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Contrasting Altitudinal Patterns and Composition of Soil Bacterial Communities along Stand Types in *Larix principis-rupprechtii* Forests in Northern China

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Abstract: Bacterial communities inhabiting the soil of mountain ecosystems perform critical ecological functions. Although several studies have reported the altitudinal distribution patterns of bacterial communities in warm-temperate mountain forests, our understanding of typical zonal vegetation dominated by *Larix principis-rupprechtii* Mayr (abbreviated as larch hereafter) and the understory elevation distribution patterns of soil bacterial communities is still limited. In this study, the Illumina NovaSeq 6000 sequencing platform was used to investigate the changes of surface and subsurface soil bacterial communities along an altitudinal gradient (from 1720 m to 2250 m) in larch forests in northern China. Altitude significantly affected the relative abundance of *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* (bacterial dominant phylum) and *Alphaproteobacteria*, *Gammaproteobacteria*, and *Actinobacteria* (bacterial dominant classes). The diversity of bacterial communities showed a concomitant increase with altitude. The variations in available nitrogen and soil temperature content at different altitudes were the main factors explaining the bacterial community structures in pure stands and mixed stands, respectively. Altitude and the contents of soil organic carbon and soil organic matter were the main factors explaining the dominant phylum (taxonomy). Our results suggest that stand type has a greater effect on the structure and composition of soil bacterial communities than elevation and soil depth, and bacterial communities show divergent patterns along the altitudes, stand types, and soil profiles.

Keywords: stand type; soil profile; bacteria; composition; Illumina NovaSeq sequencing platform

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1. Introduction

Soil bacteria are the most abundant and functionally diverse microbial taxa in forest soil and play a critical role in nutrient cycling, organic matter decomposition, and material and energy transport between above- and below-ground communities [1–4]. The altitude distribution pattern of soil bacteria is one of the important contents of the biogeographic distribution pattern. Shen et al. (2013) studied soil bacterial community composition and diversity at six elevations in six forest stand types from temperate to boreal in Eurasia and found that bacterial communities did not vary consistently across the elevation gradient, but there were significant differences. In addition, their study demonstrated that the bacterial community composition in mountain ecosystems was significantly correlated with soil moisture and soil organic carbon [5]. Zhang et al. (2015) found a monotonically decreasing trend in soil bacterial community diversity with elevation from low elevation evergreen broadleaf forests in the subtropics to subalpine scrub at high elevations [6]. Ivashchenko et al. (2021) indicated that vegetation distribution was the main factor driving the pattern of altitudinal diversity of soil bacterial communities in the Northwest Caucasus Mountains

of southern Russia, which has a temperate continental climate [7]. Ji et al. (2022), in their study of the altitudinal diversity pattern of soil bacteria in cold-temperate mountain forests in China, found that bacterial community diversity monotonically decreased with elevation. The effect of elevation on bacterial community composition was stronger than soil depth [8]. Elevation patterns of soil bacterial communities are related to soil properties [8,9] and stand types [5,6]; however, studies on the effects of the two interactions with bacteria have been ignored for a long time. Previous studies have shown that different ecosystem types, namely, e.l.s., tropical, subtropical, and boreal mountain climatic zones, may also have variability in the elevational distribution pattern of soil bacterial communities due to changes in vegetation. In addition, studies on the altitudinal distribution patterns of soil bacteria in warm-temperate mountain ecosystems in China are limited. In recent years, many scholars have found that forest stand type may briefly affect the altitudinal distribution of bacteria by altering soil C and N status [5,10,11], but it has not been further confirmed. Warm-temperate mountain forests, widely distributed in northern China, are dominated by larch trees and show wide variation in ecological processes (i.e., climate change and C/N ratio) in their vertically geographical distribution [12,13]. However, there is a lack of studies on the distribution patterns of soil bacterial communities in different stand types and soil depths along the elevation gradient, and few data on the effects of the three interactions on bacterial distribution.

How forest soil bacterial communities respond to environmental changes along elevation gradients is critical for predicting future ecosystem function and climate feedback [14,15]. Some studies have well-documented distribution patterns of soil bacterial communities along elevation gradients [16,17]. In mountain ecosystems, the high variability of vegetation communities and soil properties along altitudinal gradients inevitably leads to dramatic variations in bacterial communities. In their study on the distribution pattern of soil bacterial communities in different stand types along an altitudinal gradient, Ren et al. (2018) found that different factors affected the Alpha diversity and Beta diversity of bacterial communities, which were related to vegetation type, namely, total nitrogen in soil organic matter in the former and soil moisture and temperature in the latter, respectively [16]. Guo et al. (2015) found that the soil bacterial community structure in grasslands on the Tibetan Plateau varied significantly along the elevation gradient, which was driven by a combination of soil temperature, nutrients, soil moisture, and stand type [18]. Tian et al. (2020) confirmed that both soil bacterial abundance and Alpha diversity in the Taibai Mountains (1600–3150 m) tended to increase with increasing surface and deeper elevation [19]. In addition, soil depth is recognized as another important determinant driving the structure of soil bacterial communities [17,20,21], as it directly and indirectly affects the diffusion of soil gases and the availability of oxygen [22], which in turn affects the physiological activities of soil bacteria. In addition, soil depth is significantly correlated with the dominance of specific bacterial species [23]. Du et al. (2021) discovered that bacterial communities showed high stability in surface soil, while the opposite was true in subsurface soil [24]. By applying a national-scale metabarcoding analysis of 436 locations across seven different temperate ecosystems, George et al. (2019) found that soil bacterial Alpha diversity and composition were influenced by different ecosystems and soil properties, whereas Beta diversity was not [25]. Delgado-Baquerizo et al. (2018) revealed that only 2% of bacterial phylotypes (~500 phylotypes), e.l.s., *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Planctomycetes* consistently accounted for almost half of the soil bacterial communities worldwide, and the habitat preferences of these dominant species types are correlated with climatic and vegetation factors [26]. In addition, Staver et al. (2019) confirmed in a recent meta-analysis that changes in plant community composition do not affect carbon-acquiring enzymes but do increase the activities of nitrogen-acquiring enzymes (e.g., aminopeptidases, ureases, proteases) [27]. However, the correlation between the distribution of soil enzymes and the structure of soil bacterial communities in different stand types needs to be further investigated. Several research studies have suggested that variations in soil's physical and chemical characteristics impact the variety of bacterial

diversity and composition at different soil depths in harsh climate areas [28,29]. Uncertainty remains about warm-temperate regions with specific climatic conditions compared to the well-known information about tropical and subtropical regions. It is uncertain if soil bacterial communities exhibit distinct altitudinal distribution patterns and if temperature or other environmental factors play a major role in these variations. Our understanding of the soil bacterial communities in boreal forest ecosystems is currently limited, particularly regarding key taxa.

The vertical distribution pattern of soil bacterial communities is an important part of the biogeographic distribution pattern in mountain ecosystems that bear important ecological service functions. These ecosystems provide a variety of habitats over small-scale spatial distances in the context of rapidly changing climates, vegetation, and soil quality in mountain environments [30]. However, the distribution pattern of soil bacterial communities in larch-dominated boreal forest ecosystems has rarely been reported. Here, we investigated the distribution patterns of soil bacterial communities along an altitudinal gradient and soil depth in two stand types, including larch coniferous forests (abbreviated as pure stands hereafter) and larch–birch mixed coniferous forests (abbreviated as mixed stands hereafter) that are dominated by *L. principis-rupprechtii* in the warm-temperate mountain ecosystem in northern China. This work will yield new insights into the major ecological predictions of microbiology along an altitudinal gradient. We focused on the structure and composition of soil bacterial communities in the understory of larch forests at different elevations and stand types in two soil depths. We tested the hypotheses that (1) with increasing altitude, the diversity of the soil bacterial communities in pure and mixed stands would show consistent patterns, i.e., a monotonic increase; (2) stand types had a stronger influence than altitude and soil depth on the structure of soil bacterial communities; (3) intersecting with soil depth, altitude and stand type changed more than the main phylogenetic types of soil bacterial communities in the study area; and (4) key soil factors driving changes in soil bacterial community structure and composition differ across stand types.

2. Materials and Methods

2.1. Site Description and Soil Sampling

The study site is located in the forest zone of Guandi Mountain (37°54′ N, 111°31′ E) in Lvliangshan State Forestry Bureau, Lvliang, China (Figure 1). The main dominant tree in the study area is the *Larix principis-rupprechtii* Mayr (abbreviated as larch hereafter), with an average height of 19.71 m, with its vertical distribution varying to some extent. Basic information about the sites at different elevations is provided in Supplementary Table S1. The average annual temperature of the region is 4.3 °C and the average annual precipitation ranges from 600 mm to 822 mm. The warm-temperate mountainous climate comprises warm and rainy summers and cold winters, with the coldest month being January (−10.2 °C), the warmest month being July (17.5 °C), and the growing season generally lasting from June to September. The soils are mostly Alfisols and Oxisols, according to the United States Department of Agriculture Handbook, and have a depth of 50–70 cm.

Along altitude ranging from 1638 m to 2364 m a.s.l., the mountain forests exhibit a consistent canopy cover (between 60% and 70%), slope, and exposed area. At the end of the 1960s, the plantations of *L. principis-rupprechtii*, with an initial density of 4000–5000 trees/ha, was formed in the area after artificial afforestation. In the 25th and 40th years after afforestation, the density of the stand was adjusted to 1000–2000 plants/ha by means of artificial thinning. The main dominant tree in the study area is the *L. principis-rupprechtii*, with an average height of 19.71 m, with its vertical distribution varying significantly. Based on the vegetation composition, six altitudinal sites (1720 m, 1850 m, 1950 m, 2050 m, 2150 m, 2250 m) were identified (Figure 1). Briefly, the elevation site investigated in this study was located in two types of forest stands: pure stands were mainly covered by *L. principis-rupprechtii* and coniferous-broad-leaved mixed stands were dominated by *L. principis-rupprechtii* and *Betula platyphylla* Sukaczev (abbreviated as birch hereafter). Among

them, the pure stands were mixed with a small amount of *Picea asperata* Mast, or *Betula platyphylla* Sukaczev, while in mixed forests, 51.1%–83.9% of the total stand of the transverse sectional areas at breast height were *L. principis-rupprechtii* and 16.1%–47.8% were *Betula platyphylla*, respectively.

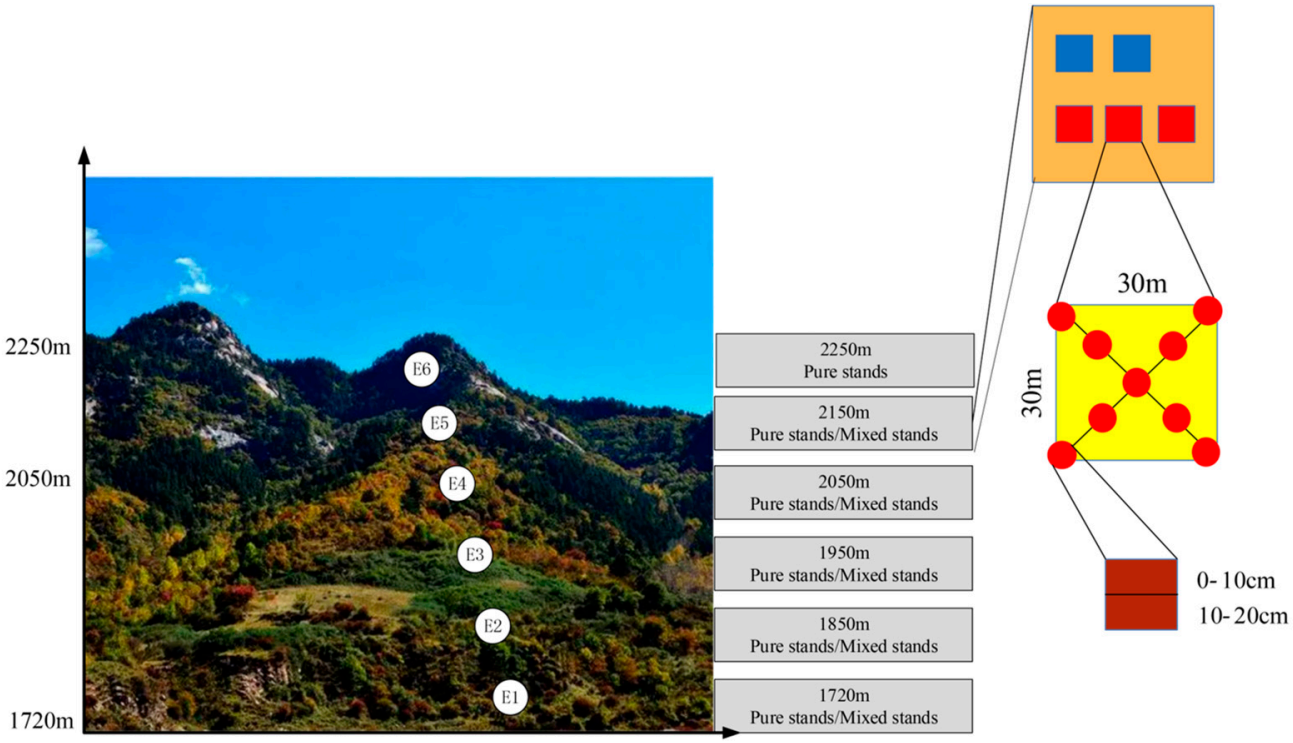

Figure 1. Location of the field experiments in this study. Note: Based on the different vegetation compositions on the elevation gradient, six elevation (E1, E2, E3, E4, E5, E6) gradients were selected on the shady slope of the study site, and 5–6 study plots of 30 m × 30 m were set up in each elevation gradient, while the soil was 0–10 cm and 10–20 cm deep in each sample plot, respectively. Blue squares represent plots of pure stands and red squares represent plots of mixed stands.

Five-to-six study plots were randomly established within each site. In every plot, nine samples of soil were collected from depths of 0–10 cm (surface soil) and 10–20 cm (subsurface soil). To comprehensively capture the diversity at the plot level, the samples were combined and mixed, while the replicated plots were utilized to analyze the variation in soil bacteria at each location. The soil samples were collected in July (mid-growing season) 2021 (N = 68) and immediately transported on ice to the laboratory. Each fresh soil sample was sifted through a 2 mm sieve to eliminate any visible roots and stones. The samples were split into two parts: one stored at −80 °C for DNA extraction and the other stored at 4 °C for analyzing soil properties.

2.2. Soil Physicochemical Properties

Using the cutting ring method, the soil moisture and bulk density (BD) were determined. The analysis of soil organic carbon (SOC) and total nitrogen (TN) levels was conducted post-tableting with a J200 Tandem laser spectroscopic element analyzer from Applied Spectra, Inc. in Fremont, CA, USA. Total phosphorus (TP) content was measured using molybdenum blue colorimetry (TU-1901, Puxi Ltd., Beijing, China) following digestion with hydrofluoric acid and perchloric acid. Soil moisture content (SW) was measured gravimetrically by drying the soil at 105 °C for 24 h. Soil temperature (ST) was measured using a multiparameter WET sensor (WET-2, Delta-T Devices Ltd., Cambridge, UK) [31]. Soil available phosphorus (AP) was extracted with 0.5 M $NaHCO_3$ and measured using the molybdenum blue colorimetric technique. The alkaline permanganate method was

utilized to determine the content quick-acting nitrogen (AN) in the soil. The activity of soil urease (URE) was measured by the colorimetric method involving sodium phenol hypochlorite, using a 10% urea solution as the substrate. Phosphatase (PHO) activity in the soil was determined by the colorimetric method using phenyl phosphate sodium. The activity of soil sucrase (INV) was measured directly by utilizing the 3,5-dinitro-salicylic acid colorimetric technique [32,33].

2.3. DNA Extraction and 16S rRNA Gene Amplicon Sequencing

Total genomic DNA was extracted from the soil samples using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, USA) and stored at −20 °C prior to further analysis. The quantity and quality of the extracted DNA were measured using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, respectively. PCR amplification of the bacterial 16S rRNA genes' V3–V4 region was performed using the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Sample-specific 7 bp barcodes were incorporated into the primers for multiplex sequencing. The PCR components contained 5 µL of buffer (5×); 0.25 µL of Fast pfu DNA Polymerase (5 U/µL); 2 µL (2.5 mM) of dNTPs; 1 µL (10 µM) of each Forward and Reverse primer; 1 µL of DNA Template; and 14.75 µL of ddH_2O. Thermal cycling consisted of initial denaturation at 98 °C for 5 min, followed by 25 cycles consisting of denaturation at 98 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 45 s, with a final extension of 5 min at 72 °C. PCR amplicons were purified with Vazyme V AHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA).

2.4. Illumina NovaSeq Sequencing and Processing of the Sequencing Data

Following the quantification of each sample, the amplicons were pooled in equal amounts, and pair-end sequencing was performed with 2 × 250 bp reads on the Illlumina NovaSeq platform using the NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd. in Shanghai, China. Microbiome bioinformatics analysis of each sample was conducted using QIIME2 software (2019.4, https//qiime2.org/, accessed on 4 September 2023) [34]. The original sequence information was separated into different categories using the demux plugin, and then the primers were removed using the cutadapt plugin [35]. The original sequencing data are available from the NCBI database (accession number: PRJNA1067874 for bacteria).

The clean tags, which were of high-quality tag data, were acquired through the DADA2 technique after the raw sequences underwent quality filtering, denoising, merging, and removal of chimeras. Assemblages were clustered at a 100% similarity level and defined as amplicon sequence variants (ASVs) using DADA2 [36]. Taxonomic annotations of the ASVs were determined using the Greengenes database for bacteria found at http://greengenes.lbl.gov (accessed on 2 August 2023) [37]. Rarefaction curves were generated based on the quantity of sequences sampled and the corresponding number of ASVs. The sparsity curve entered a plateau (Figure S1), indicating that the sequencing depth was sufficient to reflect the actual soil microbiota.

2.5. Data Analysis

The normality and chi-square of the variance of the relative abundance of the major taxa of soil bacteria, diversity indices, and soil factors were assessed using the Shapiro–Wilk test and Levene's test. Soil factors were analyzed by one-way analysis of variance (ANOVA) with Tukey's HSD test ($p < 0.05$) using SPSS 23.0 (IBM SPSS Statistics, Chicago, IL, USA) at different elevations and between stand types, and soil factors in two depths were subjected to independent samples *t*-test. Subsequently, all the soil factors were then subjected to a three-way ANOVA, with elevation, stand type, and soil depth as fixed factors.

QIIME2 software (version 2019.4) was utilized to analyze bacterial Alpha diversity indices for all sequencing data. A two-way ANOVA was used to compare the significant effects ($p < 0.05$) of elevation, stand type, and their interactions on bacterial community Alpha diversity indices (the number of observed species, Shannon, and Chao1 indices). The "Vegan" package of R language software 4.2.2 was used to perform the Weighted-Unifrac distance-based principal coordinate analysis (PCoA) to separately compare the differences in bacterial communities among samples. Afterward, a non-parametric multivariate analysis of variance (PERMANOVA) was used to compare the resemblances in bacterial communities between different elevations, stand types, and soil depths. To screen for marker microbial taxa that are differentially represented in different subgroups, we used LEfSe (LDA Effect Size) (combining the non-parametric Kruskal–Wallis and Wilcoxon rank sum tests with linear discriminant analysis) analytical method. Soil factors were analyzed for redundancy with bacterial communities using the CANOCO 5.0 software. We further identified soil factors significantly associated with community structure using the Mantal test based on the Weighted-Unifrac similarity distance, with 999 Monte Carlo tests. We then investigated the correlations between bacterial communities composition and soil variables by Spearman's analysis.

3. Results

3.1. Soil Physicochemical Properties along Altitudinal Gradients

Altitude had significant effects on the contents of soil maximum water holding capacity (SMaxW), minimum water holding capacity (SMinW), soil BD, SOC, SOM content, INV, URE, and PHO activity (Tables S2 and S3, $p < 0.05$). In surface soil, the contents of SOC and SOM showed a significant increasing trend as altitude increased. The contents of SOC and SOM at 2150 m were significantly higher than those at an altitude of 1720 m in surface soil (Table S2). The stand type had a significant effect on ST, AN content, and PHO activity (Tables S2 and S3, $p < 0.05$), and the ST and AN contents were significantly higher in larch pure stands than those in larch–birch mixed stands. Soil depth had a significant effect on ST and the contents of SOC, SOM, and AK (Table S3, $p < 0.05$). Among them, ST and the content of AK were significantly higher in surface soil than subsurface soil. The interaction of altitude and soil layer ($p < 0.001$) had a more significant effect on the contents of SOC and SOM than the interaction between stand type and soil layer (Table 1, $p < 0.05$). In larch pure stands, the INV, URE, and PHO activity in the two soil horizons showed a significant decrease and then increase with elevation gain. In larch–birch mixed stands, the INV activity showed a significant decrease with elevation gain. The three-way interaction of elevation, stand type, and soil depth had the most significant effect on the contents of SOC and SOM, followed by TN. The interaction of elevation and stand type had significant effects on all three enzyme activities (Table 1, $p < 0.05$).

Table 1. Three-way analysis of variance (ANOVA) for soil factors at different elevations, stand types, and soil depths.

Soil Factors	SW (%)	ST (°C)	SMaxW (g)	SMinW (g)	SP (%)	BD (g/cm^3)	SOC (g/kg)	SOM (g/kg)	AN (mg/kg)	TN (g/kg)	AP (mg/kg)	TP (g/kg)	AK (mg/kg)	INV (mg)	URE (mg)	PHO (mg)
ANOVA																
A	1.249 ns	1.876 ns	4.403 ***	3.704 **	2.116 ns	11.16 ***	7.801 ***	7.802 ***	0.944 ns	1.718 ns	0.427 ns	1.891 ns	1.538 ns	5.621 ***	6.291 ***	12.926 ***
F	1.025 ns	11.10 ***	2.613 ns	0.401 ns	2.162 ns	2.700 ns	1.499 ns	1.497 ns	12.236 ***	0.286 ns	0.974 ns	1.843 ns	0.035 ns	0.848 ns	2.162 ns	4.551 *
S	0.339 ns	5.542 *	0.114 ns	1.127 ns	2.628 ns	0.235 ns	6.251 *	6.259 *	0.485 ns	0.337 ns	0.137 ns	0.284 ns	9.757 **	0.941 ns	2.466 ns	3.387 ns
A × F	4.081 **	1.914 ns	1.471 ns	4.075 **	1.203 ns	4.364 **	4.743 ***	4.744 ***	2.027 ns	1.980 ns	0.435 ns	2.640 *	2.013 ns	4.943 ***	8.643 ***	5.986 ***
F × S	0.106 ns	0.070 ns	0.020 ns	0.826 ns	0.063 ns	0.009 ns	5.494 *	5.493 *	0.368 ns	1.087 ns	0.181 ns	0.157 ns	0.958 ns	1.325 ns	0.193 ns	0.168 ns
A × S	0.283 ns	0.007 ns	0.479 ns	1.059 ns	0.615 ns	0.945 ns	6.224 ***	6.226 ***	1.338 ns	1.079 ns	0.261 ns	0.254 ns	0.399 ns	0.349 ns	0.220 ns	1.514 ns
A × F × S	0.287 ns	0.024 ns	0.182 ns	0.746 ns	0.266 ns	0.567 ns	2.675 *	2.675 *	0.417 ns	2.075 ns	0.197 ns	0.134 ns	0.577 ns	0.317 ns	0.106 ns	0.255 ns

Note: A is for Altitude, F is for Stand Type, S is for Soil depth; SW: soil water content; ST: soil temperature; SMaxW: maximum soil water holding capacity; SMinW: minimum soil water holding capacity; SP: soil porosity; BD: soil bulk weight; SOC: soil organic carbon; SOM: soil organic matter; AN: alkaline nitrogen; TN: total nitrogen; AP: fast-acting phosphorus; TP: total phosphorus; AK: fast-acting potassium; INV: sucrase; URE: urease; PHO: alkaline phosphatase; * represents $p < 0.05$, ** represents $p < 0.01$; *** represents $p < 0.001$; ns represents no significant difference.

3.2. Soil Bacterial Sequencing Summary and Community Composition

The 16S rRNA genes from soil bacteria were sequenced on the Illumina NovaSeq platform. Across all soil samples analyzed, a total of 4,488,211 high-quality soil bacterial sequences were obtained by Illumina NovaSeq sequencing platform, of which 2,366,221 and 2,121,990 sequences were obtained from the pure larch forests and larch–birch mixed forests, respectively. In the 0–10 cm and 10–20 cm soil layers, 2,237,819 and 2,250,392 sequences were obtained, respectively. Each sample contained 50,567–80,704 sequences (average of 66,003 sequences). The average read length for bacteria was 377 bp, which was >99% Good's coverage. The gene rarefaction curves showed a trend towards reaching a saturation point at 100% sequence similarity in all samples, which suggested that the sequencing depth was sufficient for assessing the composition and variety of soil bacteria in the samples.

A total of 96,892 ASVs were identified that were distributed among 41 phyla, 126 classes, and 1119 genera. Proteobacteria (29.53%), Actinobacteria (22.06%), Acidobacteria (18.96%), and Chloroflexi (13.43%) were the dominant phyla, accounting for 83.98% of the total number of bacterial sequences obtained. Altitude and stand type had a significant effect on the relative abundance of Chloroflexi. Alphaproteobacteria, Gammaproteobacteria, and Actinobacteria were the dominant classes, accounting for 16.6%, 9.45%, and 9.21%, respectively (Figure 2A,B).

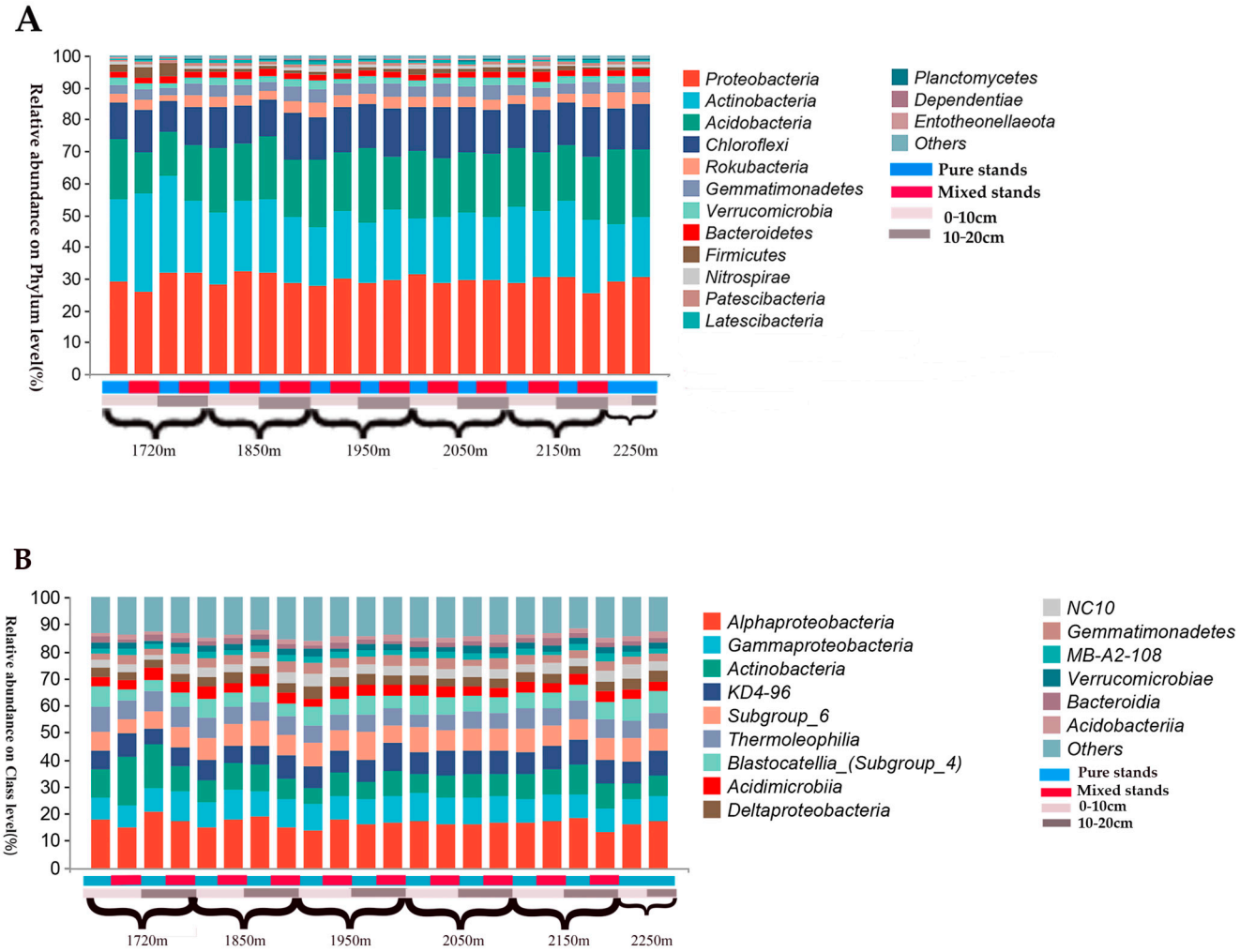

Figure 2. Relative abundance of major bacterial phyla (**A**) and classes (**B**) in soil samples at two stand types and soil depths at different elevations.

3.3. Soil Bacterial Community Diversity

Altitude and soil layer had no significant impact on the richness, Shannon, and Chao1 diversity indices of the soil bacterial communities (Figure 3A–F). The diversity of the soil bacterial communities in pure and mixed stands would show monotonic increase with increasing altitude. In surface soil, the number of observed species, Shannon, and Chao1 indices of soil bacteria at 2050 m were higher than those at 1720 m and 2250 m, respectively. In subsurface soil, the number of observed species, Shannon, and Chao1 indices of soil bacteria at 2050 m were higher than those at 1720 m and 2250 m, respectively.

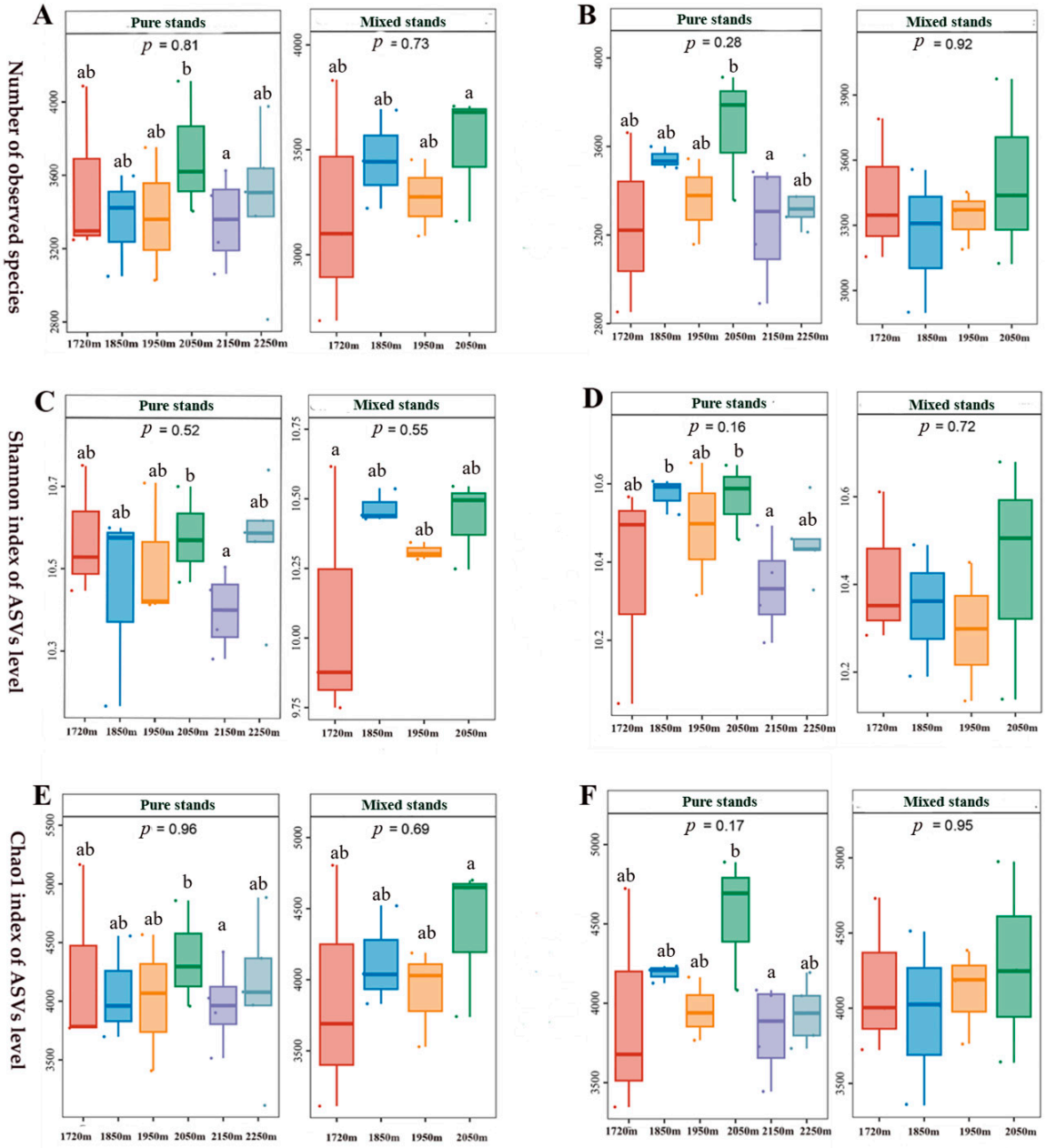

Figure 3. Alpha diversity of soil bacterial communities in two stand types and soil depths at different elevation gradients. Note: Number of observed species, Shannon, and Chao1 indices of soil bacterial communities in 0–10 cm (**A**,**C**,**E**) and 10–20 cm (**B**,**D**,**F**) soil layers along the altitudinal gradient among two stand types. Different lowercase letters indicate significant differences among altitudes in the same stand type and soil depth ($p < 0.05$).

Soil bacterial sequencing data from two different stand types at varying altitudes were analyzed using PCoA with Weighted-Unifrac distance. Soil bacteria communities were somewhat categorized by altitude, with no clear categorization by soil depth at the same altitude for bacteria (Figure 4A,B). Significant variations in the structure of soil bacterial communities were identified among different stand types based on the PERMANOVA results (Figure 3B, $p < 0.01$). Stand types had a stronger influence than altitude and soil depth on the structure of the soil bacterial communities. Compared to the pure stands, the structure of soil bacterial communities in mixed stands were more strongly clustered and more similar.

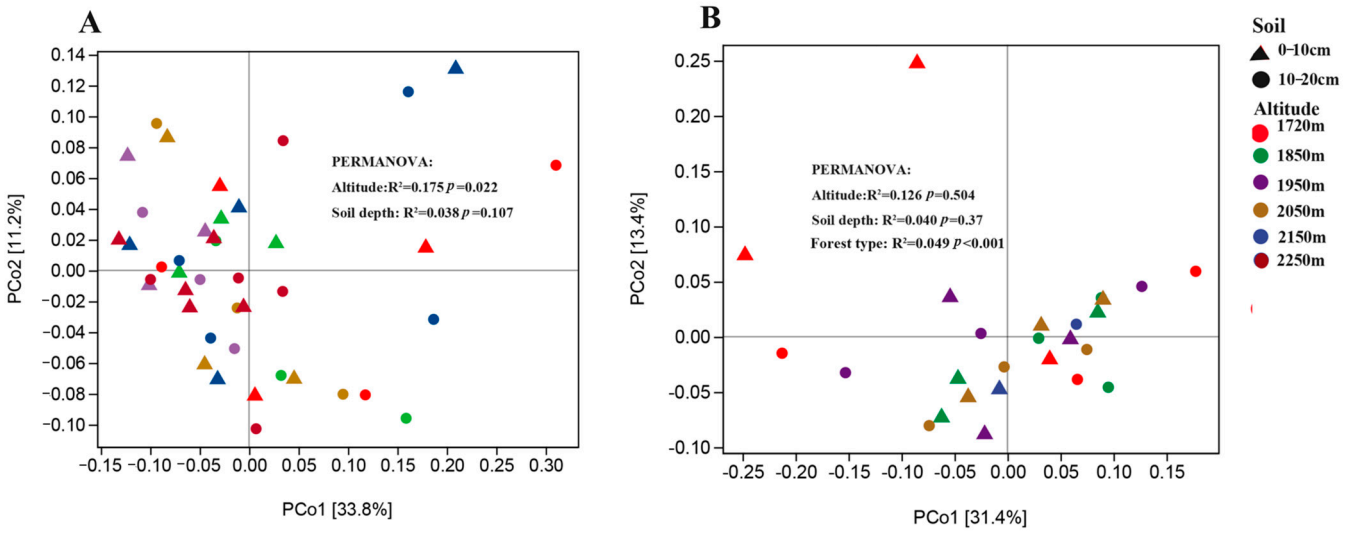

Figure 4. Non-parametric multivariate analysis of variance of soil bacterial communities based on Weighted-Unifrac distances. (**A**,**B**) indicate the soil samples in pure stands and mixed stands, respectively.

3.4. LEfSe Analysis

To determine the key phylotypes of microbiota at different levels, we used LEfSe analysis. The LDA results showed 5, 7, 1, 4, 1, and 11 discriminatory features (LDA > 2.5, $p < 0.05$) at 1720 m, 1850 m, 1950 m, 2050 m, 2150 m, and 2250 m elevations, respectively, which highlighted 2, 3, 1, 1, 1, and 5 dominant bacteria, respectively (Figure 5A,B). Pure stands and mixed stands showed 37 and 41 discriminatory features (LDA > 2.5, $p < 0.05$), which highlighted 14 and 18 dominant bacteria, respectively (Figure 5C,D). Five and two dominant microorganisms were highlighted by 11 and 4 discriminatory features (LDA > 2.5, $p < 0.05$) in surface and subsurface soil, respectively (Figure 5E,F). These results indicate that elevation and stand type more often altered the key phylogenetic types of soil bacterial communities in the study area, while soil depth remained virtually unchanged.

The major bacterial groups were then identified taxonomically through evolutionary cluster analysis maps (Figure 5A,B). For pure stands, Acidobacteria, Deltaptoteobacteria, Blastocatellales, and Pyrinomonadales had a high abundance in pure forest soil, while the abundance of Actinobacteria, Chloroflexi, Gemmatimonadetes, Latescibacteria, and Pateacibacteria was higher in mixed stands soil.

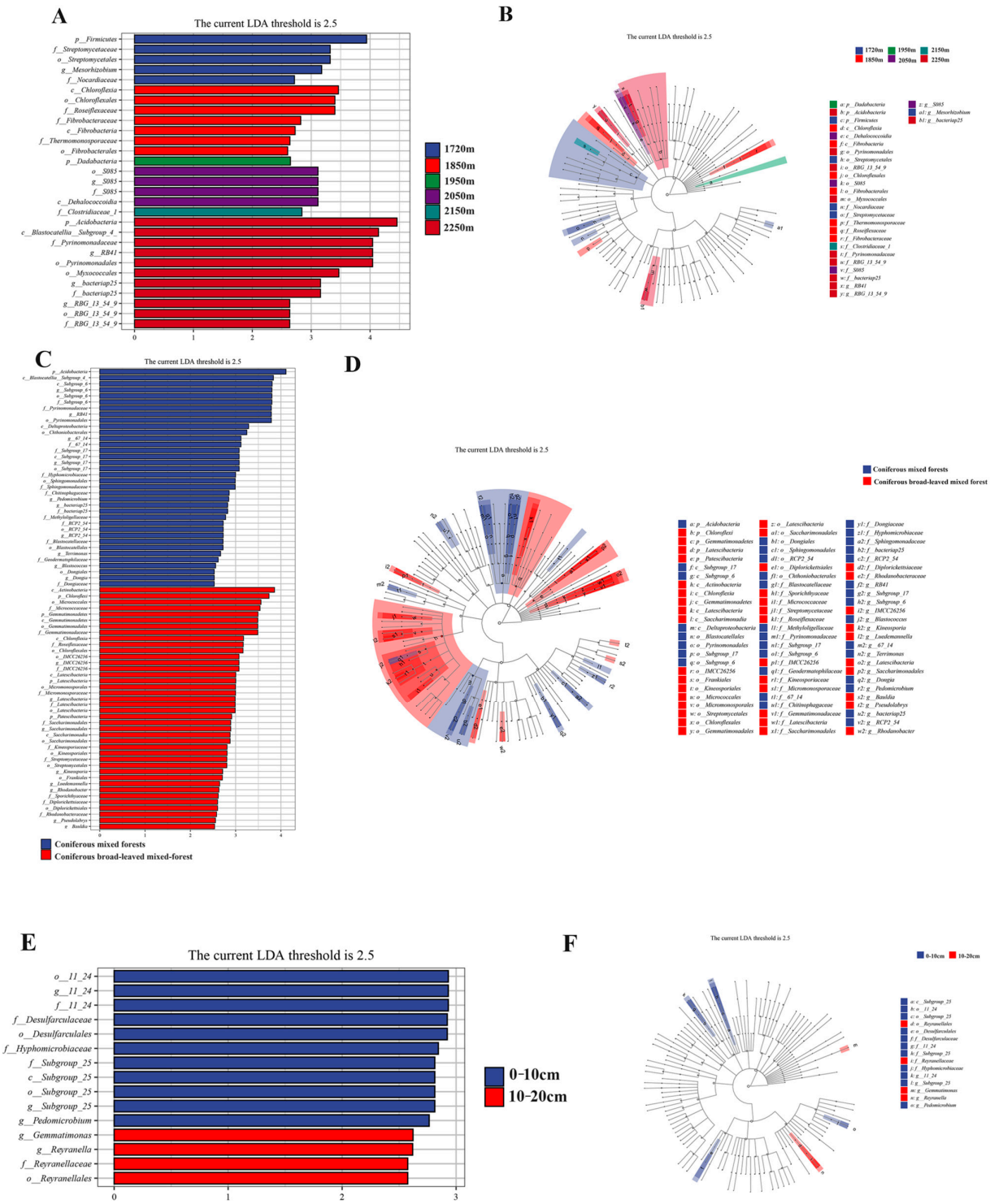

Figure 5. Plots of the results of LEfSe analysis at different altitude gradients (**A**,**B**), stand types (**C**,**D**), and soil depth (**E**,**F**); LDA score. Enriched taxa with an LDA score > 2.5 were shown in the histogram (**A**,**C**,**E**). LEfSe taxonomic cladogram (**B**,**D**,**F**).

3.5. Relationship between the Soil Bacterial Community and Soil Factors

RDA and a Mantel test were used to assess the correlation between soil characteristics and the structure of the bacterial community. Aside from the 0–10 cm soil layer in undisturbed forests, the remaining three graphs indicated that the top two axes accounted for more than 55.0% of the variability in bacterial community composition (Figure S2A–D). However, the main factors affecting bacterial community structure varied among stand

types. For pure stands, the contents of AN was the main explaining factor, followed by AK and INV. Notably, ST exerted a significant effect (R^{DON2} = 0.363) on soil bacterial community structure in the mixed stands, followed by TP (Table S4).

We then investigated the correlations between bacterial communities composition and soil variables by Spearman's correlation analysis. The correlations were stronger between the relative abundance of bacterial communities and soil variables at mixed stands than those in pure stands (Figure 6A,B). Altitude was significantly correlated with the relative abundance of Acidobacteria, Actinobacteria, Firmicutes, Rokubacteria, and Fibrobacteres at pure stands, as well as Planctomycetes at mixed stands. Altitude correlated significantly with most of the dominant bacterial phylum in pure stands (Table S5). Altitude and the contents of SOC, SOM, and AP had positive effects on the relative abundance of Acidobacteria and negative effects on the relative abundance of Actinobacteria, respectively, and the trend was more significant in mixed stands compared to pure stands (Figure 6, Table S6).

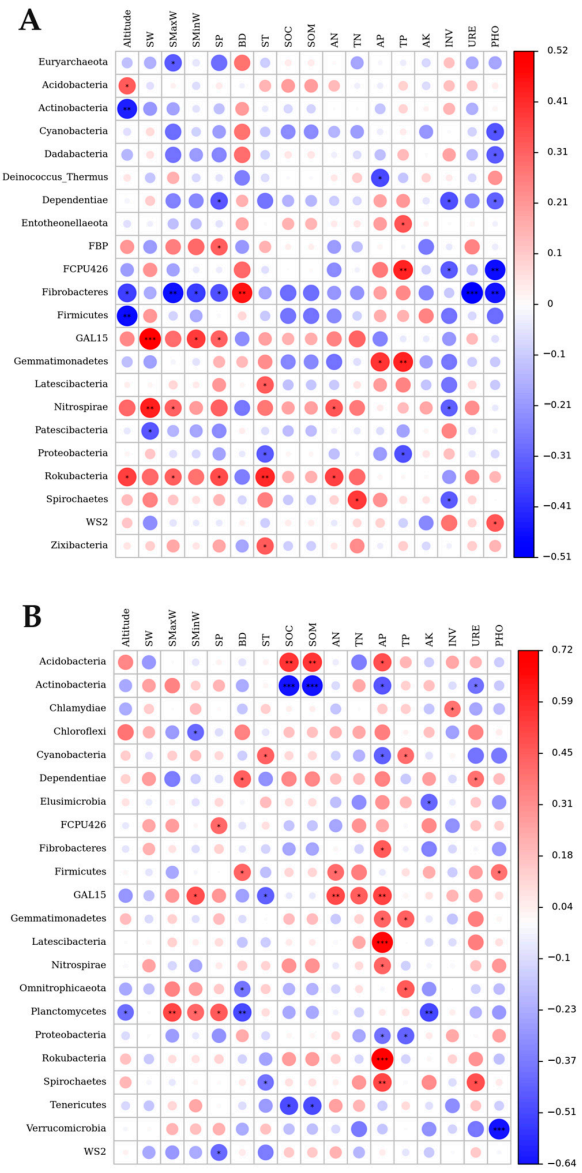

Figure 6. Spearman's correlation analysis between bacterial communities composition and soil variables in pure stands (**A**) and mixed stands (**B**), respectively. * represents $p < 0.05$; ** represents $p < 0.01$; *** represents $p < 0.001$. R-value represented by the rightmost gradient color bar.

4. Discussion

Our results highlighted several key findings related to the altitudinal distribution of soil bacterial communities under *Larix principis-rupprechtii* (Pinacrae) plantations in warm-temperate mountain ecosystems. First, inconsistent with those in temperate and tropical mountain ecosystems [5,15,38,39], the diversity of soil bacterial communities in the warm-temperate mountain ecosystem showed consistent patterns in pure and mixed stands, that is, a monotonic increase. The bacterial structures and key phylotypes were more sensitive and fragile to stand type than to altitude and soil depth, and the variation in abiotic factors along the altitudinal gradient dominated the changes in the bacterial community. Similar results were obtained in the study of Ivashchenko et al. (2021)—soil bacterial activity along an altitudinal gradient: in addition to altitudinal and soil factors, stand type is the main driver [7]. Finally, the differential soil drivers affecting the structure and composition of bacterial communities in larch pure stands and larch–birch mixed stands were captured.

4.1. Divergent Factors Controlling Bacterial Diversities and Community Compositions along an Altitudinal Gradient

Elevation diversity gradients are one of the most fundamental patterns in plant–animal biogeography. In this study, bacterial diversity showed a monotonically increasing trend across the altitudinal gradient, but there was no significant difference ($p > 0.05$). The bacterial responses were suggested by the findings of Ren et al. (2018) [16]. In contrast to the monotonically decreasing trend of soil bacterial communities along the altitudinal gradient in the cold-temperate zone [5,6,40], the increasing pattern of bacterial community diversity along the altitudinal gradient in larch forests in the warm-temperate zone was associated with an increase in soil organic matter content and moisture. Similarly, to the results of the study of Ivashchenko et al. (2021)—soil bacterial activity along an altitudinal gradient: in addition to altitudinal and soil factors, stand type is the main driver [7]. We found that soil bacterial altitude diversity showed a monotonically increasing trend in pure and mixed stands. Stand type significantly altered the structure and key phylotypes of soil bacterial communities at all elevation gradient levels, suggesting a close connection between soil bacterial and vegetation communities, which is consistent with most previous findings [5,6,41,42]. Each successional stage is characterized by specific vegetation communities, soil properties, and soil bacterial community structure [43,44]. In our study, soil bacterial community diversity was not significant between surface and subsurface soil. In addition, the soil layers had no significant effect on the relative abundance of bacterial communities of different taxa. This may be due to the fact that soil bacteria have a broader physiological range, e.g., soil bacteria can be photosynthetic autotrophs, heterotrophs, or chemoautotrophs [45]. In addition, we found that soil bacteria maintained a greater diversity at high altitude zones, which may be due to the relatively high concentrations of, e.g., SOM and AN at high altitudes promoting the growth of microorganisms [38]. Moreover, in general, we found that the diversity of bacterial communities in mixed stands was higher than that of pure stands, which is consistent with the study of Klimek et al. (2016) about temperate forests [46]. The results implied that vegetation species-rich communities can produce chemically diverse and abundant root secretions, which stimulate bacterial activity to responses to a broad spectrum of microbial substitutes, thus increasing the diversity [47].

In this study, the bacterial abundance of the different taxa showed different responses to altitude and stand type. Altitude had a marked effect on some of the more abundant bacterial phyla (Actinobacteria, Acidobacteria, Chloroflexi, Rokubacteria, Firmicutes). Our study demonstrated a greater effect of stand type on soil bacterial community diversity compared to elevation and soil depth. This is consistent with the study of Shen et al. (2013) [7]. Du et al. (2021) showed that soil depth can increase the rate of soil bacterial evolution including gene mutation, community assembly, and interactions [24]. Bacterial communities show a high degree of stability in surface soil, while the opposite is true in subsurface soil. No significant variations in community diversity were observed between

the surface and subsurface layers in bacterial communities in this research. Soil depth had no significant impact on the bacterial community diversity and richness. This may be due to the fact that soil bacteria have a wider physiological range, e.g., soil bacteria can be photosynthetic autotroph, heterotroph, or chemoautotroph organisms [45]. Based on the results of the PERMANOVA, we further verified that in this mountain ecosystem with a warm-temperate climate, the impact of stand type and altitude on bacterial community structure was more significant than soil depth.

Notably, compared to elevation and stand type, the soil depth barely altered the key phylogenetic types of the soil microbiota. In this study, a variation in vegetation composition may have contributed to this soil bacterial community variation in the response of the community between two stand types. As we expected, the SOM in this warm-temperate mountain ecosystem was a good predictor of the soil bacterial community composition; this finding is consistent with those of earlier studies [5,48,49]. Quantitative and qualitative changes in this substrate affect bacterial abundance, diversity, and community composition. Spatial heterogeneity in soil organic matter distribution has been hypothesized to drive an increase in bacterial diversity in carbon-rich soil [50]. Consistent with the findings of Huang et al. (2020) in a recent study, soil organic matter was the most important driver explaining soil microbial diversity and community composition [48]. Our study found that under limiting conditions, e.g., soil organic matter and moisture, soil bacterial communities can satisfy the demand of soil bacteria for unused resources through secretion of soil enzymes and limited allocation of energy, which is consistent with Lin et al. (2022) [51]. In addition, our study found that soil enzyme activities and soil effective nutrients such as AN, AP, and TP contents were significantly higher in pure stands than in mixed stands, which is consistent with the study of Hofmann et al. (2016) [52]. This change in effective soil nutrients and soil enzymes caused by stand types may be related to higher temperatures in pure stand soil [53–55].

4.2. Variation in Vegetation Composition May Have Contributed to the Variability in Soil Bacterial Community

Some studies have already shown that forest vegetation type can significantly influence soil bacterial community composition, structure, and its feedback on ecosystem structure and function, and is an important driver of soil bacterial community change [11,16,56–58]. In addition to vegetation composition, altitude played a key role in controlling soil bacterial composition and structure. Some factors (AN, AK, and INV for pure stands; ST and TP for mixed stands) showed distinct roles in driving the structure of soil bacterial communities. The correlation between bacterial community abundance and soil variables was stronger in mixed stands than in pure stands, which suggests that bacterial communities in the warm-temperate mountain ecosystem are more responsive to environmental changes in mixed stands than in pure stands. However, different keystone taxa existed in pure and mixed stands, which further confirmed the existence of niche differentiation among the bacterial taxa along the stands type. Edaphic factors such as higher altitudes and the contents of SOC, SOM, and AP favor acidic bacterial growth that have contrasting C use efficiency [59]. In general, the distribution of microorganisms can be explained by the oligotrophic–copiotrophic theory, corresponding to the r- and K-selected growth strategies [2,26,59,60]. Our study found that the Actinobacteria phylum (fast-growing eutrophic taxa, r-strategic bacteria) grew rapidly and had low substrate affinity, and that they responded rapidly to effective carbon and nutrient inputs, and were the most abundant at low altitudes which were enriched in labile carbon. In contrast, the abundance of Acidobacteria (slow-growing oligotrophic taxa, K-strategy bacteria) was greater at resource-rich high altitudes and had a high substrate affinity to efficiently utilize difficult-to-metabolize carbon [17], and that produced extracellular enzymes to degrade complexes when resources were scarce. Acidobacteria have been recognized as being ubiquitous and abundant in forest soil [61,62]. In this study, soil containing large amounts of organic carbon significantly promoted the growth of Acidithiobacillus while significantly inhibiting the growth of

Actinobacteria phylum during the succession from larch pure to larch–birch mixed forests, which suggested a possible shift from the r-strategy to the K-strategy.

5. Conclusions

This study described the biogeographic distribution of soil bacterial communities ranging from larch pure stands and larch–birch stands in warm-temperate mountain ecosystems in northern China, as well as more fine-resolution analysis in key bacterial groups along stand type and soil depth. Our results confirmed that soil bacterial diversity in pure stands and mixed stands showed consistent altitude distribution patterns (i.e., monotonically increasing). The significant changes in soil characteristics, such as soil organic carbon, soil organic matter, and available phosphorus across different altitudes, were the primary drivers of the differences in bacterial community composition and diversity. Bacterial structures and key phylotypes were found to be more sensitive and fragile to stand type rather than altitude and soil depth. The alterations in abiotic factors across the altitude gradient were the main drivers of changes in the bacterial community's composition. Key soil factors affecting bacterial community structure and composition varied under different stand types. Further research could focus on microbial life history strategies in larch forests, microbial interactions, and the functions of key taxa. The study of bacterial communities' characteristics between interactions of elevations, stand types, and soil depths is crucial to reveal how they regulate biogeochemical cycling in mountain ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15020392/s1: Table S1: Main characteristics of sample sites with different elevation gradients; Table S2: Variation of soil physicochemical factors under different altitudes and forest types in the 0–10 cm soil layer; Table S3: Variation of soil physicochemical factors under different altitudes and forest types in the 10–20 cm soil layer; Figure S1: Dilution curves of amplicon sequence variants (ASVs) of soil bacterial communities from two stand types and soil depths at different elevations; Table S4: Mantel test results for the correction between soil bacterial community and soil variables; Figure S2: Redundancy analysis based on pure stands (A,C) and mixed stands (B,D) at the genus level and soil factors (red and blue arrows); Table S5: Spearman's analysis of relative abundance of soil bacterial communities and environmental factors in pure stands; Table S6: Spearman's analysis of relative abundance of soil bacterial communities and environmental factors in mixed stands.

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