Contrasting altitudinal patterns and co-occurrence networks of soil bacterial and fungal communities along soil depths in the cold-temperate montane forests of China

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Abstract: Soil bacterial and fungal communities with different key ecological functions play important roles in boreal forest ecosystems. Although several studies have reported on the altitudinal distribution patterns of microbes, our understanding of the characteristics of the microbial community and the core composition of the microbiome in cold-temperate montane forests is still limited. In this study, Illumina MiSeq sequencing was used to investigate the changes in soil bacterial and fungal communities in surface and subsurface soils along an altitudinal gradient (from 830 m to 1300 m) on Oakley Mountain. The diversity of the bacterial and fungal communities showed a monotonic decrease and a monotonic increase with altitude, respectively. The influence of altitude on the bacterial and fungal community composition was stronger than that of soil depth. The variations in pH and dissolved organic nitrogen content at different altitudes were the main factors influencing the bacterial and fungal community structures, respectively. There was no obvious difference between the network structures of the surface and subsurface soil fungal communities, while the network of the subsurface soil bacterial community was more complex and intricate than that of the surface soil bacterial community. The network nodes mainly belonging to Proteobacteria and Actinobacteria were the key bacterial taxa in the two soil layers. Although the main drivers of microbial community structure are consistent for whole and sub-nerwork communities, the subnetwork community analysis revealed other important drivers (i.e. soil temperature and NO_3^--N) that do not capture by whole community analysis. Thus, the more comprehensive picture of the important factors shaping microbial community structure can be achieved by combining whole and

subnetwork community analyses. Our results demonstrated that altitude had a stronger influence on soil bacterial and fungal communities than soil depth and that bacterial and fungal communities showed divergent patterns with altitude and soil depth.

Keywords: soil depths; bacteria; fungi; cold-temperate forest; co-occurrence network; Illumina MiSeq sequencing

1 Introduction

Soil microorganisms are an important part of forest ecosystems and play a critical role in nutrient conversion, organic matter decomposition, and energy flows. The altitudinal distribution pattern of soil microorganisms is an important component of biogeographical distribution patterns but has been overlooked for a long time. In recent years, with the development of sequencing technology, many scholars have focused on the biogeography of soil microbes and found that the soil microbial community exhibit a monotonic decrease (always decreasing, and never increasing) (Bryant et al., 2008; Bahram et al., 2012; Shen et al., 2019), a “humpback” pattern (Miyamoto et al., 2014; Li et al., 2016; Peay et al., 2017) or a nonsignificant pattern along an altitudinal gradient (Fierer et al., 2011; Shen et al., 2014). However, these studies are concentrated mostly in tropical, subtropical, and temperate regions, and only limited studies have been performed on the altitudinal distribution of soil microbial communities in cold-temperate regions (Jarvis et al., 2015). Cold temperate forests are considered to be an important habitat for storing a large amount of biomass carbon (approximately 30%) (Reich, 2012). They have low productivity and nutrient cycling rates and are very sensitive to climate change, especially in terms of soil microorganisms and biochemical cycling processes (Christensen et al., 2004; Reich et al., 2012). The rapid response of microorganisms to changes in environmental conditions and their high turnover rate may provide more information about the provision of ecosystem services (Banning et al., 2011).

Given their different morphological characteristics, growth rates, environmental

sensitivity, phylogeny, and life history, soil bacteria and fungi exhibit divergent biogeographic patterns (Hannula et al., 2017). Some studies have reported that the growth rate of soil bacteria is approximately ten times higher than that of specific soil fungi, and soil fungi tend to be more resistant to low-temperature soil habitats than soil bacteria (Rousk and Bååth, 2007; Kirchman, 2018). Ma et al. (2017) found that soil bacteria and fungi had a unique biogeographic distribution in forest soils at the continent scale and that dispersal limitation and environmental variables dominated the variation in bacterial and fungal communities. In mountain ecosystems, the high variability of plant communities and soil properties along altitudinal gradient inevitably leads to dramatic variations in bacterial and fungal communities. Jarvis et al. (2015) found that temperature was the main factor affecting the ectomycorrhizal fungal community on Mt. Cairngorm in Scotland. In addition, some scholars have found that temperature has a positive correlation with the species richness of animals, plants, and microorganisms (Hawkins et al., 2003; Zhou et al., 2016). The metabolic theory of ecology explains this temperature-diversity relationship. The biochemical kinetics of metabolism predict that biodiversity will increase with increasing temperature (Brown et al., 2004). Compared to the established knowledge regarding tropical and subtropical regions, there is still uncertainty about cold temperate regions with specific climatic conditions; however, whether soil bacterial and fungal communities have obvious altitudinal distribution patterns and whether temperature or other environmental factors dominate these variations remain unclear.

In addition to abiotic factors, biotic factors (interactions among species) are

considered to be a complementary mechanism that affects the biogeographic patterns of microorganisms (Fan et al., 2017). Symbiosis, parasitism, competition and predation among different microorganisms in the community result in the formation of a complex co-occurrence network (Faust and Raes, 2012). In recent years, numerous studies have reported on the interaction and biological complexity of soil microorganisms in forest ecosystems utilizing network analysis (Xiao et al., 2018; Li et al., 2020; Tu et al., 2020). Most of studies involving co-occurrence network analysis focus on the impact of driven factors on the whole community network characteristics and topological structure of bacteria or fungi under control experiments, however, there is little published data along the altitudinal gradient on the response of different sub-networks to variations in driving factors (de Menezes et al., 2015; Li et al., 2020). Some recent studies have described the relationship between the vertical distribution and interaction of microbial communities along soil depths from the perspective of network analysis (Yang et al., 2017; Luan et al., 2020). To date, most studies have focused on the changes in the microbial community and related processes only in surface soil along altitudinal gradients (Eilers et al., 2012; Sheng et al., 2019), however, far too little attention has been given to subsurface soil. Microorganisms in subsurface soil play a key role in soil formation and biogeochemical cycling processes and exhibit different characteristics and greater variation than that of surface soil (Fritze et al., 2000). Soil depth can increase the rate of microbial evolution, including gene mutation, community assembly and interaction, and the microbial community exhibits high stability in the upper soil; the opposite conditions occur in the subsurface soil (Du et al., 2021). Some studies have

indicated that the variation in soil physicochemical properties affects microbial diversity and community composition at different soil depths in harsh climate areas (Coolen et al., 2011; Deng et al., 2015). However, due to the complexity of the soil microbiome, much less is known about the interactions between microbial members of the community, which limits our understanding of their role in ecosystem functions (Widder et al., 2016). To the best of our knowledge, information on the keystone taxa in soil microbial communities in boreal forest ecosystems is still limited. The composition of these microbial communities along soil depths and the framework affecting their community assembly have yet to be explored.

Mountain ecosystems are an important component of terrestrial ecosystems, and the regulating services of forests are of particular importance (Seidl et al., 2019). These ecosystems provide a wide variety of habitats in the context of the rapidly changing climates, vegetation and soil quality in harsh mountain environments (Sundqvist et al., 2013). Oakley Mountain has the highest peak in the northern Greater Khingan Mountains, at nearly 1520 m above sea level; however, the distribution patterns of soil microbes in this boreal forest ecosystem dominated by larch have been rarely reported. This limits our ability to predict the response of the soil microbial community to climate change in this cold-temperate region. Our recent study in Mt. Oakley suggested that altitude had stronger effects than season on fungal community structure, and fungal diversity, the soil fungal co-occurrence network exhibited obvious seasonal succession (Ji et al., 2021). Given the high variability of soil microbiomes along an altitudinal gradient and the fundamental differences in life strategies between bacteria and fungi

(Baldrian, 2017), here, we compared the diversity and co-occurrence networks of soil bacterial and fungal communities along an altitudinal gradient in a cold-temperate forest. This work will generate fresh insights into the main ecological predictors of microbiology along altitudinal gradients. We hypothesized that (1) with increasing altitude, the diversity and structure of the soil bacterial and fungal communities would show consistent patterns, i.e., a monotonic decline; (2) given previous findings on the factors that control changes in the bacterial and fungal communities, temperature and pH may be key factors affecting changes in the composition and structure of soil fungal and bacterial communities in cold-temperate ecosystems, respectively; and (3) the soil bacterial and fungal communities inhabiting surface and subsurface soils would exhibit different network topology characteristics, and the driving factors of the whole community will obscure some factors that play a pivotal role in the sub-networks community assembly.

2 Materials and methods

2.1 *Site description and soil sampling*

Oakley Mountain (51°50′N, 122°01′E) is located within the jurisdiction of the A'longshan Forestry Bureau in China. With a peak height of 1520 m, Oakley Mountain is the highest mountain in the northern Greater Khingan Mountains; it is therefore an ideal platform for investigating the biogeographical patterns of soil microbes in areas with steep topography (Figure S1). Briefly, the annual mean air temperature is -5.1 °C; the area has a cold-temperate climate with long, cold winters and short, warm summers.

The annual mean precipitation is 437.4 mm. The site is covered with snow from October to April and the deepest snowfields do not melt fully until May or June. The soils are mostly Umbric Cryosols and Gelic Podzols according to the World Reference Base and have an average depth of 20~25 cm.

Briefly, the high-elevation site investigated in this study was located in sub-alpine forests mainly covered by *Larix gmelinii* and *Pinus pumila*, and trees are gnarled, crooked and stunted, as well as Rhododendrons fairly common in this zone. The middle- and low-elevation sites were located in cold-temperate forests consisted primarily of *Larix gmelinii*, *Betula platyphylla* and *Pinus sylvestris*, characteristic plants include lichens and epiphytic mosses (Table S1). Along the altitude ranging from 796 m to 1378 m, the montane forests have the characteristic with similar canopy cover (ranging from 0.4 to 0.5), slope and exposed area. Based on the vegetation composition, the four altitudinal sites (830 m, 950 m, 1100 m, and 1300 m) were identified where not any anthropogenic disturbance. Triplicate study plots were randomly established within each site. We used a button-activated temperature sensor (HOBO H8 Pro, Onset Complete Corp., Bourne, MA, USA) to record the soil temperature (ST) in each plot. Nine soil samples were taken from 0~10 cm (surface soil) and 10~20 cm (subsurface soil) depths in each plot. The samples were pooled and homogenized in order to fully capture the diversity at the plot level, and the replicated plots were used to characterize the variation in the soil microbiota at each site. The soil samples were collected in July (mid-growing season) 2019 (N=24) and immediately transported on ice to the laboratory. All fresh soil samples were sieved to 2 mm, and visible roots and stones

were removed. Each sample was divided into two subsamples: one that was stored at -80 °C until DNA extraction and one that was stored at 4 °C for the measurement of soil properties. Basic information about the sites at the different elevations is provided in Supplementary Table S1.

2.2 *Soil physicochemical properties*

The soil pH was measured using a digital pH meter (MT-5000, Shanghai) in a soil water (1:5 w/v) suspension that had been shaken for 30 min. The soil moisture and bulk density (BD) were measured by the cutting ring method. The soil organic carbon (SOC) and total nitrogen (TN) contents were analysed after tableting using a J200 Tandem laser spectroscopic element analyser (Applied Spectra, Inc., Fremont, CA, USA), and the total phosphorus (P) content was determined by molybdenum blue colorimetry (TU-1901, Puxi Ltd., Beijing, China) after digestion with hydrofluoric acid and perchloric acid. The soil dissolved organic carbon (DOC) content was measured using a total organic carbon (TOC) analyser (Analytik Jena, Multi N/C 3000, Germany), and the soil nitrate (NO_3^--N), ammonium (NH_4^+-N), and total dissolved nitrogen (DTN) contents were determined using a continuous flow analytical system (AA3, Seal Co., Germany). The soil dissolved organic nitrogen (DON) was calculated from the soil NO_3^--N, NH_4^+-N, and DTN contents. The soil microbial biomass carbon and nitrogen (MBC and MBN) were determined by the chloroform fumigation method (Brookes et al., 1985; Joergensen, 1996).

2.3 *DNA extraction and PCR amplification*

Bacterial and fungal DNA was extracted from the soil samples using an E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, U.S.) according to the manufacturer's instructions. The DNA extracts were then mixed together and quantified with a 1.0% (w/v) agarose gel using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). The bacterial 16S and fungal ITS genes were amplified. For bacteria, the primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the V3-V4 region. For fungi, the primers ITS3F (5'-GCATCGATGAAGAACGCAGC-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the ITS2 region (Lee et al., 2012; Gade et al., 2013). All bacterial and fungal primers were performed with a thermocycler PCR system (GeneAmp 9700, ABI, USA). PCR was carried out in triplicate in a 20 μL mixture composed of 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The thermal cycling programs for bacteria and fungi were previously described elsewhere (Chen et al., 2021).

2.4 *Illumina MiSeq sequencing and processing of the sequencing data*

A 2% agarose gel was used to extract the PCR products, which were pooled together in equimolar amounts and then purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The products were quantified using a QuantiFluor™-ST fluorometer (Promega, USA) based on the manufacturer's protocols. Then, the amplicons were merged on the Illumina MiSeq platform (Illumina,

San Diego, USA) in equimolar amounts and paired-end sequenced (2 × 300 bp) following the standard protocols of Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The original sequencing data are available from the NCBI database (accession number: PRJNA721110 for bacteria, PRJNA721105 for fungi).

Trimmomatic was used to demultiplex the raw fastq files and conduct quality filtering, and the reads were merged with FLASH (Caporaso et al., 2012). UPARSE (version 7.1, http://drive5.com/uparse/) was used to cluster the sequences into operational taxonomic units (OTUs) based on 97% similarity (Edgar, 2013), and UCHIME was used to identify chimeric sequences. The taxonomic identities of the gene sequences for each 16S and ITS were assigned by BLAST against the SILVA bacterial and UNITE fungal ITS databases, respectively.

2.5 *Co-occurrence network analysis*

The co-occurrence networks for the different soil depths were constructed using the molecular ecological network analysis method (http://ieg4.rccc.ou.edu/mena/). According to the detailed descriptions of the algorithms and procedures by Deng et al. (2012) and Zhou et al. (2011), Spearman rank correlation was used to establish co-occurrence networks of the soil bacterial and fungal communities. We applied the same similarity threshold (*St*) to ensure that the co-occurrence networks at two contrasting depths could be compared with each other. Subsequently, the same network size and average number of links were used to generate 100 corresponding random networks. The *Z*-test was carried out to test for differences between the empirical network and the

248 random networks. The simplified classification and evaluation criteria applied were
249 those described in Deng et al. (2016) and Olesen et al. (2007). To show the results more
250 clearly, Cytoscape (version 3.7.1) was used to visualize the co-occurrence networks of
251 the soil bacteria and fungi (Cline et al., 2007).

252 2.6 *Data analysis*

253 We rarefied all samples based on the lowest sequence depth (49,674 sequences for
254 bacteria and 46,434 sequences for fungi) to minimize the impact of read-count variation
255 among the different samples. The alpha diversity indices (observed number of OTUs
256 (Sobs), Chao1, Faith's phylogenetic diversity (PD), and Simpson index) of the Illumina
257 MiSeq sequencing data were analysed with QIIME (Caporaso et al., 2012). The
258 Shapiro-Wilk test and Levene test were used to evaluate the normality of the data and
259 the homogeneity of variance. Nonmetric multidimensional scaling analysis (NMDS) of
260 the beta diversity based on Bray-Curtis distances was conducted with the 'vegan'
261 package in R (version 3.6.1) to analyse bacterial and fungal community similarity.
262 Analysis of similarities (ANOSIM) and permutation multivariate analysis of variance
263 (PERMANOVA) of the Bray-Curtis distances were conducted to test for differences in
264 the properties of the soil bacterial and fungal communities among different altitudes
265 and soil depths. Redundancy analysis (RDA) was performed to identify the major
266 factors driving the bacterial and fungal distribution along the altitudinal gradient
267 between two contrasting depths. A Mantel test with a Monte Carlo simulation consisting
268 of 999 randomizations was performed. Variation partitioning analyses (VPA) were

performed to explore the individual and joint effects of three sub-grouping factors (soil variables, plant community and altitude) on explaining the variations in community composition (R Core Team, 2013). The RDA and VPA were conducted in R package vegan. We extracted the sub-network and calculated each microbial sub-network community composition using principal component analysis (PCA) using R software. Based on the previous studies (Purahong et al., 2016), the first two axes scores of PCA were used to represent the microbial community composition. Spearman correlation analysis was used to analyse the relationship between the first two axes scores of PCA of each microbial sub-network and soil variables.

3 Results

3.1 Soil physicochemical properties along altitudinal gradients

Altitude had significant effects on the soil BD, soil moisture, ST, TP, pH, inorganic nitrogen, MBC, MBN and DON ($P<0.05$, Table 1). As the altitude increased, BD showed a significant decreasing trend, and soil moisture showed a significant increasing trend. The ST and soil pH at 830 m were significantly higher than those at altitude of 1300 m. Soil nitrate and ammonium nitrogen were the highest at 1300 m and 1100 m, respectively. The MBC at 1100 m was the highest and was 242.78% higher than that at 830 m; the MBN at 950 m was the highest and was 274.64% higher than that at 830 m. Soil depth had a significant effect on soil moisture and ST ($P<0.05$), and the soil moisture and ST of the surface soil were significantly higher than those of the subsurface soil. The interaction of altitude and soil depth had no significant effect on

any of the soil factors.

3.2 Soil bacterial and fungal sequencing summary and community composition

The 16S rRNA genes from soil bacteria and ITS genes from fungi were sequenced on the Illumina MiSeq platform. Across all soil samples analysed, 1,472,023 high-quality soil bacterial and 1,527,911 high-quality soil fungal sequences were obtained by Illumina MiSeq sequencing. A total of 49,674~74,237 (mean = 61,334) soil bacterial and 46,434~74,407 (mean = 63,662) soil fungal sequences were obtained per sample. The average read lengths for bacteria and fungi were 411 bp and 317 bp, which were larger than 99% Good's coverage for the 16S and ITS gene regions, respectively. The rarefaction curves of the genes tended to approach a saturation plateau at 97% sequence similarity for all samples (Fig. S2), which indicated that the sequencing depth was adequate to evaluate the structure and diversity of soil bacteria and fungi across all samples.

For soil bacteria, a total of 6577 OTUs were identified that were distributed among 31 phyla, 91 classes and 646 genera. Proteobacteria, Acidobacteria and Actinobacteria were the dominant phyla, accounting for 75.8% of the total number of bacterial sequences obtained (Fig. 1A). Altitude and soil depth had a significant effect on the relative abundances of Chloroflexi, Planctomycetes and Firmicutes (Table S2). Alphaproteobacteria, Acidobacteriia and Actinobacteria were the dominant classes, with relative abundances of 27.0%, 20.0% and 18.2%, respectively (Figure 1B). The interaction between altitude and soil depth had no significant effect on the relative

abundance of any of the bacterial phyla or classes (Table S2).

For soil fungi, a total of 2739 OTUs were identified that were distributed among 14 phyla, 51 classes and 548 genera. At the phylum level, fungal communities were dominated by Ascomycota and Basidiomycota, with relative abundances of 60.8% and 35.8%, respectively (Figure 1C). Altitude had a marked effect on the abundances of Ascomycota, Basidiomycota and Mucoromycota, and with increasing altitude, the relative abundance of Ascomycota showed a gradually decreasing trend (Table S3, Figure 1C). The dominant fungi at the class level were Agaricomycetes, Eurotiomycetes and Leotiomycetes, and their relative abundances accounted for 83.4% of the total number of fungal sequences (Figure 1D). Soil depth, altitude and their interaction had no significant effect on the relative abundance of any of the fungal phyla or classes (Table S3).

3.3 Soil bacterial and fungal community diversity

Altitude had a significant impact on the Sobs, Chao1 and Faith's PD diversity indices of the soil bacterial communities (Figure 2A-2D). In general, the diversity of bacterial communities decreased with increasing altitude. In the 0~10 cm soil layer, the Sobs, Chao1 and Faith's PD indices of soil bacteria at 830 m were 23.5%, 25.4% and 28.9% higher than those at 1300 m, respectively (P<0.05). In the 10~20 cm soil layer, the Sobs, Chao1 and Faith's PD indices of soil bacteria at 830 m were 21.1%, 23.3% and 26.2% higher than those at 1300 m (P<0.05). The soil depth had no significant effect on the alpha diversity of the soil bacterial community.

The fungal community alpha diversity index showed a potential increasing trend with altitude. In the 0~10 cm soil layer, the fungal Sobs, Chao1 and Faith's PD indices at 1300 m were 42.7%, 40.6% and 50.8% higher than those at 830 m, respectively, while there was no significant difference in the 10~20 cm soil layer (Figure 2E, 2G and 2H). The soil depth had no significant effect on the alpha diversity of the fungal community (Figure 2E-2H).

NMDS analysis based on Bray-Curtis distance was performed on the soil bacterial and fungal sequencing data corresponding to the different altitudes for two contrasting soil depths. The bacterial and fungal communities were partially grouped based on altitude, while there was no evident grouping by soil depth at the same altitude for bacteria or fungi (Figure 3A and 3B). Compared to the soil fungal communities, the bacterial communities at 950 m, 1100 m and 1300 m were more strongly clustered and more similar. ANOSIM and PERMANOVA revealed significant differences in the structures of the soil bacterial and fungal communities among altitudes (P<0.01, Figure 3). The results of the PERMANOVA of all samples demonstrated that altitude had a stronger influence than soil depth on the structure of the soil bacterial and fungal communities (P<0.01, Table S4).

3.4 Relationship between the soil microbial community and soil factors

The relationships between soil factors and the microbial community structure were evaluated by RDA and the Mantel test. The biplots showed that the first two axes explained more than 55.0% of the variation in both bacterial and fungal community

structure (Figure 4A and 4B). However, there were differences in the main factors affecting the bacterial and fungal community structure. For soil bacteria, pH was the main influencing factor, followed by BD and soil moisture. Notably, DON exerted a significant effect (R_{DON}^2=0.708) on the soil fungal community structure, followed by soil moisture, ammonium nitrogen and BD (Table 2).

Variance partitioning analysis (VPA) was performed to test the relative explanation of soil variables, plant community, and altitude on the bacterial and fungal communities (Figure 4C). It showed that soil variables and plant community accounted for the larger explanation for the variance of bacterial community composition in both two soil depths (Figure 4C, 4D). Compared with the results in surface soil, the combined effects (23.0%) of soil variables, plant community and altitude accounted for the highest explanation for bacterial community composition in subsurface soil (Figure S3B). For fungal community composition, the individual effect of soil variables explained 22.1% and 22.7% of the variation in surface and subsurface soil, respectively (Figure 4E, 4F).

3.5 Soil bacterial and fungal co-occurrence patterns

Bacterial and fungal co-occurrence networks were constructed for the different soil depths. For soil bacteria, the nodes of OTUs in the network belonged mainly to Proteobacteria, Actinobacteria, Chloroflexi and Acidobacteria, and the nodes of the bacterial community were divided into 11 and 21 modules in the surface and subsurface soil, respectively (Figure 5A, 5B). Compared with that at the surface soil layer, the number of nodes and connections of the bacterial community in the subsurface soil was

significantly higher, and the network topological characteristics for the subsurface soil included a higher average degree, average clustering coefficient and average path length (Table 3). In the surface and subsurface soil, 92.9% and 90.8% of the interaction connections were positive, respectively. For fungi, most of the nodes belonged to Ascomycota and Basidiomycota, and 12 modules were generated for each soil layer (Figure 5C, 5D). The two soil layers had similar numbers of nodes, links, average degrees and average clustering coefficients; moreover, the proportions of positive and negative connections in the two soil layers were similar. Compared with the 0~10 cm soil layer, the 10~20 cm layer soil fungal network had a higher average path length and degree of modularity (Table 3).

Based on the *Zi* and *Pi* values of the networks, we defined the peripheral nodes, network connectors, module hubs and network hubs in the network. *Zi-Pi* scatter plots for all bacterial and fungal nodes in the two contrasting soil layers were generated based on the module network. No node was both a module hub and a network connector. Of all the nodes, 98.3% and 97.7% were peripheral nodes in the bacterial and fungal networks, respectively, and the peripheral nodes were highly connected within their respective modules (Figure 6A, 6B). For the bacterial network, 11 nodes (belonging mainly to Proteobacteria and Acidobacteria) were classified as module hubs, and these nodes had strong associations with many nodes in their modules. Twelve nodes were specifically classified as connectors between modules (Figure 6A). In the fungal network, 10 nodes (belonging to Ascomycota and Basidiomycota) and 4 nodes (belonging to Ascomycota, Basidiomycota, and Mucoromycota) were classified as

module hubs and network connectors, respectively (Figure 6B).

We then investigated the correlations between sub-networks communities and soil variables by Spearman's correlation analysis. The strength of the correlations between the bacterial sub-networks communities and soil variables was higher than those in fungal sub-networks (Table S5 and S6). The altitude, soil BD and soil moisture correlated significantly most of bacterial sub-networks in both two soil depths (Table S5). For the fungal sub-networks, the largest module I in surface soil showed low correlations compared with module V. The module I and module V from fungal community in subsurface soil exhibited no correlations with any soil variables (Table S6). Soil BD and soil moisture were the main factors driving the most of fungal sub-networks. The sub-network community analysis revealed other important drivers (i.e. ST for bacteria and fungi; NO_3^--N for bacteria) that do not capture by whole community. ST was highly correlated with all bacterial modules II in surface soil and module II, IV, and V in subsurface soil, as well as in fungal module V in surface soil and module II and III in subsurface soil. The NO_3^--N had a significant effect on bacterial module II and IV in surface soil and module I and II in subsurface soil. Specific responses of sub-network communities were also detected for NH_4^+-N (for bacteria), and TN, TP, pH, DOC and MBN (for fungi).

4 Discussion

Our results highlighted several key findings related to the altitudinal distribution of soil bacterial and fungal communities in cold-temperate zones. First, similar to those

in temperate and tropical climates (Bahram et al., 2012; Miyamoto et al., 2014; Peay et al., 2017; Shen et al., 2019), the bacterial and fungal communities in the cold-temperate mountain ecosystem showed inconsistent patterns, that is, a monotonic decline and a monotonic increase, respectively. The bacterial and fungal community structures were more sensitive and fragile to altitude than to soil depth, and the variation in abiotic factors along the altitudinal gradient dominated the changes in the microbial community. Finally, the co-occurrence network of bacteria in the subsurface soil had high complexity and modularity, while the complexity of the fungal network did not change with increasing soil depth. In contrast to the whole community composition of microbes, the differential drivers were captured affecting the sub-networks (modules) communities of bacteria and fungi.

4.1 Divergent factors controlling bacterial and fungal diversities and community compositions along an altitudinal gradient

Previous studies of microbial diversity in mountain ecosystems reported different altitude-diversity patterns (Shen et al., 2013; Shen et al., 2014; Singh et al., 2014; Peay et al., 2017; Ren et al., 2018; Guo et al., 2020; Shen et al., 2020). Similar to the results of most studies performed with high-throughput sequencing technology (Li et al., 2018; Shen et al., 2015; Shen et al., 2019), we found that soil bacterial diversity decreased with increasing altitude; however, the fungal diversity increased with altitude, which partially supported our first hypothesis, that is, soil bacterial diversity showed a monotonically decreasing. Some recent studies have also emphasized the inconsistency

of bacterial and fungal biogeographical patterns (Peay et al., 2017; Bahram et al., 2018; Shen et al., 2020). Peay et al. (2017) pointed out that due to the notable differences in the life and evolutionary histories of different taxa, soil bacteria (single peak) and fungi (linear increase) on Mt. Hawaiian show different altitudinal distribution patterns. In general, the harshness of the environment increases with altitude, so it is expected that the abundance of bacteria and fungi would decrease along an altitudinal gradient (Margesin et al., 2009). However, we found that soil fungi maintained a higher diversity at high altitudes; this may be due to the higher soil nutrient levels (DON and ammonium nitrogen) at high altitudes promoting the growth of microorganisms (Peay et al., 2017). In addition, we found that the diversity of bacterial communities was higher than that of fungal communities, which is consistent with the study of Meng et al. (2013) in subtropical mountain ecosystems, this result implied that niche differentiation occurred for the different microbial groups along the altitudinal gradient in the cold-temperate zone (Prosser et al., 2007). In this study, the microbial abundances of the different taxa showed different responses to altitude and soil depth. Altitude had a marked effect on some of the more abundant bacterial phyla (Actinobacteria, Chloroflexi, Planctomycetes) and fungal classes (Agaricomycetes, Leotiomycetes, Pezizomycetes, Umbelopsidomycetes). Shen et al. (2020) recently conducted a more fine-resolution comparison of the diversity of bacterial and fungal communities on Mt. Kilimanjaro in East Africa and pointed out that the diversity patterns of taxonomic groups (phyla or classes) in bacterial and fungal communities were different and similar, respectively. Due to the uneven distribution of microbe-available nutrients and plant roots along soil

460 depths, soil depth may have a greater influence on soil microbial communities than
461 geographic location (Rousk et al., 2010). In this study, there were no significant
462 differences in community diversity between the surface layer and the subsurface layer
463 for either fungal or bacterial communities. Soil depth had a significant impact on the
464 bacterial community richness but had no significant effect on the fungal community
465 richness. This was probably because soil fungi have a narrower physiological range
466 than bacteria. For example, soil fungi are heterotrophic organisms, while soil bacteria
467 can be photosynthetic autotrophic organisms, heterotrophic organisms or
468 chemoautotrophic organisms (Lladó et al., 2017). Based on the results of the
469 PERMANOVA, we further verified that in this cold-temperate mountain ecosystem, the
470 influence of altitude on the community structure of bacteria and fungi was stronger than
471 that of soil depth.

472 Vegetation community and soil heterogeneities may have potential effects on soil
473 microbial communities (Curd et al., 2018), we found that soil variables and plant
474 community had larger explanations in bacterial community composition in both two
475 soil depths than those of altitude (Figure S3A and S3B). Indeed, different plant
476 identities harbored potential bacterial community in soil (Berg and Smalla 2009). As
477 we expected, the soil pH in this cold-temperate mountain ecosystem was a good
478 predictor of the soil bacterial community composition; this finding is consistent with
479 those of earlier studies (Shen et al., 2013; Bahram et al., 2018; Shen et al., 2019). The
480 pH range in this study (value 3.92~4.74) was similar to that in a study on Changbai
481 Mountain (value 3.89~6.31) (Shen et al., 2013), although the pH variability in our study

was very small. A previous study reported the effect of soil pH variations within a small range on bacterial community structure (Sagova-Mareckova et al., 2015). Rousk et al. (2010) pointed out that the composition of bacterial communities was affected mainly by the soil pH rather than by diffusion limitations among microbial communities or other environmental factors (Tian et al., 2018). Although many studies have reported on the relationship between pH and bacterial community composition and diversity, in our study, soil moisture also played an important role in influencing the bacterial community. The study of Shen et al. (2020) on Mt. Kilimanjaro pointed out that the average annual rainfall was the second most important factor in predicting soil bacterial diversity; rainfall indirectly affects soil bacterial communities by regulating pH and plant productivity (Tian et al., 2018). Although many studies have reported on the relationship between temperature and soil fungal communities (Jarvis et al., 2015; Newsham et al., 2016; Shen et al., 2020), our results were not in line with our expectation that temperature would be the main factor affecting the diversity and composition of the fungal community. In our study, the soil variables had a higher explanation in fungal community compositions in both two soil depths, DON played the most important role in affecting the composition of the soil fungal community, followed by soil moisture. Dissolved organic matter (DOM) is an important component of soil organic matter and provides organic substrates and resources for heterotrophic microorganisms (Benner, 2011; Huang et al., 2020). Huang et al. (2020) found in a recent study that DOM quality was the most important driving factor explaining the diversity and community composition of soil fungi. In this study, altitude had a

504 significant impact on DON. Vegetation types at different altitudes have specific effects
505 on soil physical and chemical properties. In particular, the composition of plant litter
506 can lead to certain differences in the composition of soil organic matter (Quideau et al.,
507 2001), resulting in the development of different soil microclimates (Knelman et al.,
508 2012). Shen et al. (2016) reported that DOC can be used to predict the functional genetic
509 diversity of microorganisms in the Changbai Mountain ecosystem, from the forest to
510 the tundra. Additionally, in a study along a small-scale altitudinal gradient on the
511 Changbai Mountain tundra, Ni et al. (2018) found that the abundances of Ascomycota
512 and ectomycorrhizal fungi were significantly correlated with the contents of DON and
513 NH_4^+-N, respectively.

514 *4.2 Potentially more connected network of soil bacteria in surface soil than that in*
515 *subsurface soils*

516 The microbial community is composed of a complex combination of highly
517 interactive taxa (Fuhrman, 2009). Understanding the correlations among microbial taxa
518 is essential for predicting the responses of microbial communities to climate change,
519 and microbial co-occurrence networks with lower complexity are considered to be
520 easily stressed by the environment (Banerjee et al., 2019). It is worth noting that
521 although soil depth had only a small effect on the composition and diversity of bacterial
522 and fungal communities, the co-occurrence network of bacteria and fungi showed
523 differential patterns to soil depth; this result was consistent with our third hypothesis,
524 that is, the soil bacterial and fungal communities in surface and subsurface soils exhibit

different network topology properties. For the bacterial community, the differences of the network between different soil layers were more obvious; that is, the network of subsurface soil had greater modularity and density and more highly connected nodes than the surface layer. Inversely, the fungal co-occurrence networks in the different soil layers were not obviously different. To the best of our knowledge, this is the first reported study of the co-occurrence network of microorganisms along soil depths in the cold-temperate zone of China. In a recent study, de Vries et al. (2018) found that the network of soil fungi was more stable in response to extreme conditions than the bacterial network; in addition to vegetation composition, soil moisture played a key role in controlling these networks. In this study, the soil moisture and temperature were highly variable along the soil depths, which may lead to the differences in the response of these sub-networks of different microbial groups in the two contrasting soil layers. A recent study by Tu et al. (2020) on six forests in the United States found that the ST and soil water content were highly correlated with the modularity of the microbial co-occurrence network. In addition, one possible mechanism to explain the more connected network was the reduction in root input, metabolites and the number of available substrates in the subsurface soil, which would have caused more competition for or co-metabolism of substrates of a wide variety of bacterial communities (Upton et al., 2020). Despite no significant effects of several soil variables on the whole bacterial and fungal community composition, interestingly, some factors (ST, NO_3^--N and NH_4^+-N for bacteria; ST, TN, TP, pH, MBN, and DOC for fungi) showed distinct roles in driving the sub-network communities. These environmental variables will select

547 microorganisms with similar niche adaptability to form sub-networkcommunities (de
548 Menezes et al., 2015; Purahong et al., 2016). We infer that the analysis of the modular
549 networks could provide more detailed pictures and fine-resolution of microbial
550 community assembly.

551 Compared with the fungal network, the bacterial network was more complex, and
552 the correlations between the soil variables and bacterial sub-networks were higher,
553 which also implied that the bacterial communities in the cold-temperate mountain
554 ecosystem are more sensitive to the variation in environmental factors along soil depths.
555 In contrast to our results, Xiao et al. (2018) compared *Phyllostachys edulis* plantations
556 and noted that the degree of connectivity of the bacterial network was lower than that
557 of the fungal community network, which might imply that the interaction patterns of
558 microorganisms vary between these different habitats. In this study, OTUs belonging to
559 Proteobacteria and Actinobacteria functioned mainly as modular hubs and network
560 connectors in the bacterial networks and thus played a critical role in the bacterial co-
561 occurrence networks of the different soil layers. Proteobacteria is usually the dominant
562 nitrogen-fixing bacterial phylum in soil ecosystems (Gaby and Buckley, 2011).
563 Actinobacteria exhibit a mycelial growth pattern in the soil that allows plants to expand
564 their surface area into a deeper soil layer to absorb nutrients; these mycelia form soil
565 aggregates and act as active components that preserve water and nutrients (Fierer et al.,
566 2013; Upton et al., 2020). However, the bacterial networks of the surface and subsurface
567 soil layers had different keystone taxa, which further confirmed the existence of niche
568 differentiation among the bacterial taxa along the soil depths.

5 Conclusions

This study describes for the first time the biogeographic distribution of soil microbial communities in cold-temperate mountain ecosystems in China, as well as more fine-resolution analysis in modules community along the soil depths. Our results confirmed that soil bacterial (monotonically decreasing) and fungal (monotonically increasing) diversity showed inconsistent altitudinal distribution patterns. The dramatic variations in soil properties along the altitudinal gradient were the main factors driving the variation in the community composition and diversity of bacteria (driven by pH) and fungi (driven by DON). Although the soil microbial community was affected more by the altitudinal gradient than by the soil depth, the network analysis further emphasized the obvious differences in the bacterial and fungal communities between the two contrasting soil layers. The soil bacterial communities were more sensitive to changes in soil variables along the soil depths than fungal communities, and bacterial networks in subsurface soils exhibited more complex and compact topological features. Further research could focus on specific taxa, microbial interactions, and the functions of keystone taxa in forest ecosystems. Such work is essential for achieving a better understanding of the mechanisms that affect microbial diversity and functions in this fragile ecosystem.

CRediT authorship contribution statement

Li Ji: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Visualization, Funding acquisition. **Fangyuan Shen:** Writing -review &

editing. **Yue Liu:** Investigation, Visualization. **Yuchun Yang:** Conceptualization. **Jun Wang:** Investigation. **Witoon Purahong:** Conceptualization, Visualization, Writing - review & editing. **Lixue Yang:** Conceptualization, Writing - review & editing, Funding acquisition. All authors helped to edit and complete the manuscript.

Declaration of competing interest

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

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Table 1 The soil physicochemical property for surface and subsurface soils in different altitudes

Table 2 Mantel test results for the correlation between relative abundance of bacterial and fungal genera and soil variables in different soil depths along the altitudinal gradient.

Table 3 The topological properties for soil bacterial and fungal co-occurrence networks in different soil depths

868 **Table 1 The soil physicochemical property for surface and subsurface soils in different altitudes**

Soil variables	830 m		950 m		1100 m		1300 m		Two-way ANOVA		
	Surface soil	Subsurface soil	Surface soil	Subsurface soil	Surface soil	Subsurface soil	Surface soil	Subsurface soil	Altitude	Depth	A×D
BD ($g \cdot cm^{-3}$)	0.89±0.13Aa	1.18±0.16Aa	0.63±0.10ABa	0.81±0.08Ba	0.58±0.13ABa	0.64±0.04Ba	0.34±0.02Ba	0.45±0.04Ba	***	ns	ns
Soil moisture (%)	30.75±3.48Ca	20.34±1.31Cb	37.48±1.75BCa	30.7±3.41BCb	47.14±3.85Ba	41.71±5.89Bb	71.01±3.96Aa	58.32±3.55Ab	***	**	ns
ST (℃)	11.12±0.17Aa	7.00±0.58Bb	10.41±0.19ABa	9.02±0.29Ab	10.25±0.35ABa	9.48±0.84Ab	9.01±0.87Ba	9.23±0.28Ab	*	*	ns
SOC ($mg \cdot g^{-1}$)	61.28±2.65Aa	62.08±3.75Aa	65.62±3.26Aa	62.03±0.23Ba	63.14±1.45Aa	61.22±1.91Ba	61.67±2.54Aa	63.83±2.62Aa	ns	ns	ns
TN ($mg \cdot g^{-1}$)	7.02±0.18Aa	6.87±0.32Aa	6.64±0.30Aa	6.97±0.01Aa	6.88±0.15Aa	7.06±0.12Aa	6.97±0.17Aa	6.75±0.20Aa	ns	ns	ns
TP ($mg \cdot g^{-1}$)	0.48±0.05Aa	0.56±0.03Aa	0.19±0.03Ba	0.62±0.04Aa	0.44±0.14Aa	0.34±0.07Aa	0.52±0.17Aa	1.59±0.17Aa	*	ns	ns
pH	4.55±0.02Aa	4.74±0.36Aa	4.31±0.02ABa	4.21±0.18Aa	4.23±0.04ABa	4.28±0.06Aa	4.01±0.45Ba	4.23±0.07Aa	*	ns	ns
NO_3^--N ($mg \cdot kg^{-1}$)	5.39±0.08Ba	5.35±0.25Ba	5.92±0.18Ba	5.62±0.04Ba	5.57±0.09Ba	5.39±0.24Ba	6.97±0.32Aa	6.43±0.31Aa	***	ns	ns
NH_4^+-N ($mg \cdot kg^{-1}$)	63.25±5.23Ba	63.34±5.25Ba	61.27±2.32Ba	58.59±0.53Ba	94.68±4.35Aa	87.72±2.44Aa	88.09±14.84ABa	82.02±3.88Aa	***	ns	ns
DOC ($mg \cdot kg^{-1}$)	163.33±7.96Ba	148.13±4.80Ba	269.60±17.07Aa	295.87±9.12Aa	291.47±7.54Aa	262.80±11.31Aa	213.07±4.92Aa	233.33±12.41ABa	ns	ns	ns
DON ($mg \cdot kg^{-1}$)	15.92±2.31Ba	11.48±1.75Ba	17.57±1.61Ba	15.34±1.22Ba	33.33±2.59Aa	36.57±2.12Aa	32.61±6.74Aa	33.73±1.85Aa	***	ns	ns
MBC ($mg \cdot kg^{-1}$)	456.84±26.86Ba	307.02±11.88Bb	602.81±59.38Ba	538.95±45.01Ba	1565.96±98.72Aa	1515.79±24.21Aa	785.26±54.04Ba	885.26±75.11ABa	*	ns	ns
MBN ($mg \cdot kg^{-1}$)	16.05±1.36Ba	10.11±1.79Ba	60.13±5.40Aa	34.69±1.89Ab	37.78±1.86Ba	36.23±1.15Aa	34.29±1.15Ba	34.59±1.53Aa	*	ns	ns

869 A: Altitude; D: Soil depth. BD, Bulk density; ST, Soil temperature; SOC, Soil organic carbon; TN, Total nitrogen; NO_3^--N, Nitrate nitrogen; NH_4^+-N, Ammonium nitrogen; DOC, Dissolved organic carbon; DON,
870 Dissolved organic nitrogen; MBC, Microbial biomass carbon; MBN. Microbial biomass nitrogen. Data with different uppercase letters were significantly difference at 5% level among different altitudes in the same soil
871 layer (P<0.05), while different lowercase letters indicate significant differences among different soil layers in the same altitude (P<0.05); ns, not significant; *, P<0.05; **, P<0.01; All data were mean ± standard error
872 (Mean ± SE)

Table 2 Mantel test results for the correlation between relative abundance of bacterial and fungal genera and soil variables in different soil depths along the altitudinal gradient.

Soil variables	Bacteria		Fungi	
	R^2	*P*	R^2	*P*
BD	0.414	**0.004**	0.396	**0.008**
Soil moisture	0.376	**0.005**	0.567	**0.001**
ST	0.090	0.358	0.112	0.279
SOC	0.123	0.240	0.007	0.934
TN	0.004	0.961	0.129	0.211
TP	0.300	0.051	0.043	0.591
pH	0.442	**0.002**	0.210	0.061
NO_3^--N	0.227	0.079	0.259	**0.046**
NH_4^+-N	0.196	0.107	0.510	**0.002**
DOC	0.182	0.112	0.048	0.623
DON	0.273	**0.043**	0.708	**0.001**
MBC	0.197	0.102	0.346	**0.012**
MBN	0.344	**0.014**	0.065	0.451

BD, Bulk density; ST, Soil temperature; SOC, Soil organic carbon; TN, Total nitrogen; NO_3^--N, Nitrate nitrogen; NH_4^+-N, Ammonium nitrogen; DOC, Dissolved organic carbon; DON, Dissolved organic nitrogen; MBC, Microbial biomass carbon; MBN. Microbial biomass nitrogen.

Table 3 The topological properties for soil bacterial and fungal co-occurrence networks in different soil depths

Network features		Bacteria		Fungi	
		Surface soil	Subsurface soil	Surface soil	Subsurface soil
Empirical network	Similarity threshold (St)	0.890	0.890	0.840	0.840
	Number of nodes	558	764	306	304
	Number of links	595	1092	424	416
	R^2 of power-law	0.948	0.916	0.911	0.884
	Number of positive correlations	553 (92.9%)	992 (90.8%)	359 (84.7%)	352 (84.6%)
	Number of negative correlations	42 (7.1%)	100 (9.2%)	65 (15.3%)	64 (15.4%)
	Average degree (avgK)	2.133	2.859	2.771	2.737
	Average clustering coefficient (avgCC)	0.097	0.133	0.154	0.163
	Average path distance (GD)	6.083	6.352	6.756	7.633
	Modularity	0.858	0.770	0.785	0.800
Random network	avgCC±SD	0.004±0.002	0.007±0.002	0.011±0.004	0.012±0.005
	GD±SD	6.115±0.148	4.995±0.060	4.991±0.111	4.809±0.106
	Modularity±SD	0.791±0.006	0.648±0.005	0.651±0.008	0.650±0.008

Figure 1 Relative abundances of main soil bacterial and fungal phyla (A, C) and classes (B, D) for surface and subsurface soils in different altitudes. 830_T, 950_T, 1100_T and 1300_T indicate the surface soil in 830 m, 950 m, 1100 m and 1300 m, respectively. 830_S, 950_S, 1100_S and 1300_S indicate the subsurface soil in 830 m, 950 m, 1100 m and 1300 m, respectively.

Figure 2 Sobs, Shannon, Chao1 and Faith's PD indices of soil bacterial (A~D) and fungal (E~H) communities for surface and subsurface soils in different altitudes. OTUs were delineated at 97% sequence similarity. These indices were calculated using bacterial and fungal random subsamples of 49674 and 46343 sequences per sample. Two-way ANOVA for altitude and soil depth was conducted. Data with different uppercase letters were significantly difference at 5% level among different altitudes in the same soil layer (P<0.05), while different lowercase letters indicate significant differences among different soil layers in the same altitude (P<0.05).

Figure 3 Nonmetric multidimensional scaling analysis of soil bacterial (A) and fungal (B) communities based on Bray-Curtis distances.

Figure 4 Redundancy analysis based on soil bacterial (A) and fungal (B) community at the genus level and soil factors (red arrows). The variation partition analysis of bacterial (C, D) and fungal (E, F) community. C, E: Variation was partitioned in surface soil; D, F: Variation was partitioned in subsurface soil. The top 20 most abundant classified bacterial and fungal genera (97% sequence similarity) in the soil samples. Direction of arrow indicates the soil factors associated with changes in the community structure, and the length of the arrow indicates the magnitude of the association. The asterisk represents the significant soil factors associated with the bacterial or fungal community. The percentage of variation explained by RDA 1 and 2 is shown. BD, Bulk density; SM, Soil moisture; ST, Soil temperature; NH4, Ammonium nitrogen; DON, Dissolved organic nitrogen; MBC, Microbial biomass carbon; MBN. Microbial biomass nitrogen.

Figure 5 Overview of the co-occurrence networks for bacterial (A, B) and fungal (C, D) communities in surface (A, C) and subsurface (B, D) soils. Node size is proportional to the relative abundance. Major phylum (with nodes > 5) were randomly colored. Positive links between nodes were colored red and negative links were colored blue. **Spearman's correlations between sub-networks and environmental variables in bacterial (E) and fungal (F) communities.** Significant correlation coefficients (P < 0.05) are shown in bold. BD, Bulk density; SM, Soil moisture; ST, Soil temperature; SOC, Soil organic carbon; TN, Total nitrogen; NO_3^--N, Nitrate nitrogen; NH_4^+-N, Ammonium nitrogen; DOC, Dissolved organic carbon; DON, Dissolved organic nitrogen; MBC, Microbial biomass carbon; MBN. Microbial biomass nitrogen.

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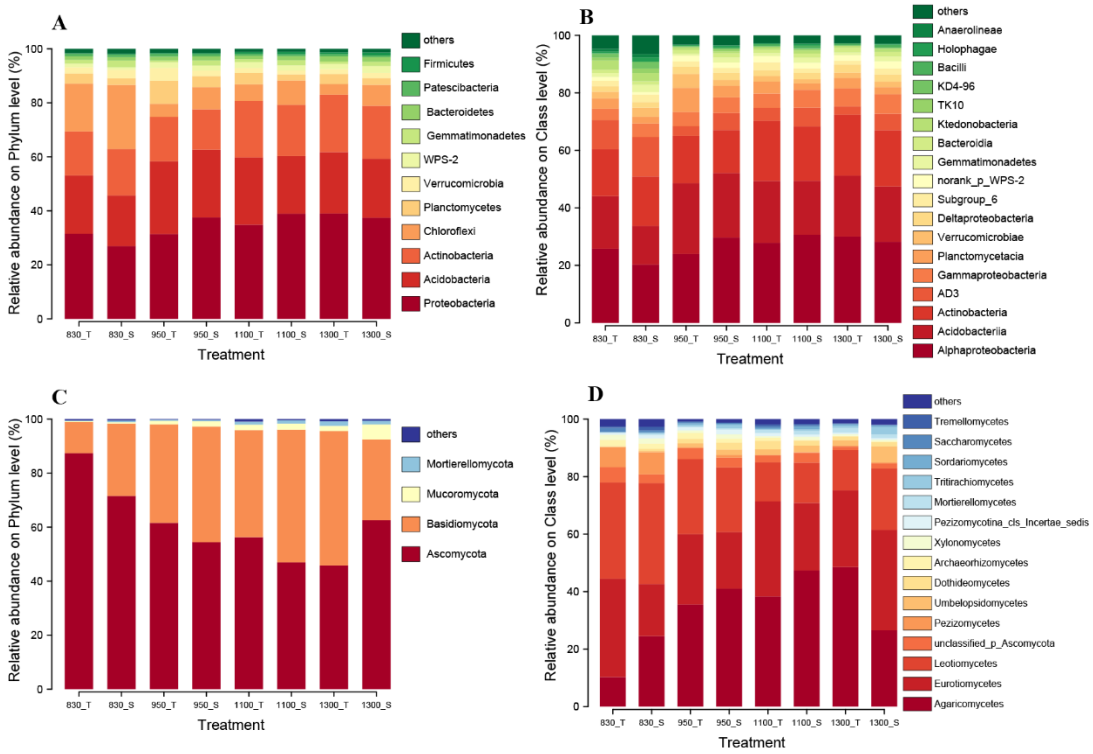
Figure legends



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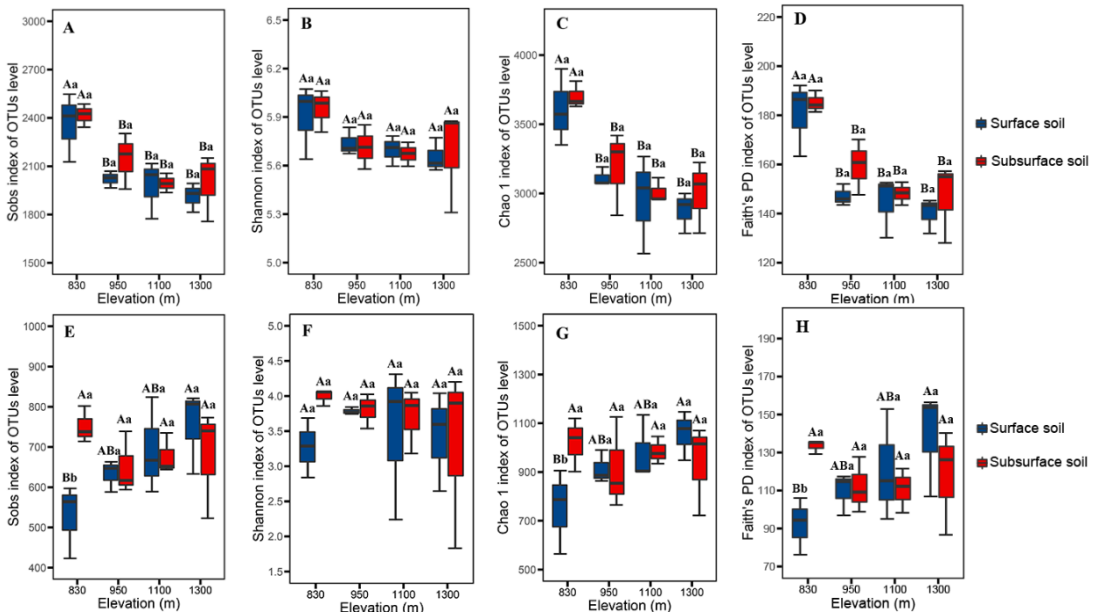

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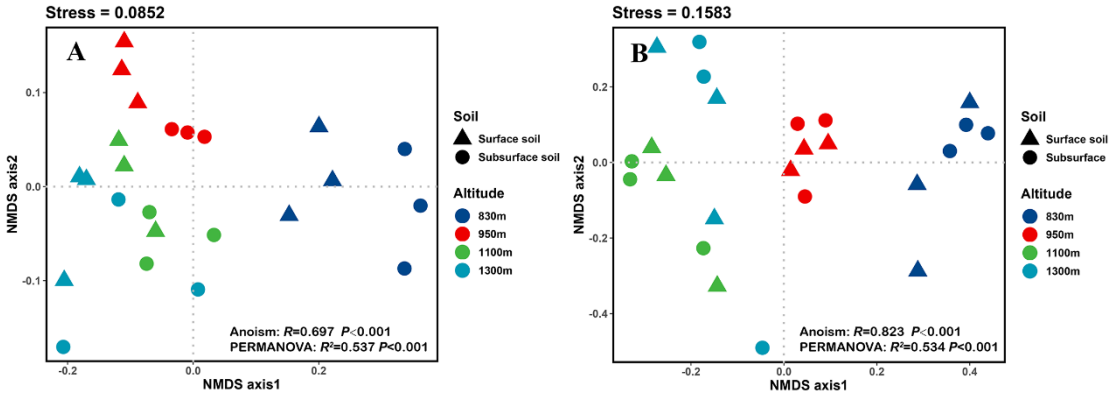

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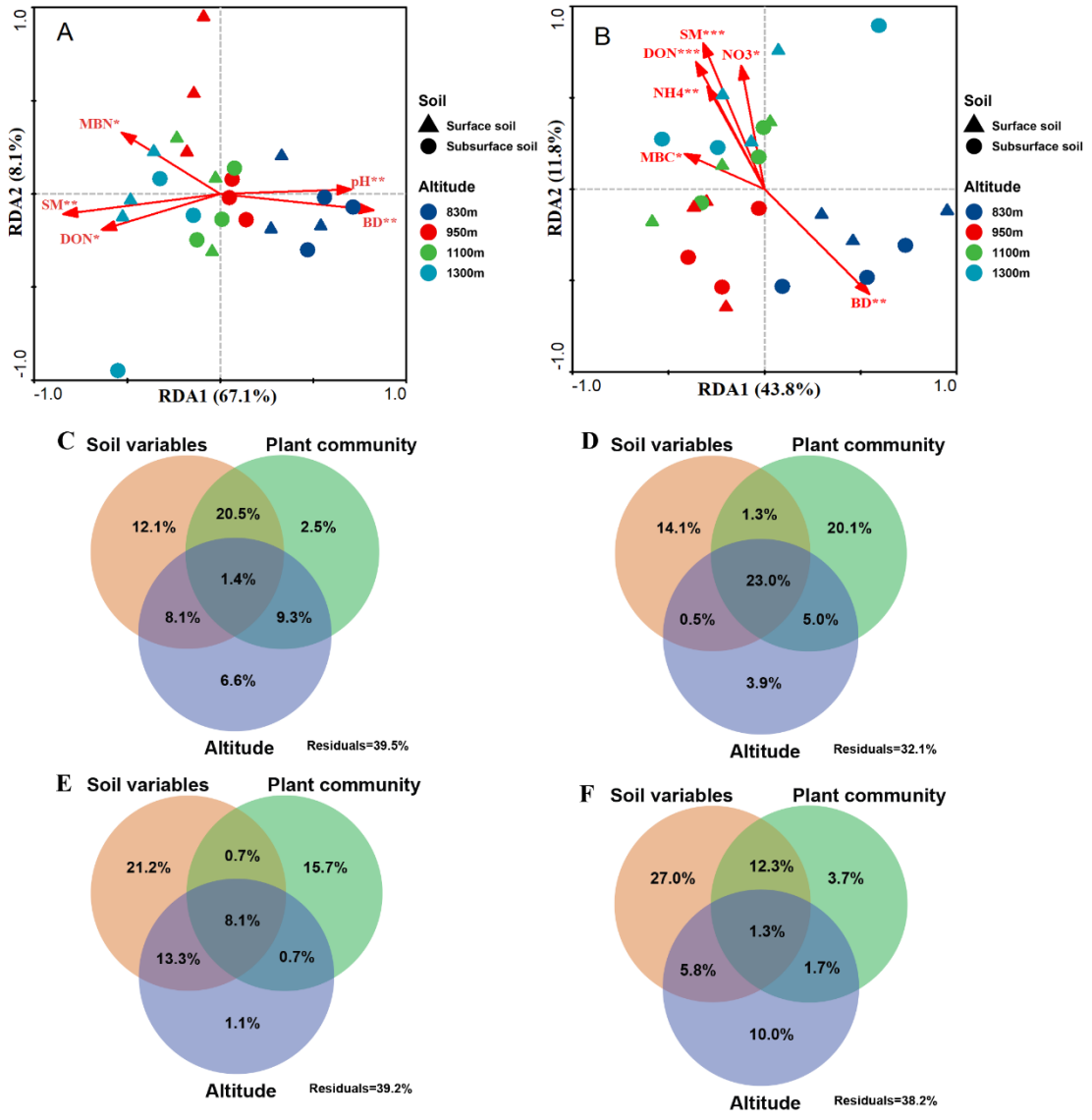

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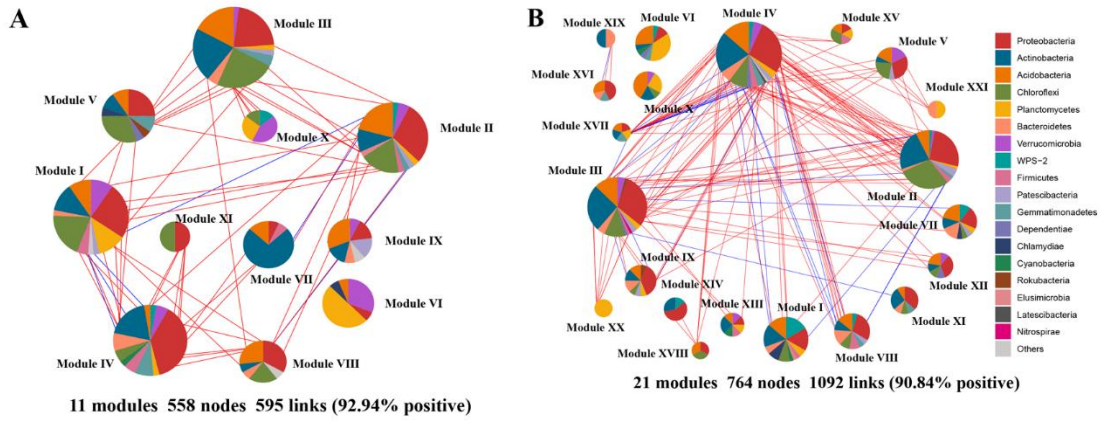

E

	Surface soil					Subsurface soil				
	Module I	Module II	Module III	Module IV	Module V	Module I	Module II	Module III	Module IV	Module V
Altitude	**0.94**	**0.94**	**0.76**	**0.94**	**0.71**	**0.98**	**0.84**	**0.82**	**0.87**	**0.72**
BD	**0.73**	**0.81**	**0.72**	**0.75**	0.64	**0.82**	**0.77**	**0.94**	**0.93**	**0.95**
SM	**0.80**	**0.96**	0.66	**0.87**	0.54	**0.91**	**0.85**	**0.79**	**0.84**	**0.73**
ST	0.64	**0.73**	0.66	0.67	0.53	0.65	**0.82**	0.67	**0.79**	**0.72**
SOC	0.27	0.34	0.43	0.29	0.44	0.29	0.15	0.51	0.35	0.15
TN	0.36	0.29	0.39	0.27	0.36	0.26	0.11	0.47	0.25	0.08
TP	0.38	0.20	0.32	0.36	0.50	0.60	0.65	0.32	0.21	0.18
pH	**0.85**	**0.84**	**0.79**	**0.83**	**0.75**	0.47	**0.72**	**0.70**	**0.78**	0.68
NO_3^--N	0.66	**0.88**	0.67	**0.73**	0.50	**0.85**	**0.89**	0.39	0.32	0.35
NH_4^+-N	0.62	0.53	0.50	0.63	0.48	0.62	0.47	0.44	**0.78**	0.39
DOC	0.37	0.46	0.45	0.38	0.61	0.23	0.52	0.60	0.58	0.65
DON	0.66	0.64	0.54	**0.75**	0.51	**0.81**	0.64	**0.79**	**0.88**	**0.73**
MBC	0.33	0.24	0.41	0.42	0.33	0.46	0.52	0.43	0.44	0.33
MBN	0.66	0.39	0.59	0.40	0.60	0.43	0.60	**0.70**	0.61	0.51

F

	Surface soil					Subsurface soil				
	Module I	Module II	Module III	Module IV	Module V	Module I	Module II	Module III	Module IV	Module V
Altitude	0.52	0.61	0.23	0.40	**0.85**	0.26	**0.91**	**0.86**	**0.76**	0.22
BD	**0.68**	0.49	0.41	0.43	**0.67**	0.31	**0.80**	**0.85**	**0.79**	0.36
SM	0.46	0.65	0.16	0.47	**0.82**	0.30	**0.80**	**0.89**	**0.74**	0.31
ST	0.51	0.20	0.15	0.45	**0.79**	0.53	**0.86**	**0.74**	0.40	0.61
SOC	0.44	0.65	0.64	0.35	0.18	0.15	0.37	0.24	0.36	0.21
TN	0.36	**0.70**	0.66	0.40	0.17	0.22	0.34	0.36	0.48	0.31
TP	0.37	0.53	0.44	0.26	0.16	0.13	0.20	**0.77**	0.58	0.24
pH	0.54	0.21	0.37	0.48	**0.80**	0.31	**0.78**	0.58	0.29	0.43
NO_3^--N	0.48	**0.82**	0.18	0.64	**0.77**	0.10	0.41	**0.84**	0.42	0.35
NH_4^+-N	0.63	0.30	0.34	0.23	**0.72**	0.55	**0.86**	0.38	0.54	0.48
DOC	0.65	0.13	0.65	0.64	0.33	0.54	0.34	**0.69**	**0.83**	0.48
DON	0.61	0.20	0.33	0.32	**0.71**	0.47	**0.96**	0.64	**0.67**	0.46
MBC	0.56	0.02	**0.80**	0.40	**0.81**	0.23	0.63	0.48	0.33	0.12
MBN	0.57	**0.75**	**0.81**	**0.69**	0.35	0.36	0.57	0.66	**0.70**	0.41

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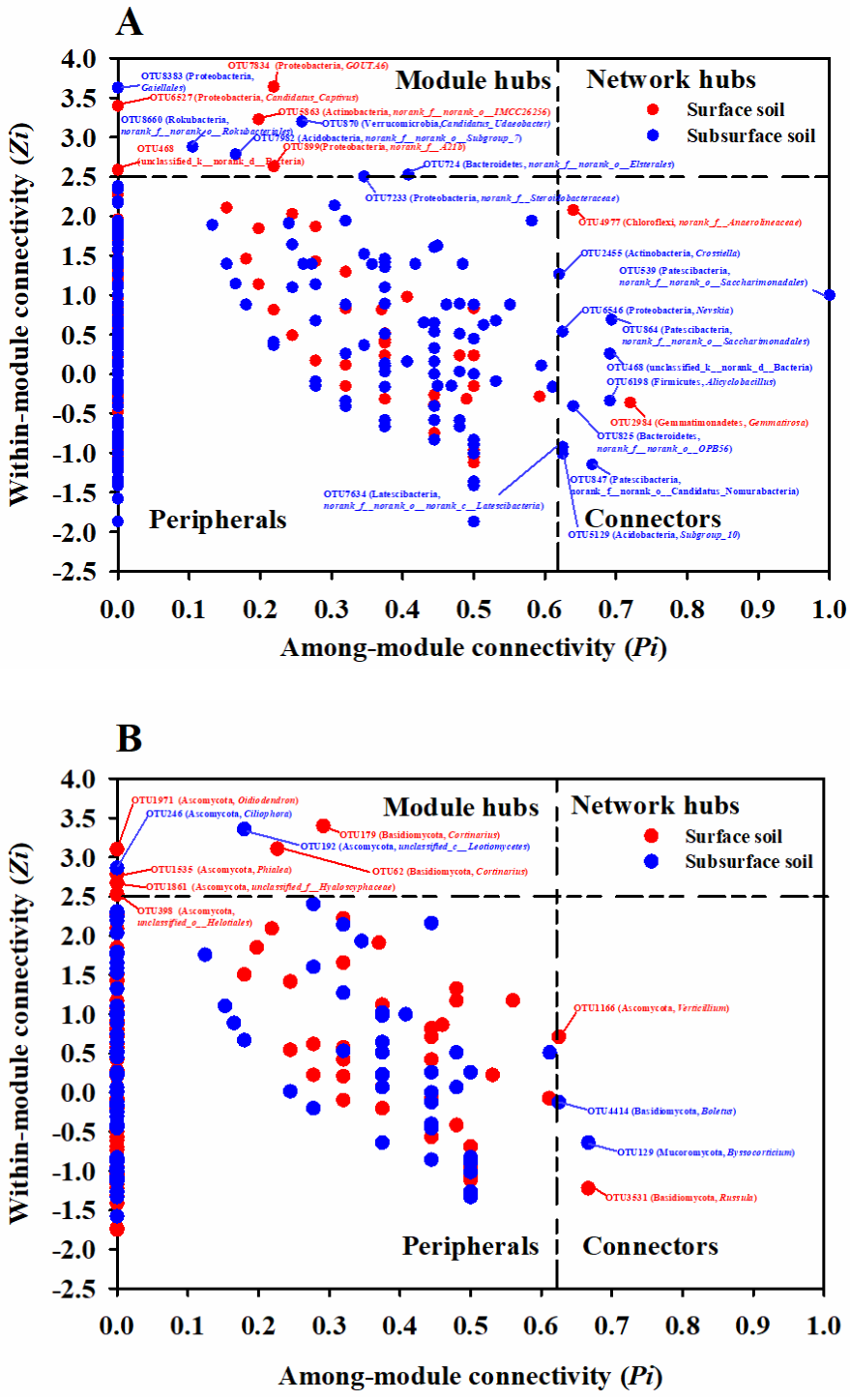

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Supplementary information

Supplementary Table 1 Site characteristics of different altitudes

Altitude (m, a.s.l.)	Coordinates	Soil type	Vegetation type	Dominant taxa
830	N 51°47′41″ E 122°5′3″	Umbric Cryosols	Cold temperate coniferous forest	*Larix gmelinii*、*Vaccinium vitis-idaea*、*Ledum palustre*、*Rosa davurica*、*Lonicera caerulea*、*Rubus arcticus*、*Pyrola incarnata*、*Deyeuxia angustifolia*
950	N 51°49′42″ E 122°3′34″	Umbric Cryosols	Cold temperate coniferous forest	*Larix gmelinii*、*Betula platyphylla*、*Vaccinium vitis-idaea*、*Ledum palustre*、*Rhododendron dauricum*、*Spiraea dahurica*、*Rubus sachalinensis*、*Sambucus williamsii*、*Rosa davurica*、*Artemisia lagocephala*、*Cimicifuga foetida*、*Vicia ramuliflora*、*Pyrola incarnata*
1100	N 51°49′89″ E 122°2′76″	Gelic Podzols	Cold temperate coniferous forest	*Larix gmelinii*、*Pinus sylvestris*、*Pinus pumila*、*Betula platyphylla*、*Vaccinium vitis-idaea*、*Ledum palustre*、*Rhododendron dauricum*、*Spiraea dahurica*、*Rubus sachalinensis*、*Sambucus williamsii*、*Artemisia lagocephala*、*Clematis sibirica*、*Cimicifuga foetida*、*Vicia ramuliflora*
1300	N 51°50′14″ E 122°2′19″	Gelic Podzols	Cold temperate coniferous forest	*Larix gmelinii*、*Pinus pumila*、*Betula ermanii*、*Rhododendron dauricum*、*Vaccinium vitis-idaea*、*Ledum palustre*、*Artemisia lagocephala*、*Aquilegia viridiflora*、*Saxifraga bronchialis*、*Polygonum alpinum*

a.s.l. = above sea level

Supplementary Table 2 Two-way analysis of variance of relative abundance of dominant bacterial phyla and classes

Taxonomy		Altitude		Soil depth		Altitude×Soil depth	
		F	*P*	F	*P*	F	*P*
Phyla	Proteobacteria	3.146	0.054	0.213	0.651	1.236	0.329
	Acidobacteria	1.431	0.271	1.276	0.275	0.084	0.968
	Actinobacteria	4.928	**0.013**	0.965	0.341	0.390	0.762
	Chloroflexi	61.919	**<0.001**	19.336	**<0.001**	0.610	0.618
	Planctomycetes	8.590	**0.001**	16.985	**0.001**	1.956	0.161
	Verrucomicrobia	2.161	0.133	1.003	0.331	1.961	0.161
	WPS-2	2.957	0.064	0.038	0.849	0.601	0.623
	Gemmatimonadetes	3.094	0.057	26.875	**<0.001**	0.453	0.719
	Bacteroidetes	1.334	0.298	0.187	0.671	0.170	0.915
	Patescibacteria	5.807	**0.007**	3.676	0.073	0.559	0.650
	Firmicutes	16.033	**<0.001**	6.011	**0.026**	0.886	0.469
Classes	Alphaproteobacteria	1.822	0.184	0.021	0.886	1.312	0.305
	Acidobacteriia	3.282	**0.048**	2.909	0.107	0.149	0.929
	Actinobacteria	4.928	**0.013**	0.965	0.341	0.390	0.762
	AD3	29.807	**<0.001**	16.862	**0.001**	0.240	0.867
	Gammaproteobacteria	1.946	0.163	1.403	0.253	0.060	0.980
	Planctomycetacia	8.801	**0.001**	17.336	**0.001**	1.958	0.161
	Verrucomicrobiae	2.161	0.133	1.003	0.331	1.961	0.161
	Deltaproteobacteria	2.134	0.136	0.066	0.801	0.208	0.889
	Subgroup_6	0.483	0.699	0.004	0.951	1.412	0.276
	norank_p_WPS-2	2.957	0.064	0.038	0.849	0.601	0.623
	Gemmatimonadetes	3.094	0.057	26.875	**<0.001**	0.453	0.719
	Bacteroidia	1.112	0.373	0.185	0.673	0.162	0.920
	Ktedonobacteria	11.820	**<0.001**	0.071	0.793	0.301	0.824
	Saccharimonadia	3.782	**0.032**	6.279	**0.023**	0.621	0.611
	TK10	61.669	**<0.001**	28.633	**<0.001**	1.667	0.214
	KD4-96	25.943	**<0.001**	3.668	0.074	1.886	0.173
	Bacilli	19.137	**<0.001**	6.165	**0.024**	1.004	0.417
	Holophagae	25.750	**<0.001**	31.642	**<0.001**	2.966	0.063
	Anaerolineae	57.998	**<0.001**	4.146	0.059	0.914	0.456
	Blastocatellia_Subgroup_4	12.031	**<0.001**	4.791	**0.044**	2.389	0.107

Supplementary Table 3 Two-way analysis of variance of relative abundance of dominant fungal phyla and classes

Taxonomy		Altitude		Soil depth		Altitude×Soil depth	
		F	P	F	P	F	P
Phyla	Ascomycota	4.127	**0.024**	0.388	0.542	1.299	0.309
	Basidiomycota	4.249	**0.022**	0.265	0.614	2.028	0.150
	Mucoromycota	3.284	**0.048**	2.357	0.144	1.162	0.355
	Mortierellomycota	3.232	0.050	0.090	0.767	0.241	0.867
	Rozellomycota	2.201	0.128	2.570	0.128	0.788	0.518
Classes	Agaricomycetes	4.838	**0.014**	0.107	0.747	2.473	0.099
	Eurotiomycetes	0.260	0.853	0.583	0.456	0.501	0.687
	Leotiomycetes	9.501	**0.001**	0.250	0.624	0.608	0.619
	unclassified_p_Ascomycota	2.448	0.101	0.195	0.664	0.898	0.464
	Pezizomycetes	12.362	**<0.001**	0.190	0.669	0.034	0.991
	Umbelopsidomycetes	3.394	**0.044**	2.301	0.149	1.212	0.338
	Dothideomycetes	3.807	**0.031**	0.055	0.817	1.044	0.400
	Archaeorhizomycetes	0.959	0.436	0.245	0.628	0.047	0.986
	Xylonomycetes	5.949	**0.006**	1.722	0.208	0.597	0.626
	Pezizomycotina_cls_Incertae_sedis	9.129	**0.001**	3.539	0.078	3.430	0.052
	Mortierellomycetes	3.234	0.050	0.091	0.767	0.239	0.868
	Tritirachiomycetes	3.968	**0.027**	3.563	0.077	1.279	0.315
	Sordariomycetes	2.881	0.068	3.403	0.084	1.229	0.332
	Saccharomycetes	3.150	0.054	2.026	0.174	0.597	0.626
	Tremellomycetes	9.458	**0.001**	1.049	0.321	2.981	0.063
	unclassified_p_Rozellomycota	2.535	0.093	2.641	0.124	0.775	0.525

Supplementary Table 4 Non-parametric multivariate analysis (PERMANOVA) of soil bacterial and fungal community by altitude and soil depth

		Df	Sums of Sqs	Mean Sqs	F.Model	R^2	Pr(>F)
Bacteria	Altitude	3	0.760	0.253	7.732	0.537	**0.001**
	Soil depth	1	0.121	0.121	2.073	0.086	0.076
Fungi	Altitude	3	2.457	0.819	7.650	0.534	**0.001**
	Soil depth	1	0.137	0.137	0.675	0.029	0.727

Supplementary Table 5 Heatmap of the correlations between individual bacterial sub-network communities (top five sub-network) and soil variables.

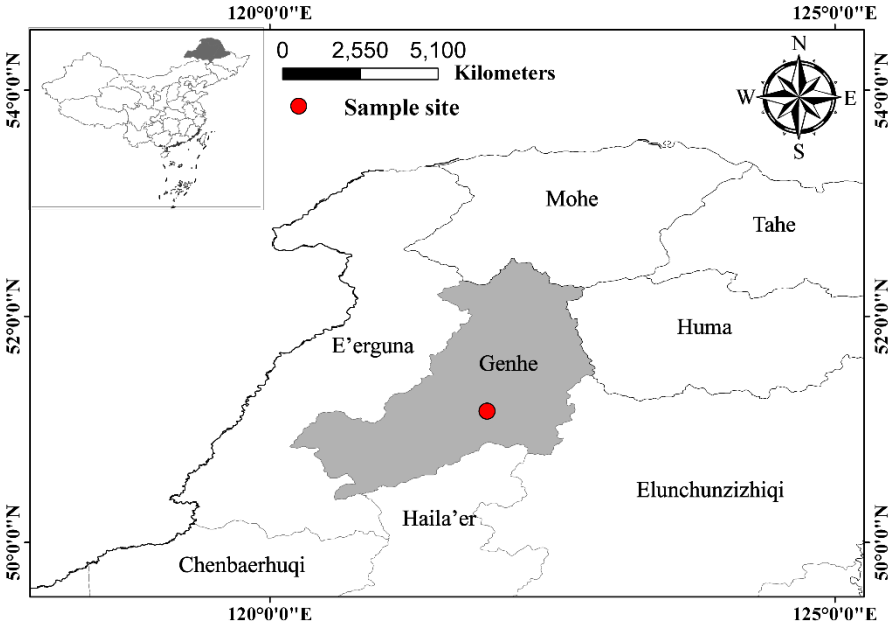
Soil variables	Surface soil										Subsurface soil									
	Module 1		Module 2		Module 3		Module 4		Module 5		Module 1		Module 2		Module 3		Module 4		Module 5	
	PC1 (61.3 %)	PC2 (30.3 %)	PC1 (69.6 %)	PC2 (14.8 %)	PC1 (97.8 %)	PC2 (0.8 %)	PC1 (71.4 %)	PC2 (11.2 %)	PC1 (92.3 %)	PC2 (4.7 %)	PC1 (68.3 %)	PC2 (18.0 %)	PC1 (84.2 %)	PC2 (7.3 %)	PC1 (82.6 %)	PC2 (7.3 %)	PC1 (69.6 %)	PC2 (15.1 %)	PC1 (89.7 %)	PC2 (5.3 %)
Altitude	**0.97**	0.13	**0.86**	0.19	**0.82**	0.09	**0.97**	0.28	**0.82**	0.07	**0.80**	**0.63**	**0.73**	0.28	0.50	**0.78**	**0.91**	0.09	**0.71**	0.01
BD	**0.85**	0.06	**0.90**	0.15	**0.74**	0.04	**0.81**	0.32	**0.68**	0.01	0.56	**0.68**	**0.87**	0.27	**0.64**	**0.65**	**0.94**	0.16	**0.87**	0.01
SM	**0.90**	0.08	**0.87**	0.27	**0.73**	0.06	**0.91**	0.27	**0.71**	0.02	**0.71**	0.53	**0.75**	0.43	0.50	**0.71**	**0.85**	0.11	**0.76**	0.08
ST	**0.81**	0.15	**0.78**	0.11	**0.84**	0.04	**0.69**	0.34	**0.84**	0.09	**0.74**	0.09	0.54	0.31	0.46	0.52	**0.60**	0.18	**0.65**	0.10
SOC	0.03	0.33	0.19	0.04	0.05	0.37	0.01	0.27	0.01	0.34	0.27	0.27	0.17	0.07	0.07	0.45	0.13	0.37	0.31	0.15
TN	0.07	0.39	0.18	0.08	0.08	0.32	0.06	0.29	0.00	0.36	0.22	0.32	0.27	0.01	0.02	0.34	0.14	0.36	0.32	0.22
TP	0.28	0.29	0.16	0.23	0.16	0.32	0.29	0.35	0.25	**0.66**	0.06	0.21	0.18	**0.58**	0.08	0.14	0.06	0.24	0.27	0.20
pH	**0.86**	0.03	**0.80**	0.01	**0.73**	0.05	**0.81**	0.33	**0.86**	0.13	0.26	0.03	0.30	0.10	0.32	0.28	0.19	0.45	0.42	0.02
NO_3^--N	**0.76**	0.10	**0.83**	0.06	**0.84**	0.08	**0.70**	0.32	**0.70**	0.15	0.54	0.03	0.33	**0.85**	0.12	0.25	0.27	0.06	0.14	0.36
NH_4^+-N	**0.66**	0.12	0.39	0.31	0.41	0.32	**0.66**	0.25	**0.65**	0.11	**0.58**	0.34	0.33	0.04	0.25	0.47	**0.68**	0.40	0.27	0.24
DOC	0.20	0.32	0.18	0.46	0.13	0.34	0.03	0.26	0.41	0.34	0.01	0.06	0.11	0.21	0.30	0.01	0.11	0.18	0.25	0.32
DON	**0.66**	0.17	0.56	0.25	0.42	0.22	**0.80**	0.08	**0.59**	0.10	0.52	**0.71**	0.57	0.20	0.41	**0.73**	**0.84**	0.18	**0.71**	0.09
MBC	0.11	0.32	0.28	0.20	0.05	0.20	0.32	0.22	0.11	0.23	0.55	0.57	**0.62**	0.18	0.47	**0.65**	**0.69**	0.11	**0.68**	0.17
MBN	0.16	0.07	0.28	0.14	0.41	0.36	0.30	0.03	0.15	**0.70**	0.55	0.18	0.36	0.13	0.55	0.43	0.54	0.24	0.30	0.34

Significant correlation coefficients ($P < 0.05$) are shown in bold

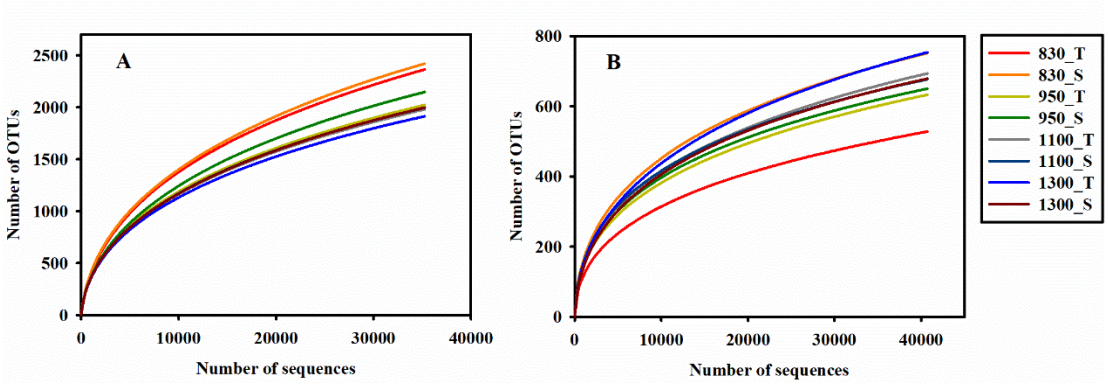
Supplementary Table 6 Heatmap of the correlations between individual fungal sub-network communities (top five sub-network) and soil variables.

Soil variables	Surface soil										Subsurface soil									
	Module 1		Module 2		Module 3		Module 4		Module 5		Module 1		Module 2		Module 3		Module 4		Module 5	
	PC1 (85.8 %)	PC2 (9.9 %)	PC1 (83.6 %)	PC2 (13.9 %)	PC1 (77.3 %)	PC2 (12.3 %)	PC1 (80.7 %)	PC2 (16.2 %)	PC1 (52.2 %)	PC2 (28.4 %)	PC1 (75.2 %)	PC2 (18.7 %)	PC1 (97.1 %)	PC2 (1.6 %)	PC1 (85.4 %)	PC2 (8.0 %)	PC1 (67.3 %)	PC2 (28.6 %)	PC1 (72.5 %)	PC2 (18.6 %)
Altitude	0.43	0.22	0.52	0.13	0.43	0.26	0.52	0.02	**0.84**	0.52	0.24	0.45	**0.86**	0.17	**0.78**	0.19	**0.65**	0.13	0.41	0.04
BD	0.32	0.05	0.36	0.33	0.35	0.15	0.41	0.06	**0.64**	**0.63**	0.36	0.46	**0.68**	0.08	**0.82**	0.19	**0.78**	0.11	0.51	0.07
SM	0.45	0.15	0.38	0.22	0.44	0.21	0.41	0.04	**0.79**	0.55	0.34	0.42	**0.66**	0.03	**0.85**	0.29	**0.68**	0.12	0.41	0.06
ST	0.43	0.13	0.27	0.09	0.46	0.29	0.48	0.06	0.53	**0.64**	0.51	0.55	0.49	0.41	**0.68**	0.02	0.53	0.13	0.03	0.39
SOC	0.39	0.11	0.18	0.51	0.12	0.38	0.18	0.06	0.17	0.09	0.07	0.02	0.29	0.38	0.25	0.14	0.21	0.10	0.23	0.06
TN	0.31	0.18	0.25	**0.58**	0.13	0.48	0.27	0.17	0.13	0.11	0.08	0.15	0.26	0.41	0.28	0.21	0.27	0.27	0.31	0.06
TP	0.21	0.57	0.34	0.15	0.06	0.25	0.13	0.25	0.19	0.08	0.10	0.15	0.33	0.08	0.46	0.52	0.51	0.08	0.12	0.36
pH	0.45	0.19	0.40	0.03	0.47	0.33	0.49	0.07	**0.85**	0.38	0.20	0.24	0.22	0.41	0.35	0.05	0.16	0.22	0.08	0.14
NO_3^--N	0.08	0.33	0.52	0.15	0.19	0.35	0.55	0.36	0.56	**0.63**	0.01	0.04	0.19	0.05	**0.60**	**0.67**	0.14	0.48	0.19	0.47
NH_4^+-N	**0.70**	0.50	0.20	0.29	0.54	0.18	0.36	0.25	**0.78**	0.14	0.11	0.57	**0.75**	0.57	0.18	0.21	**0.62**	0.39	0.22	0.18
DOC	0.32	0.46	0.15	0.04	0.36	0.16	0.18	0.46	0.22	0.02	0.51	0.11	0.13	0.25	0.20	0.30	0.33	**0.58**	0.31	0.49
DON	**0.64**	**0.59**	0.32	0.20	0.57	0.23	0.39	0.15	**0.77**	0.11	0.33	0.55	**0.88**	0.31	0.43	0.26	**0.66**	0.55	0.33	0.26
MBC	0.34	0.29	0.32	0.41	0.44	0.29	0.40	0.17	0.46	0.11	0.34	0.49	**0.84**	0.13	0.48	0.20	0.51	0.22	0.23	0.01
MBN	0.34	0.36	**0.61**	**0.58**	0.42	**0.81**	**0.62**	0.56	0.21	0.47	0.43	0.44	0.49	0.15	0.48	0.26	**0.76**	0.36	0.28	0.01

Significant correlation coefficients ($P < 0.05$) are shown in bold



Supplementary Figure 1 Location of the Oakley Mountains in Greater Khingan Mountains



Supplementary Figure 2 The Rarefaction curves of the number of operational taxonomic units (OTUs) for soil bacterial (A) and fungal (B) communities. Random subsamples of 49674 and 46434 gene per sample were used to generate the rarefaction curves. OTUs were delineated at 97% sequence similarity. 830_T, 950_T, 1100_T and 1300_T indicate the surface soil in 830 m, 950 m, 1100 m and 1300 m, respectively. 830_S, 950_S, 1100_S and 1300_S indicate the subsurface soil in 830 m, 950 m, 1100 m and 1300 m, respectively.