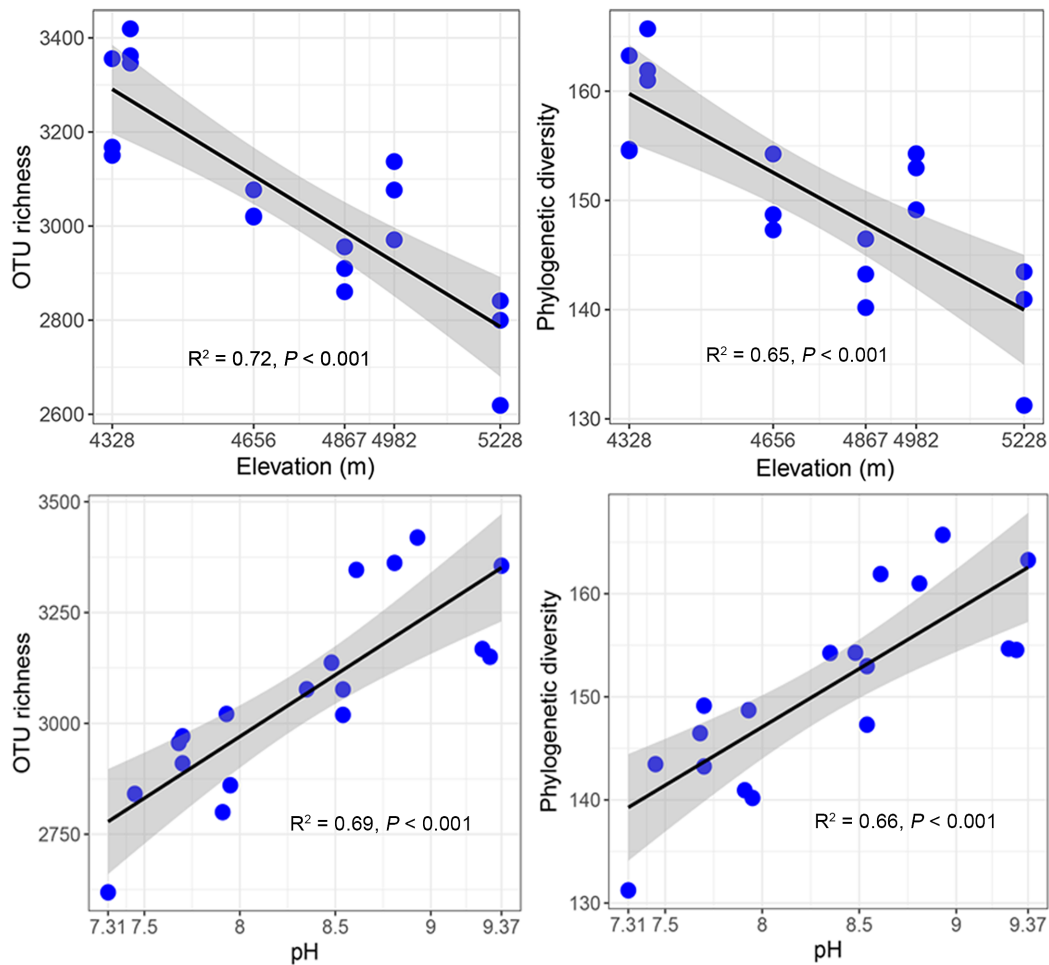


Figure 2. Relationships between bacterial diversity and elevation and soil pH. Diversity indices were calculated using random selections of 29 000 sequences per soil sample. The strength of each relationship is based on the linear regression equation.

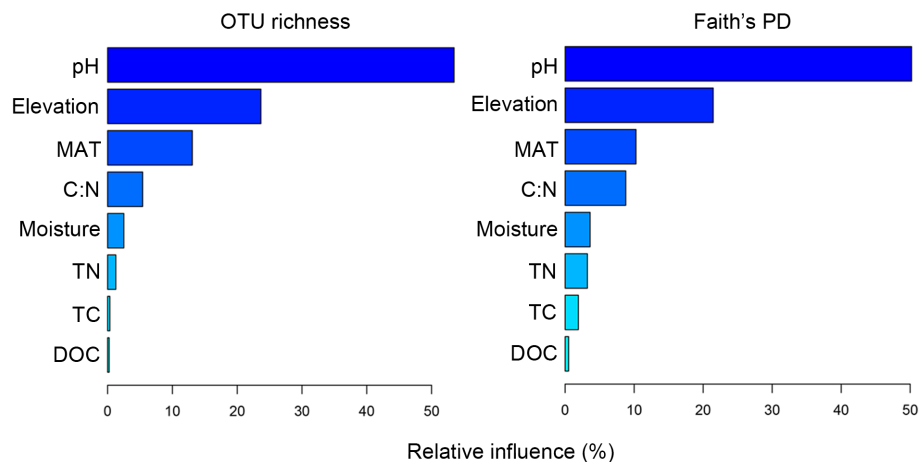


Figure 3. Relative importance of predictors for alpha diversity with the optimized aggregated boosted tree (ABT) model. Faith's PD, Faith's phylogenetic diversity. TN, total nitrogen. TC, total carbon. C:N, carbon: nitrogen ratio.

study. Besides pH, we also found the secondary role of other environmental factors (e.g. MAT and C:N). Temperature, which is often correlated with aboveground biodiversity of macroorganisms, may also strongly influence microbial metabolic rates and species richness according to metabolic theory of ecology

(Hendershot et al. 2017). For instance, under the condition of relatively constant soil pH, one study has revealed the dominant effect of temperature in driving bacterial decreasing elevational diversity patterns (Nottingham et al. 2018). Thus, we infer that the influence of temperature was overwhelmed by pH in

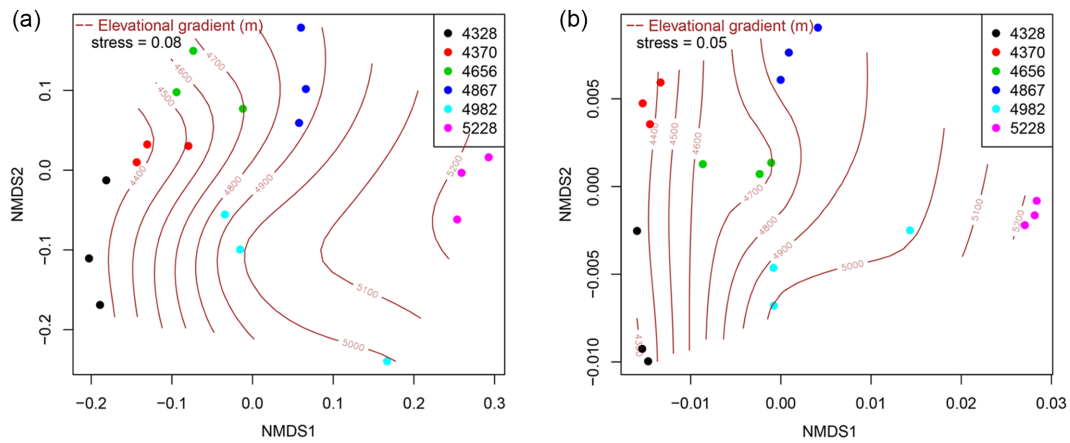


Figure 4. Non-metric multidimensional scaling (NMDS) ordinations of the taxonomy-based (a) and phylogeny-based (b) dissimilarities of the bacterial communities at different elevations. Sites were coded with different colors according to the elevations.

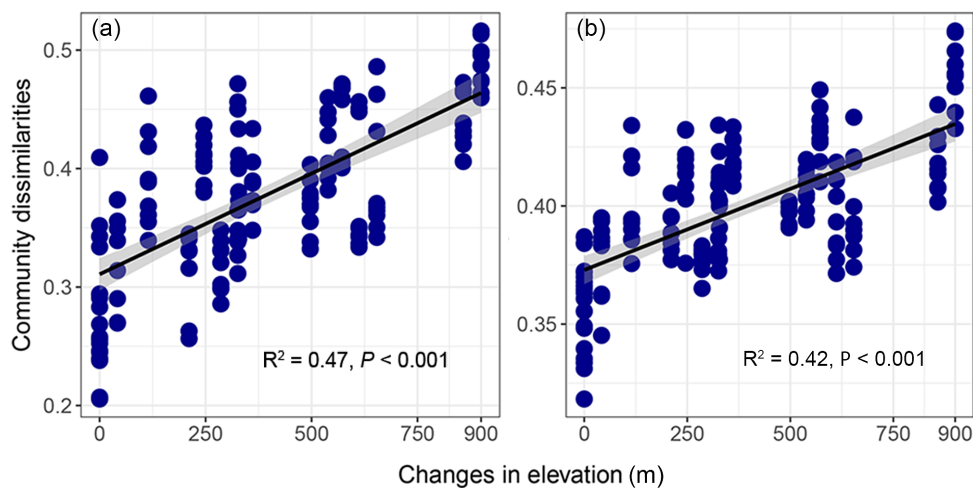


Figure 5. Bacterial community dissimilarities based on the Bray-Curtis distances (a) and unweighted Unifrac distances (b) in relation to elevational distances. The strength of each relationship is based on the linear regression equation.

most cases. A wide pH range among sampling sites is an important precondition for finding pH driving bacterial diversity: if pH range had been narrow throughout the transect then another factor such as temperature would presumably have dominated. The important role of pH in elevational gradients is apparently not limited to the soil environment: a recent study identified pH as a major factor influencing the decreasing bacterial diversity along elevation in freshwater lake sediments (Zeng *et al.* 2016).

It is interesting that the present study and others, which begin above the treeline and extended upwards (Shen *et al.* 2015), tend to show a decreasing bacterial diversity trend with elevation—whereas others that extend across the treeline show other trends (Singh *et al.* 2012; Li *et al.* 2016). For example, our previous study showed that there was no apparent elevational gradient in bacterial diversity across a complete elevational gradient (530–2200 m) on Changbai Mountain. However, at a relatively small scale (2000–2500 m) above the treeline (about 1950 m), we found a monotonically decreasing diversity pattern (Shen *et al.* 2015). Likewise, no evident trend in soil bacterial diversity was found along a complete elevational gradient (1020–2280 m) in Dongling Mountain. Below the treeline (about 1770 m), a U-shaped diversity-elevation pattern was found, whereas there was decreasing diversity pattern with increasing elevation (Li

et al. 2016). This may in part relate to the less complex environmental variation above the treeline, although it remains unclear what factors actually produce the gradient. Vegetation types such as conifer forest can strongly alter soil pH, and this may explain the more complex pattern below the treeline (Shen *et al.* 2013; Li *et al.* 2016).

Another aspect to consider is that elevational series, which start at high elevations and extend upwards are less likely to be disturbed by humans, allowing ecological trends to show themselves against simpler gradients. From the ten studies which observed decreasing a elevational diversity pattern summarized in Table S9 (Supporting Information), the lowest elevations selected were all above 2000 m (except one at 1770 m). Distinct from that alpine meadow or tundra ecosystems in high-elevations (Shen *et al.* 2015; Li *et al.* 2016), forest ecosystems in low-elevation areas might be easily disturbed by human activities (e.g. logging and fires) (Nogués-Bravo *et al.* 2008).

Elevation has been reported as an indicator of bacterial community differentiation in soil, aquatic and sediment environments (Singh *et al.* 2014; Wang *et al.* 2011; Zeng *et al.* 2016). As expected, significant dissimilarities of bacterial community among elevational gradients were observed in the present study (Figs 4 and 5), which reinforced the role of elevation per se

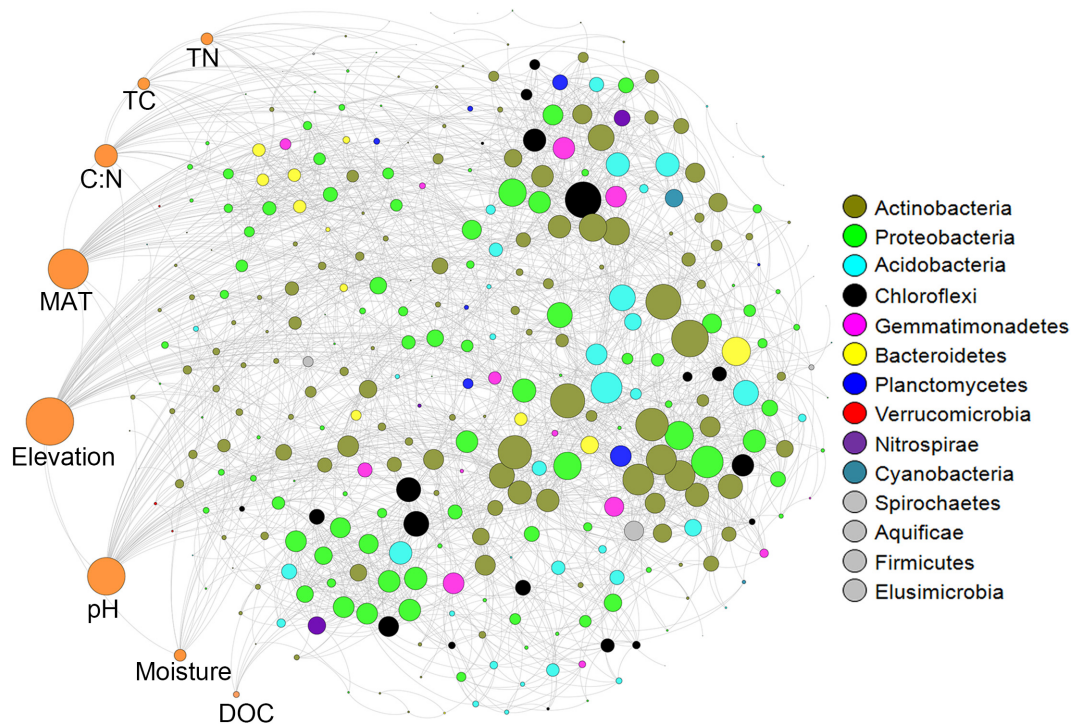


Figure 6. Network co-occurrence analyses of bacterial phylotypes in all soils. Each node represents a bacterial phylotype (an OTU clustered at 97%). A connection stands for statistically significant ($P < 0.05$) SparCC correlation with a magnitude $>+0.75$ (positive correlation-red edges) or <-0.75 (negative correlation-green edges). The size of each node is proportional to the number of connections (i.e. degree). Each node is labeled at the phylum level.

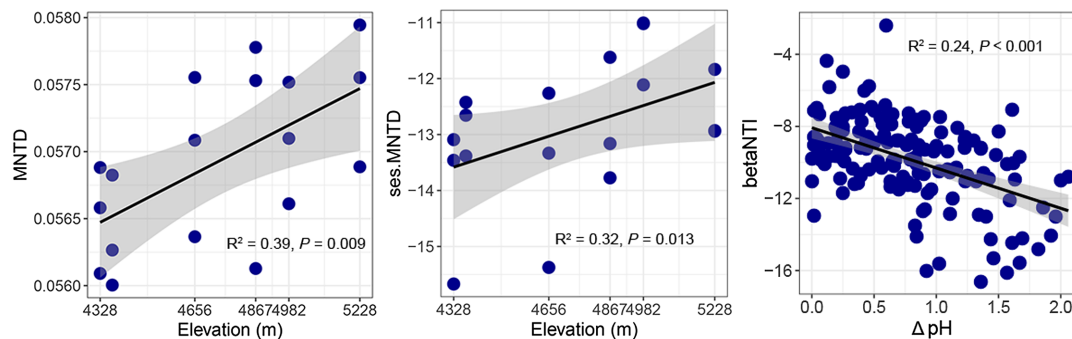


Figure 7. Mean nearest taxon distance (MNTD) and standardized effect size of the MNTD (ses.MNTD) associated with elevation. Relationships between betaNTI and Δ pH (euclidean distance). The strength of each relationship is based on the linear regression equation.

as a geographical isolation. Besides elevation, partial Mantel analyzes showed that pH significantly correlated with bacterial community composition, followed by MAT (Table S7, Supporting Information). Intriguingly, previous elevational studies on different mountains showed that soil pH range was consistently acidic, whereas the pH range in our study is alkaline (7.31–9.37), likely reflecting the relatively arid conditions of these very high elevations and of the Tibetan Plateau in general. The relative abundance of the phylum *Chloroflexi* significantly correlated with pH (Fig. 1). Despite that, the influence of other soil characteristics (e.g. C:N ratio, TC and TN) on bacterial community composition should not be ignored. For example, at the phylum or OTU level, C:N ratio closely interacted with bacterial community composition based on correlation and network analyzes (Figs 1 and 6). Evidence from one study in the Tibetan Plateau also showed a strong effect of carbon/nitrogen ratio on bacterial community composition (Chu et al. 2016).

Deterministic processes underlying the decreasing elevational diversity pattern

Phylogenetic structure analysis permits insights into the phylogenetic relatedness of bacterial communities and potential assembly processes (Fan et al. 2017). Previous studies observed an increasing phylogenetic clustering pattern of bacterial communities in stream and sediments towards higher elevations, owing to an enhanced environmental filtering effect (e.g. acidity and C:N) at higher elevations (Wang et al. 2012; Zeng et al. 2016). By contrast, we found the increasing pattern of phylogenetic clustering towards lower elevations based on MNTD and ses.MNTD. This contradictory result implies that high pH (alkaline) can also lead to phylogenetic clustering among bacterial organisms (Tripathi et al. 2018 also found this in relation to pH). Nonetheless, both ses.MNTD and betaNTI were significantly below -2 , indicating that bacterial community assembly processes were dominated by deterministic processes (environmental filtering) along

the elevational gradient. A recent meta-analysis demonstrated that the influence of soil pH on bacterial community assembly processes generates within different succession stages and occurs globally (Tripathi et al. 2018). Here, we found the betaNTI was significantly correlated with variation in pH (Fig. 7). Thus, deterministic processes in relation to pH effect might be the driving force shaping the decreasing elevational diversity pattern observed in the present study.

SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](https://femsec.org) online.

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Conflicts of interest. None declared.

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