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Soil depth exerts stronger impact on bacterial community than elevation in subtropical forests of Huangshan Mountain

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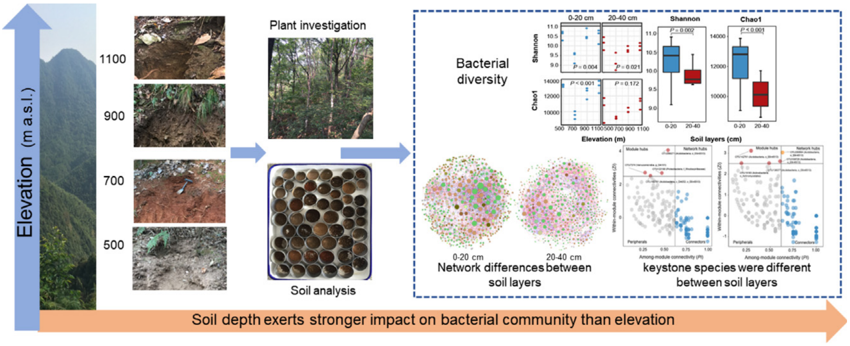
HIGHLIGHTS

- Soil bacterial diversity showed a U shape along elevation in both soil layers.
- Soil depth exerted stronger influence on the soil bacterial community than elevation.
- LOC mainly drove the bacterial community composition in surface soils.
- Elevation was the main driver of bacterial community composition in subsurface soils.
- Bacterial network properties and keystone species differ between soil layers.

GRAPHICAL ABSTRACT



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ABSTRACT

The elevational distribution of bacterial communities in the surface soil of natural mountain forests has been widely studied. However, it remains unknown if microbial communities in surface and sub-surface soils exhibit a similar distribution pattern with elevation. To do so, Illumina HiSeq sequencing was applied to study the alterations in soil bacterial communities of different soil layers, along an altitudinal gradient from 500 to 1100 m on Huangshan Mountain in Anhui Province, China. Our results revealed a significant higher diversity of the bacterial communities in surface soil layers than in subsurface layers. Adonis analysis showed that soil layer had a greater influence on the composition of the bacterial communities than the elevation. The distance-based multivariate linear model suggested that soil labile organic carbon and elevation were the main element influencing the bacterial community composition in surface and subsurface soils, respectively. A remarkable difference appeared between the co-occurrence network structures of bacterial communities in different soil layers. Compared with the subsurface soil, surface soil had more edges, average degree, and much higher clustering coefficient. The two-way ANOVA results highlighted the significant impact of soil layers on the topological properties of the network compared with that of elevation. The keystone species belonged to Rhodospirillaceae in the surface soil, while the OTUs belonged to Actinomycetales in the subsurface soil. Collectively, our results demonstrate that the effects of soil depth on soil bacterial community composition and network properties of subtropical forest in Huangshan Mountain were significantly higher than those of elevation, with

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different keystone species in different soil layers. These findings can be served as an important basis for better understanding the microbial functions influencing the maintenance of habitat heterogeneity, biodiversity, and ecosystem services in forests ecosystems.

1. Introduction

The search for generalizable biodiversity patterns is a core task in ecology research, in which elevational diversity patterns are indispensable for comprehensively understanding the influence of climate change on ecosystems (Gotelli et al., 2009; Malhi et al., 2010). Although there is no consensus on these patterns, several hypotheses have been proposed to explain the observed elevational patterns over the past century, such as climatic factors and the mid-domain effect (Wang et al., 2010). Soil bacteria are an important component of below-ground biota, and their diversity pattern with elevation has been reported by many researches, but the observed elevational patterns were quite different, including decreasing, unimodal, or regular patterns (Bryant et al., 2008; Fierer et al., 2011; Shen et al., 2015). However, these literatures have solely focused on the near surface soil layers.

Apparently, deep soil microbial communities undoubtedly play crucial roles in regulating nutrient cycling and biogeochemical processes due to the root distribution (Chu et al., 2016; Fierer et al., 2003; Zhou et al., 2014). Differences in soil properties with soil depths may lead to the different ecological processes and underlying mechanisms (Stegen et al., 2012), as well as the different degrees of environmental filtering or interspecific competition (Wang et al., 2013). Basic ecological processes can be classified into two general sections: deterministic and stochastic processes (Luan et al., 2020). Zhu et al. (2020) observed that bacterial community assemblies were mainly controlled by deterministic processes, elevation and seasonal changes had no effect on bacterial community. The fungal community at the soil surface is predominantly dispersal limitations and decreases significantly with soil layer, while stochastic processes (ecological drift) showed a contrary trend (Hussain et al., 2021). Previous studies have shown that soil physicochemical properties (e.g., pH, C/N ratio and nutrients' availability) influenced the community structure of microorganisms through controlling soil nutrient cycling (Nakayamaa et al., 2019). In the surface soil of Taibai Mountain (Shaanxi Province, China), pH was found to be the main factor affecting bacterial diversity, while pH and soil carbon availability were the main driving factors in deep soil (Tian et al., 2021). So far, most of studies have focused on surface layer, or have only singularly studied microbial variation with elevation or with soil layers, with less researches on the interactive effect elevation and soil layers. The vulnerability of subsurface microbial communities to environmental change can be predicted by the relationship between above-ground vegetation and subsurface microorganisms (Ding et al., 2022). This can enhance the function of keeping soil healthy in the face of constant future climate change, as well as stabilizing ecosystems.

It is well known that bacteria are not individuals that live alone, they play an essential role in regulating the structure of soil microbial communities and ecosystem function by forming complex co-occurrence networks with fungi and plants through indirect and direct interactions (Hallam and McCutcheon, 2015). Recent works have rarely applied the network analysis to address the vertical distribution of microbial communities in relation to the soil depth (Luan et al., 2020; Yang et al., 2017a, 2017b). Soil depth can alter microbial evolution rates such as community assembly. In most cases, the microbial community of subsurface soil is relatively simple, whereas that of, surface soil has a high degree of complexity (Du et al., 2018). Moreover, keystone species are highly connected taxa that, regardless of their abundance, have a strong influence on the compositional structure and function of microbial communities (Banerjee et al., 2018). For example, Fan et al. (2019) identified the main keystone species *Geobacter* spp. with nitrogen fixation under long-term fertilization by constructing a co-occurrence network of all samples. However, the keystone taxa of soil microbial communities in subtropical forest ecosystems are poorly investigated. Combined with current studies on the structure of microbial communities at elevation, studying microbial interactions with the elevation in different soil layers will provide new directions and perspectives for studying soil microbial patterns.

Forests are an integral part of terrestrial ecosystems, providing vital ecological services (Šimpraga et al., 2019). In this study, investigates the diversity of soil layers at different elevations of soil bacteria, community structure, community assembly mechanisms, and bacterial co-occurrence network that reflect changes in biological interactions, in subtropical forests. Considering that the reduction of soil organic matter's transport with increasing soil depth, we assumed that bacterial diversity would be significantly higher in surface soils than in subsurface soils (Yang et al., 2017a, 2017b).

Based upon which, we developed two hypotheses for the community composition and co-occurrence networks. One is that soil bacteria have divergent community driving factors and assembly processes in different soil layers due to environmental and competitive interactions for resources or the variability of dispersal barriers across soil layers. The other is that the network structure of soil surface is more complex, with a significantly higher number of edges, average degree, and graph density, leading to the enrichment of organic matter content and more active microbial communities.

2. Materials and methods

2.1. Site selection and soil sampling

Huangshan national positioning observation and research station of forest ecosystem is located on Jiulongfeng Nature Reserve (117°58′–118°04′ E and 30°04′–30°08′ N), Anhui Province, China (Fig. S1). This region has a humid subtropical monsoon climate with rich flora and fauna species. Across an elevational gradient ranging from 500 to 1200 m a.s.l., in this area, the average annual temperature and precipitation are 15.5 °C and 1759 mm, respectively. Soil was sampled from four sites along an elevation gradient (500 m, 700 m, 900 m and 1100 m a.s.l) on a south-facing slope. At each elevation, a representative soil sample was collected composed of five subsamples from three sampling points. At each sampling point, a total of 24 soil samples were collected at depths (0–20 and 20–40 cm). All samples were immediately shipped to the laboratory in ice-packed boxes. Prior to the analyses, we passed all the fresh samples through 2 mm sieve, and then store one quarter of the sieved sample at 4 °C for physical and chemical properties, and the remaining three quarters of the other part at −80 °C for subsequent DNA extraction. The plant species composition was measured at every elevation, including the species and number. Please see Table S1 for the detailed information of the vegetation and soil at each elevation.

2.2. Measurement of soil properties

A total of nine soil variables were measured. Soil moisture (SM) was measured gravimetrically by drying the samples in an oven at 105 ± 5 °C to a constant mass. Soil pH and electrical conductivity (EC) were measured using pH meter and conductivity meter (soil-to-water ratio, 1:2.5), respectively. The total organic carbon (TOC) was measured via a total organic carbon analyzer. (Analytik Jena AG, Multi N/C3100, Germany). Soil dissolved organic carbon (DOC) was extracted via deionized water at water-soil ratio of 2:1 by shaking the mixture for 30 min then mixture the centrifuged. The suspension was filtered, and the organic carbon content in the filtrate was determined with a TOC-TN analyzer (Shimadzu Corp., Kyoto, Japan). The soil labile organic carbon (LOC) was determined using a sequential fumigation-incubation method (Stanford and Smith, 1972). Soil carbon and nitrogen were measured using a 2400 II CHN elemental analyzer, and then the C/N ratio was calculated. Soil available K (AK) was analyzed using a flame photometer. The summary of the soil variables from the four elevations are listed in Table S2.

2.3. DNA extraction, amplicon sequencing and bioinformatic analyses

According to the manufacturer's instructions, bacterial DNA was extracted from the soil samples using FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, USA). Purified DNA was quantified by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA). Amplification of extracted DNA for the V4–V5 variable region of bacterial 16S rDNA, amplified using the primer 515F (5′ - GTGCCAGCMGCCGCGGTAA - 3′) and 907R (5′ - CCGTCAATTCMTTTRAGTTT - 3′). Please see Liu et al. (2021) for more details. The PCR products of all samples were normalized to equimolar amounts and then sequenced via an Illumina HiSeq PE250 platform (Bainuosheng Bio-pharm Technology Co., Ltd., Hefei, China).

We employed FLASH V1.2.7 (http://ccb.jhu.edu/software/FLASH) to assemble the paired-end sequences (Magoc and Salzberg, 2011). The data were then processed mainly using Qiime 1.9.0 to conduct quality filtering, length trimming, chimera checking, OTU clustering, and taxonomy assignment (Rideout et al., 2014), and then clustered into OTUs according to a 97 % similarity threshold based on the Usearch algorithm (Edgar, 2010). Comparison of SILVA bacterial database by BLAST and then the gene sequences of each 16S were identified taxonomically. To avoid sequencing and sampling bias, 29,000 sequences were randomly selected per sample.

2.4. Network analyses

By using non-random co-occurrence network method to elucidate the bacterial interactions in different soil layers and elevations, co-occurrence network structures were determined using the SPIEC-EASI R packages in *R* 4.0.4 to build SparCC networks (Layeghifard et al., 2017). Retain the 1000 most dominant OTUs for SparCC analysis. The correlation coefficients were restricted to those having correlation >0.65 or <-0.65 ($P < 0.001$, two-sided) (Friedman and Alm, 2012). Visualization was done with Gephi 0.9.2, using the ForceAtlas 2 layout (Jacomy et al., 2014). Using the igraph package to obtain the sub-network topological network properties, the network nodes are defined using the method proposed by Deng and Wu (2012). Zi and Pi values were also calculated using the igraph package in *R*. Two-way ANOVA was applied to detect the effect of elevation and soil layer on the network topological properties. Calculate the Spearman correlation coefficient to assess the relationship between network properties and environmental parameters, and to assess the impact of different biogeographic parameters on co-occurrence network parameters. Network plots were visualized using the interactive platform Gephi 0.9.2 with an undirected network layout (Kurtz et al., 2015).

2.5. Statistical analysis

The Chao1 and Shannon-Wieners diversity indices were used to show bacterial α-diversity in different soil layers and elevation. The data were first tested for the normal distribution (Table S3). Then the difference in α-diversities between two soil layers was determined using the independent *t*-tests and one-way ANOVA tests. The significance of the effects of elevation and soil layers on the bacterial alpha diversity indices was determined by Two-way ANOVA tests. Spearman's correlation analysis was applied to constrain the relationship between the soil bacterial α-diversity and environmental variables and between the relative abundances of phyla/network parameters and environmental variables. Permutational multivariate analysis of variance (Adonis) was evaluated the impact of environmental factors of different soil layers the bacterial community. The relationship between the community dissimilarity and the elevation was studied using non-metric multidimensional scaling analyses (NMDS) in vegan package. A permutation multivariate analysis of variance (PerMANOVA) using Bray-Curtis distances was applied to examine the differences in soil bacterial communities among different soil depths. Adonis was used to detect whether soil layer or elevation contributed more to the community. To determine the relationship between the community composition and environmental variables, a distance-based multivariate analysis was performed using a linear model (McArdle and Anderson, 2001). The normalized stochasticity ratio (NST) was conducted to distinguish the different soil bacterial community assembly processes (Ning et al., 2019). Distance-based multivariate linear model (DistLM) forward 3 software was used to test the correlation between soil bacterial communities and soil properties (Anderson, 2001). All statistical analyzes were conducted in *R* 4.0.4.

3. Results

3.1. Bacterial diversity and community composition

A lower alpha diversity index was observed at 700 m elevation compared with those at other elevations, and one-way ANOVA revealed significant differences in the bacterial Shannon and Chao1 indices among altitudes (Fig. 1a). The Shannon and Chao1 indices were significantly higher at 0–20 cm soil layer than those at 20–40 cm (Fig. 1b). Two-way ANOVA showed that Chao1 was significantly influenced by soil layers and elevations ($P = 0.004$ and $P = 0.026$, Table S4).

The collected soil layers had different phylum levels in terms of community composition. In accordance, Proteobacteria phylum and Acidobacteria prevailed at 0–20 and 20–40 cm, respectively (Fig. 2a and b). Non-metric multidimensional analysis based on the Bray-Curtis distance showed that the microbial community of different soil layers was clearly divided (Fig. 3). The PerMANOVA analysis further confirmed that the bacterial community of soil surface significantly differed from that in subsurface ($P = 0.002$, Fig. 3). We found significant variation along with altitude in the subsurface soil, and weaker variation with elevation in the surface soil. And, the Adonis analyses showed that soil depth explained 12.4 % of the variation in bacterial community composition, whereas elevation accumulatively explained 7.9 % of the variation (elevation, $P < 0.007$, $R^2 = 0.079$; layer, $P < 0.001$, $R^2 = 0.124$).

3.2. Effects of environmental variables on soil bacterial community and possible assembly mechanisms of community construction

The Adonis results in the surface soil showed that the bacterial community composition was significantly correlated with soil pH, EC, TOC, and LOC. In the surface soil, LOC made the greatest contribution to the bacterial community, and elevation contributed most to the variance in the subsurface soil (Table S5). In addition, the linear model DistLM analysis revealed that soil LOC (13.52 %) was the most significant driver factor influencing the composition of the soil bacterial community in surface soil, but that elevation (15.76 %) was the primary driver factor in the subsurface (Table S6). Analysis of the correlation between environmental factors and diversity showed that surface bacterial alpha diversity was significantly correlated with soil pH and EC, while in subsurface soil, it was only correlated with AK (Table S7). Relative abundance of dominant taxa as related to environmental variables by Spearman correlation analysis (Fig. S2). The relative abundances of Firmicutes were found to be negatively associated with DOC, TOC, LOC, elevation, and soil electrical conductivity in the surface soil, and positively associated with pH. In contrast, the relative abundance of Chloroflexi was significantly positively correlated with an elevation in the subsurface soil.

The Jaccard distance index-based NST demonstrated that bacteria in both surface and subsurface soils were primarily controlled by stochastic processes that mirrored β-diversity patterns (Fig. S3). The magnitude of the stochasticity varied from 83.17 % to 80.57 % in different soil layer

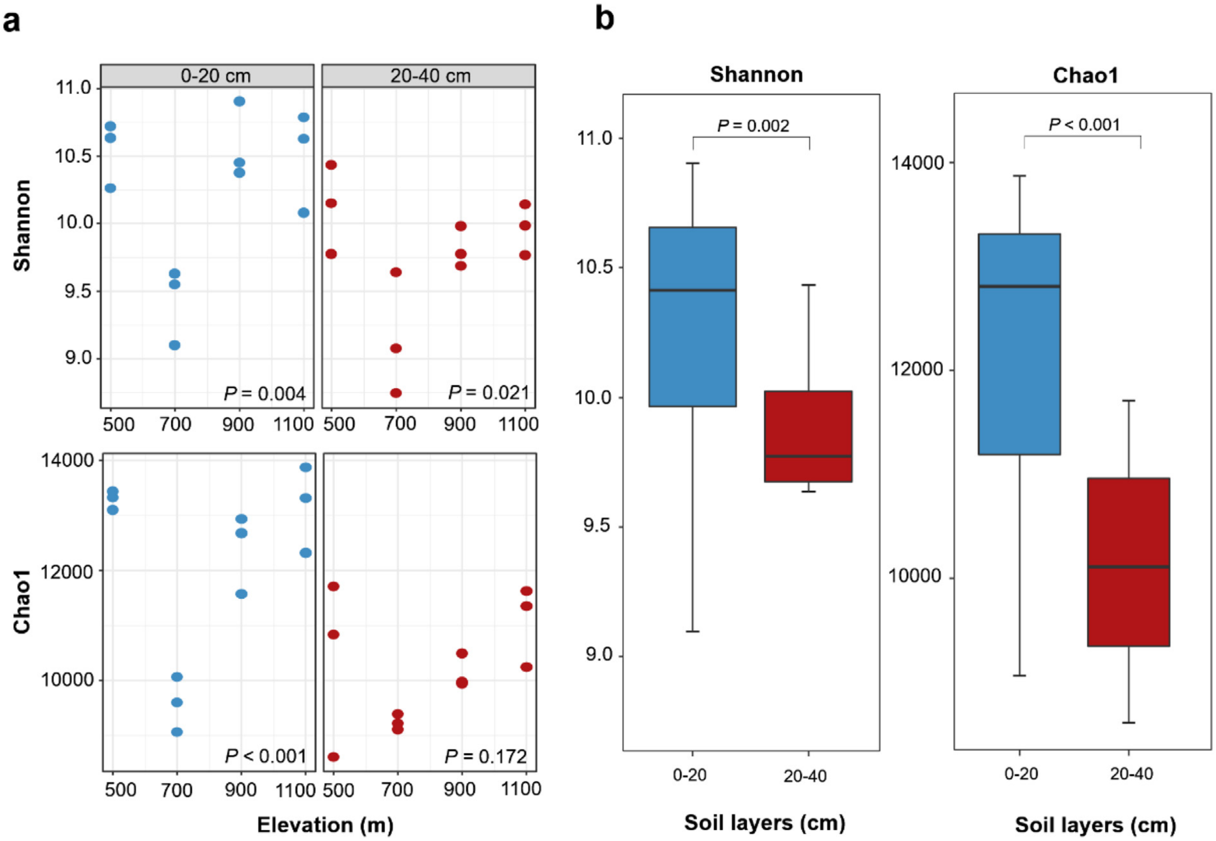

Fig. 1. One-way ANOVA followed by Student's *t*-Test significant difference test was applied to identify significant differences in subtropical forests of Huangshan Mountain bacterial alpha diversity (Shannon and Chao1) among the four elevation (a) and two soil layers (b), respectively. $P < 0.05$ indicates a significant difference.

communities. Fig. S4 shows the deterministic versus stochastic processes among bacterial communities of the different soil layers at elevation. Correspondingly, the responses of surface soil and subsurface soil to the bacterial communities ranged from 52.91 % to 97.92 % and 49.41 % and 96.31 %, respectively.

3.3. Co-occurrence patterns in networks

The co-occurrence patterns were analyzed to explore the potential roles of bacterial interactions at different elevations and soil layer communities using network analysis. The co-occurrence networks of soil bacterial

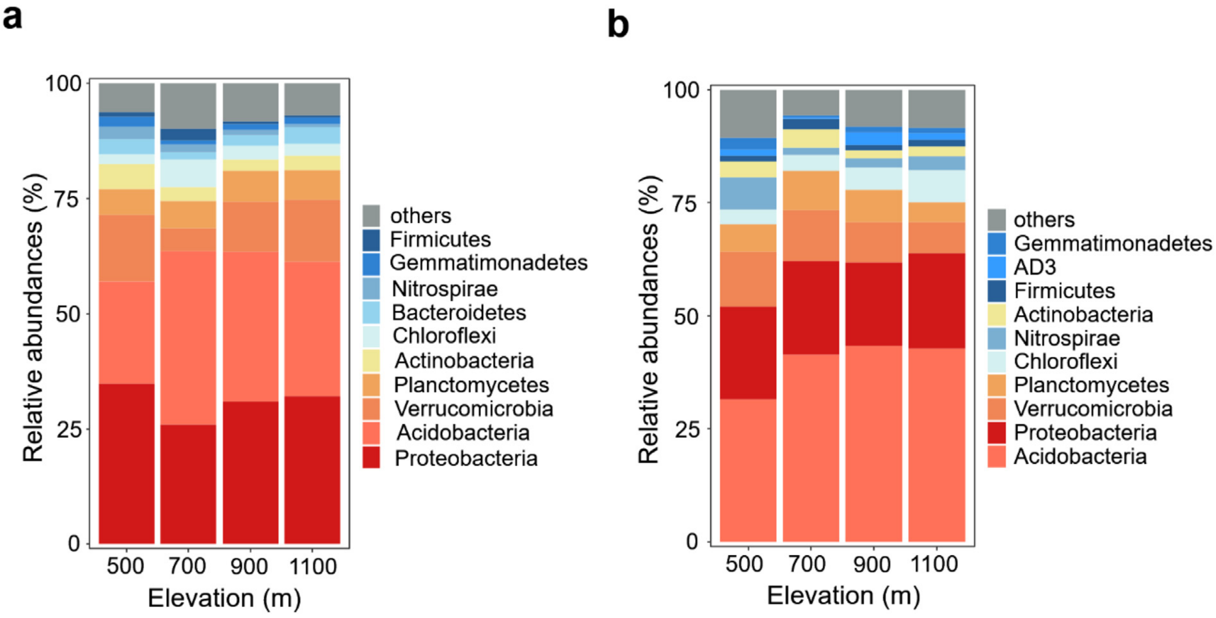

Fig. 2. Relative abundances of dominant bacterial phylum levels for 0–20 cm (a) and 20–40 cm (b) soils in different elevations. Relative abundances are based on the proportional frequencies of the reads that could be classified at the phylum level. We take the top ten phyla to show, the rest were assigned to “others” for simplicity.

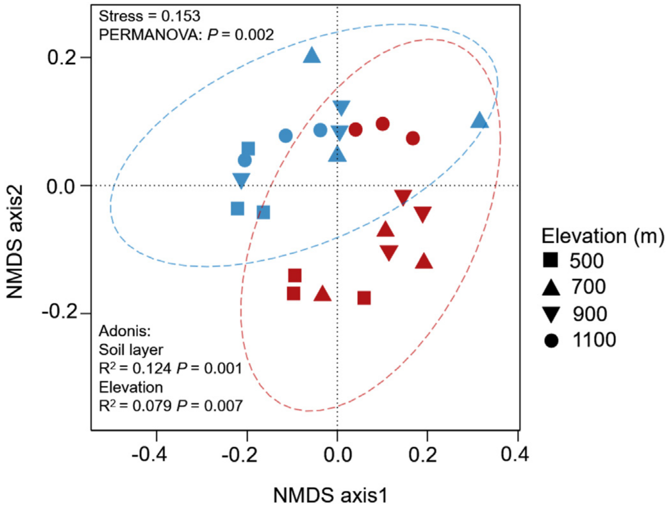

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plot of bacterial communities based on pairwise Bray-Curtis distances. Ellipses indicate 95 % confidence intervals around centroids for each soil depths of blue and red colored circles indicate surface and subsurface soil, respectively. Permutation multivariate analysis of variance (PERMANOVA) of the Bray-Curtis distances was conducted to test for differences soil in bacterial community among different soil layers. Permutational multivariate analysis of variance (Adonis) was tested to differences in soil bacterial community among different elevations and soil layers. *P* < 0.05 indicates a significant difference.

communities at different elevations had subtle differences in topological properties (Fig. S5, Table S8), while the different soil layers showed notable differences in the network and topological properties (Fig. 4a, Table S9). We further performed two-way ANOVA analysis to explore effects of both elevation and soil layer on the network topological properties. The result indicated that the effect of soil layer on the bacterial network topological properties was greater than that of elevation (Table S10). The 0–20 cm soil network included 1994 (57.43 %) positive links and 1478 (42.57 %) negative links, whereas the 20–40 cm soil network included 1423 (59.37 %) positive links and 974 (40.63 %) negative links (Fig. 4a). In addition, edges, degree, clustering coefficient, graph density, and degree centralization remarkably decreased in subsurface soil (Table. S9). Results of the Spearman's correlation analysis suggested that the topological features of the surface network were extensively correlated with the environmental variables (Fig. 4b). For example, the number of nodes was positively correlated to the soil EC, TOC, DOC, LOC, TN, and AK, and negatively correlated to the soil pH. However, the graph density showed the opposite trend compared with the number of nodes. In the subsurface soil network, the network diameter was the only variable with a significant ($P < 0.05$) relationship with EC, although the average degree had a positive correlation with the EC and C/N (Fig. 4b).

We applied the Zi-Pi relationship analysis to identify the keystone taxa of the ecological network through node classification (Fig. 5). Accordingly, all nodes were divided into four parts: peripherals, module hubs, network hubs, connectors, and most of which were peripheral at two soil layers. Phylum Acidobacteria Order Ellin6513 (OTU200894) as the network hubs were identified as network hubs in the subsurface network, however, in the surface network was no network hub. Multiple module hubs were identified by us (four nodes in the surface network, three nodes in the

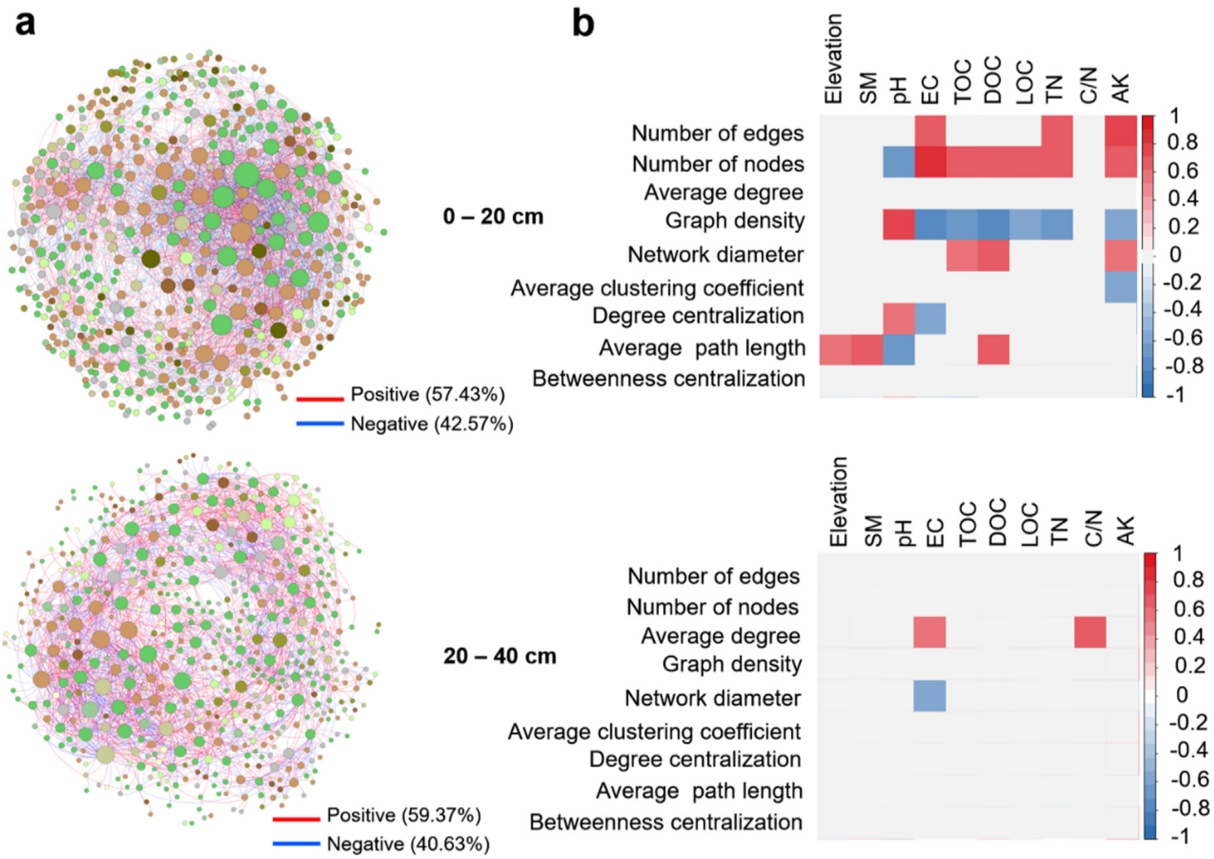

Fig. 4. Co-occurrence network analyses of bacteria in different soil layers, 0–20 and 20–40 cm. Each node represents a bacterial phylotype. Correlation coefficients (ρ) with statistical significance ($P < 0.05$) and an absolute value over 0.65 were retained in the network analysis, following the ForceAtlas 2 settings in Gephi software. The size of each node is proportional to the number of bacteria. Each node is labeled at the phylum level. A positive correlation is shown as red edges and a negative correlation as blue edges (a). Linkage between bacterial co-occurrence network topological parameter and soil properties. Red indicates positive correlations, and blue indicates negative correlations assessed by the Spearman's rank correlation rho (b).

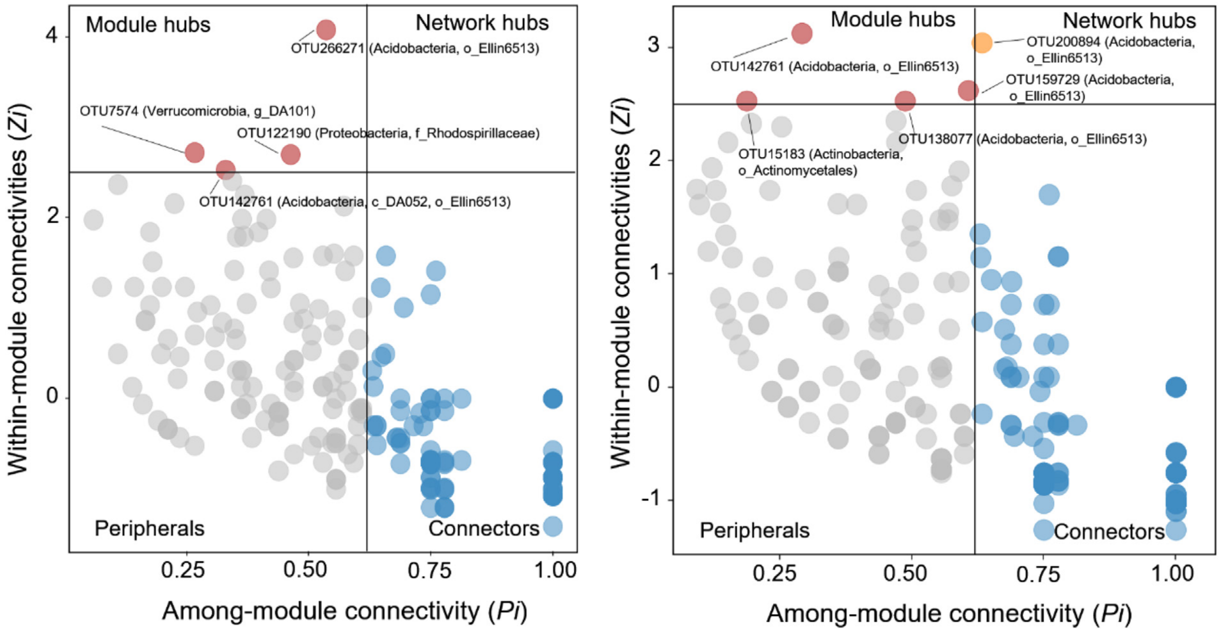

Fig. 5. Zi-Pi plots showing the distributions of OTUs based on their topological roles in the 0–20 and 20–40 cm soil layer networks. Sub-datasets consisting of three samples were extracted from the total network. The threshold values of Zi and Pi used to categorize the OTUs were 2.5 and 0.62, respectively.

subsurface network) belonging mainly to the Phylum Proteobacteria Family Rhodospirillaceae (OTU122190); Phylum Acidobacteria Order Ellin6513 (OTU142761 and OTU266271); Phylum Verrucomicrobia Genus DA101 (OTU7574); Phylum Acidobacteria Order Ellin6513 (OTU142761, OTU159729, and OTU138077); and Phylum Actinobacteria Order Actinomycetales.

4. Discussion

4.1. Bacterial diversity along altitude and soil layers

The trend in the distribution of soil bacterial diversity in this area showed a concave shape pattern, which is different from the unimodal and monotonic pattern of distribution found in previous studies (Singh et al., 2011; Shen et al., 2015). This concave shape of the elevational diversity pattern has rarely been observed in nature (Rahbek, 2004; Singh et al., 2014). Our results suggested that the bacterial diversity was influenced by vegetation, both had similar trends (Table S1). Previous works have confirmed that the bacterial diversity is mainly related to the plant Shannon index and that bacterial variation could reflect the changes in plant community and soil properties across an altitudinal gradient (Bragazza et al., 2014; Bryant et al., 2008; Ren et al., 2018). In addition, soil bacterial diversity showed apparent differences between the soil layers, with a sharp decline from surface to subsurface soil, supporting our first hypothesis. Bacterial diversity generally decreased with increasing soil depth, a result that is compatible with previous report (Eilers et al., 2012). This is due to the limited nutrient input from surface litter as soil depth increases, resulting in a reduction in soil nutrients such as C, N and P, which affects soil microbial diversity (Naylor et al., 2022). On the contrary, Du et al. (2018) found no reduction in bacterial richness with increasing depth in forest soils. These inconsistencies suggest the involvement of other factors, influencing the bacterial diversity with soil depth, such as the type of various nutrients. Some bacteria in deeper soil layers might require nutrients from organic matter decomposing and or the exudation of abundant tree roots (Clemmensen et al., 2013; Du et al., 2018).

Our results demonstrated that the drivers of bacterial diversity and community composition varied between soil layers. Bacterial diversity was mainly correlated with pH in the surface soil, which is consistent with previous studies (Shen et al., 2014; Shen et al., 2019a, 2019b), while in the subsurface soil, AK was the major influencing factor on soil bacterial diversity, which is likely attribute to the intra-species competition resulting from a lack of resources with increasing soil depth (Wang et al., 2021). Also, this might be caused by the high selection forces involved in environmental heterogeneity and biological interactions in surface soil (Luan et al., 2020).

4.2. Bacterial community composition along elevation and soil layers

Not only are there differences in diversity, but there are also significant differences in the bacterial composition of the upper and lower soil layers. For example, we found that the predominant bacterial community in the surface layer was the Proteobacteria phylum, which accounted for 30.9 %, and in subsurface, soil bacterial community was dominated by the Acidobacteria phylum accounting for 39.7 %. Hu et al. (2017) revealed that Proteobacteria exhibited a remarkable correlation with soil organic carbon, suggesting that plant litter decomposition products and root secretions have strong effects on their growth. However, Acidobacteria are considered oligotrophs that more likely survives in a poor carbon environment (Fierer et al., 2007). Moreover, Brewer et al. (2019) also revealed that the characteristics of the candidate phylum Dormibacteraeota (formerly AD3) may allow its survival in resource-limited soil environments.

Although our study had a vertical control of only 600 m, the bacterial communities differed significantly between soil layers. The impact of soil layer on the bacterial diversity and community was greater than that of elevation, which was consistent with the situation in the western Tibetan Plateau in Ngari, China (Chu et al., 2016). This points out, at the regional scale, bacterial communities were strongly divided in soil layers. However, our results differed from the soil bacterial distribution in the Oakley Mountain, China (Ji et al., 2021b), which may attribute to the differences among the forests in different latitudinal climate zones. Climate has been found to be a key factor in determining microbial distribution (Bardgett et al., 2008; Melillo et al., 2017). Climate change could lead to significant changes in the main ecological drivers controlling below-ground microbial communities, for example, reduction of vegetation cover and increase in soil pH (Margesin and Niklinska, 2019).

4.3. Community assembly processes and divergent driving factors in different soil layers

The results indicated that the bacterial community assembly process was mainly stochastic at elevation and soil layers, suggesting that homogenizing dispersal is the most important part of the bacterial community assembly process in the subtropical forests of Huangshan. Microbial assembly processes are dominated by stochastic rather than the deterministic processes in each soil profile due to homogeneous diffusion leading to similar composition of a single soil profile (Stegen et al., 2012). Although some studies have shown that soil bacterial community assemblies are primarily governed by deterministic processes (Zhu et al., 2020), literature suffers from the lack of data on microbial community assembly of subsurface soil (Xu et al., 2021). Soil properties and dispersal limitations do not explain surface variation in fungal communities (Yang et al., 2017a, 2017b), but dispersal limitation affected the bacterial communities in both the surface and subsurface soils in Ngari drylands of the Asiatic Plateau (Chu et al., 2016). Another important reason attributed to the lower environmental heterogeneity is that assemblies are more broadly governed by stochastic processes, which may include ecological drift, probabilistic dispersal, and random births or deaths (Chase, 2010; Luan et al., 2020).

Soil properties have also been long recognized as the crucial determinants of the soil bacterial community (Li et al., 2019). Bacterial community composition was found to mainly correlate with soil LOC in the surface soil, to correlate with elevation in the subsurface soil. This supports our second hypothesis. Although several studies have found that soil pH is significantly correlated with soil bacterial community composition in mountain ecosystems (Bahram et al., 2018; Shen et al., 2020). In this study LOC was found more influential due to the small range of variation in pH over the inter-altitude range (Ni et al., 2018). Another important reason is that the LOC has not been studied in many studies. Recent studies have reported a significant correlation between the soil carbon contents and bacterial community structure, but studies of soil LOC have rarely been reported (Li et al., 2019). Farrell et al. (2013) found that LOC is the most key factor for explaining significant and rapid changes in soil microbial communities. Altitude directly modulates bacterial diversity and composition through speciation, competition, and the dispersal of microbial communities, or indirectly by climatic factors and vegetation affecting soil properties, which in turn drive different patterns of bacterial change in the surface and subsurface layers (Delgado-Baquerizo et al., 2016; Singh et al., 2014; Tian et al., 2021). In contrast to the areas with a large altitude span, our sampling elevation sample sites are long-term sample sites and the maximum altitude of the sampling site is only 1281 m a.s.l., which results in a narrow pH range. Langenheder and Prosser (2008) also suggested that resource availability is the main factors determining the composition of soil microbial community along a depth gradient. These results further reveal a nutrient effect on microbial communities along with soil depth layers.

4.4. Bacterial network properties and keystone species differ between soil layers

Through network analysis, we can uncover previously unnoticed patterns of microbial co-occurrence to decipher complex relationships among microbial taxa (Liu et al., 2021; Zhang et al., 2018). Our results indicated that the surface soil network had a greater average degree, graph density, and highly connected edges than the subsurface soil, which is in line with our third hypothesis. The average degree of bacterial communities was the highest in the surface soil and decreased with soil depth, indicating a higher degree of habitat heterogeneity for microbes in surface soils (Tian et al., 2015). Similarly, the higher average clustering coefficient in the surface soil indicates that the number of intra-cluster links was higher than the number of inter-cluster links. This suggests that the connections between soil bacteria in similar niches were stronger linked than those in different niches (Ji et al., 2021a, 2021b). Moreover, the average path length of networks was smaller in the surface soil than in subsurface soil. Networks with small path lengths are considered small-world networks (Watts and Strogatz, 1998) and they are linked to the quick responses of an ecosystem to perturbations (Shu et al., 2018). The surface network was found more complex than the subsurface network, with stronger correlation to the soil variables. This also implies that the bacterial communities are more to changes in environmental factors with soil depth, in Huangshan. In this area, depth had a greater impact on the microbial co-occurrence network and topology than the elevation across the soil profile. The changes with increasing soil depth might attribute to a gradual reduction of the soil nutrient contents with soil depth. Because of the humus and higher contents of nutrients, topsoil provides a better environment for the development of root system and bacterial communities in forest ecosystems (Scheu and Parkinson, 1995; Spohn et al., 2016; Tang et al., 2018).

Keystone species play a crucial role in maintaining ecological community structure and have a greater impact on the community than would be expected based on their relative abundance or total biomass (Banerjee et al., 2018; Banerjee et al., 2021; Ma et al., 2016). We found that the keystone nodes were different among the co-occurrence networks in the different soil layers. The most dominant keystone species taxa included (Phylum Acidobacteria Order Ellin6513; Phylum Verrucomicrobia Genus DA101; Phylum Proteobacteria Family Rhodospirillaceae) at the surface soil. While in the subsurface soil, the dominant OTUs included (Phylum Acidobacteria Order Ellin6513; Phylum Actinobacteria Order Actinomycetales) from the different layers, exerting important soil functions in Huangshan Mountain forests. Ellin 6513 taxa have been reported as dominant groups in previously described cold environments at high elevations (Pershina et al., 2020; Wu et al., 2017). Based on a pure culture method, Verrucomicrobia was found to participate in methane oxidation (Sharp et al., 2014), nitrogen fixation (Khadem et al., 2010), and polysaccharide degradation (Nixon et al., 2019). The dominant genus DA101 occupies distinct ecological niches and executes oligotrophic life-history strategies in soil environments (Shen et al., 2017). Rhodospirillaceae are shown to be the microorganisms that drive greenhouse gas fluxes (Morrison et al., 2020). Actinobacteria because they grow in the soil in the form of mycelium, which enables plant roots to spread to deeper soil layers to absorb nutrients and water (Upton et al., 2020). The bacterial network exhibits different keystone species between different soil layers, which supports the existence of ecological niche differentiation between bacterial taxa. Although Ellin6513 was found to be the key dominant species in our study, we do not know what ecological role it plays in the bacterial community. Future research on the cultivation of new strains should be intensified, which will be critical to understanding the role these keystone species play in the ecosystem.

It was notable that we did not start from the lowest elevation because we selected four of the original 13 sample sites at 200 m intervals. The purpose of this approach was to reach as large of an elevation span as large and to obtain more reasonable results. Furthermore, the relatively small sample size may limit the reliability of our results. More samples could provide more specifics, leading to a better understanding of the elevational patterns of soil microbial communities in different soil layers and their drivers. Although our results are not generalizable, they are complementary to existing altitudinal microbial distribution patterns for subtropical forests in China.

5. Conclusion

Our results reveal that the soil bacterial diversity showed a concave distribution along the altitude gradient, and the soil bacterial community significantly more affected by soil depth than by elevation gradient in the forest of Huangshan Mountain. Bacterial networks differed significantly between soil layers. In comparison to subsurface soil, surface soil network was more sensitive to the changes of soil environmental variables and had complex topological characteristics. Stochastic processes that take place in the soil layers and elevations dominated the bacterial communities. In the future, study should concentrate keystone taxa and their functions in ecosystems. This kind of research is essential to comprehending the mechanisms that keep soil ecological function and microbial diversity in forests.

CRediT authorship contribution statement

Chao Ma and Haiyan Chu jointly conceived and designed the experiments. Luyao Song and Shangguang Xia performed experiments and analyzed the data. Luyao Song, Teng Yang, Xu Liu, Zhong Yin, Shaopeng Li, Ruibo Sun, Hongjian Gao, Haiyan Chu, Chao Ma participated to the writing, drafting, and revising the manuscript.

Data availability

Data will be made available on request.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.158438.

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