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Vertical Patterns of Soil Bacterial and Fungal Communities along a Soil Depth Gradient in a Natural *Picea crassifolia* Forest in Qinghai Province, China

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Abstract: Soil bacterial and fungal communities play different roles in maintaining the ecosystem structure and functions. However, these differences, which are related to soil depths, remain unclear and are the subject of this study. We selected six sample plots (20 m × 50 m) in a natural *Picea crassifolia* forest in an alpine meadow to determine the vertical patterns (0~10 cm, 10~20 cm, 20~30 cm, and 30~50 cm) of soil bacterial and fungal communities, and to predict their potential functions. The phyla Verrucomicrobia, Acidobacteria, and Proteobacteria dominated the soil bacteria, with more than 50% of the relative abundance, while the fungi Basidiomycota and Ascomycota dominated the soil fungi. The potential functions of bacteria, including metabolism and transcription, increased with soil depth, and corresponded to specific bacterial taxa. The functional guilds of fungi, including endophytes, arbuscular mycorrhiza, and ectomycorrhiza, did not change with soil depth. The structural equation modeling analysis revealed that soil organic carbon (SOC) and pH were the key drivers shaping the soil bacterial communities and potential functions in the 0–50 cm soil layer. SOC was also a key driver of soil fungal α diversity. The sample plot, namely, its geographic locations, was another key driver shaping soil fungal *β* diversity and potential functions, but soil depth was not. Our results differentiate the importance of SOC and geographic location in shaping soil bacterial and fungal communities, respectively, and indicate that examining soil microbial composition and corresponding functions concomitantly is important for the maintenance and management of forest ecosystem functions.

Keywords: soil profile; fungal diversity; bacterial composition; potential functions; alpine forest

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1. Introduction

Soil bacteria and fungi are crucial decomposers that regulate the ecosystem structure and function [1], as they are important for organic matter decomposition and soil structure. Soil microorganisms are sensitive to changes in external environments, and it has been reported that they are influenced by vegetation types and diversity [2], elevation [3], and soil physical and chemical properties [4]. Notably, soil microbial community composition and functions, such as carbohydrate metabolism and transcription, are stable characteristics in a natural, undisturbed ecosystem. The main influence on the structure of soil microbial communities are the microenvironmental properties of their habitats, including organic matter, bulk density, soil nutrients, and pH. These variables change with soil depth [5–7]; consequently, soil microbial communities and soil properties usually display covariational vertical patterns along soil depths.

Recent studies have focused on the vertical patterns of soil microbial communities along soil depths [8–10]. Generally, soil microbial biomass and diversity, including bacteria and fungi, decrease with an increase in soil depth [9,10]. For example, the soil bacteria Cyanobacteria, Planctomycetes, and Proteobacteria are more abundant [10–12], and soil fungal biomass and diversity are greater [13,14] in surface soil than in subsoils. In addition, the vertical patterns of soil bacteria and fungi at the kingdom, family, or phylum levels are related to soil depth. The abundance of copiotrophic bacteria is substantially greater than oligotrophic bacteria in the organic soil layer [15]. Furthermore, soil microbial communities are affected mainly by soil matter content in the topsoil (0~10 cm), but are affected mainly by processes of soil development in deeper layers [16]. Therefore, specific bacterial taxa have exhibited different responses to soil depth; however, the soil microbial taxa and their drivers along soil depths remain unclear.

A global analysis of terrestrial ecosystems reported that up to 60% of the soil microbial biomass is stored in deep soils [17]. Soil microbial communities in deep soil layers generally play key roles in nutrient cycling [8], as the priming effect and enzymatic activities of the microbial community increase with soil depths [18]. Therefore, soil microbial communities at different soil depths also show distinct potential roles in regulating the ecosystem structure and functions.

The main soil microbial taxa are bacteria and fungi, and they display different responses and potential functions because of their capacity to adapt to the microenvironment along soil depths. In general, soil bacteria have a relatively narrow tolerance to changes in soil pH [19,20], whereas soil fungi tolerate a much wider soil pH range [21]. Also, soil fungi have lower nitrogen (N) requirements than bacteria [22]; thus, the fungi to bacteria ratio increases in subsoil with low C and N availability [6]. Moreover, soil bacteria require abundant nutrients, while fungi survive in soils with low available nutrients [23]. These key variables (soil pH and nutrient contents) change substantially with soil depth and are drivers of soil bacterial and fungal communities. In addition, some soil bacteria and fungi are involved in distinct ecological functions. For example, the soil bacteria Verrucomicrobia and Euryarchaeota are responsible for anaerobic processes (anaerobic methane oxidization and methanogenesis) related to soil microbial metabolism [24,25]. Soil fungi are decomposers of dead and living organic material, and some functional guilds (e.g., mycorrhizal fungi) form beneficial associations with plant species. Additionally, some fungi are important plant or animal pathogens [26]. Therefore, the functional guilds of fungi are vital in maintaining soil health and ecosystem stability. However, the dynamics of specific groups of microorganisms along soil depths and their ecological functions are uncertain. For example, analyses of soil microbial communities are generally based on phospholipid fatty acid, without information on the specific taxa [17,27,28].

Alpine forests in the Qinghai–Tibetan Plateau have important ecological functions, including erosion prevention, sand-fixing, and carbon storage. A detailed understanding of soil microbial communities and ecological functions along soil depths would promote the protection and maintenance of forest soils and forest ecosystem functions. In this study, we used high-throughput sequencing techniques to examine the shifts in soil bacterial and fungal communities along a soil depth gradient in a *Picea crassifolia* forest. We aimed to determine: (1) the vertical patterns of soil bacterial and fungal communities and their potential functions from 0 to 50 cm soil depth; and (2) the key factors shaping soil bacterial and fungal communities.

2. Material and Methods

2.1. Study Area

The study site was located in the Beishan National Forest Park (36°42′–37°06′ N, 102°00′–102°43′ E), Huzhu County, at the junction of the Qinghai–Tibetan Plateau and Loess Plateau. This region has a typical plateau continental climate with distinct cold and warm and wet and dry seasons. The mean annual air temperature is 3.4 °C, and the mean daily air temperature of the coldest month, January, is −8 °C and of the warmest

month, July, is 16 °C. The average annual precipitation is 400~500 mm, with more than 70% occurring between June and September. Cold temperate coniferous forests, dominated by *Picea crassifolia*, are the main forest type. The study area consisted of natural *P. crassifolia* forests at the middle of succession with a mean canopy density of 0.68 and a mean tree layer height of 14.2 m. The plants *Potentilla fruticose* and *Polygonumb viviparum* were the dominant shrub and grass species of the forests. The soil type was classified as haplic Luvisol according to the FAO taxonomy [29]. More details are presented in Table 1.

Table 1. Geographical and plant community characteristics in the *Picea crassifolia* forest of six sampling plots.

Sample Site	Geographical Location	Altitude (m)	Canopy Density (%)	Number of Trees	Mean Forest Height (m)	Mean Forest DBH (cm)	Tree Biomass ($t \cdot ha^{-1}$)	Shrub Biomass ($t \cdot ha^{-1}$)	Herb Biomass ($t \cdot ha^{-1}$)	Litter Biomass ($t \cdot ha^{-1}$)
Plot A	36°52′13.80″ N 102°26′36.60″ E	2835	70	112	6.44	11.6	115.95	0.82	0.51	8.53
Plot B	36°52′16.20″ N 102°26′36.60″ E	2817	60	89	6.98	11.6	40.63	1.22	0.72	0.99
Plot C	36°52′16.20″ N 102°26′40.80″ E	2816	80	88	6.48	10.9	32.62	0.72	0.64	1.37
Plot D	36°52′22.80″ N 102°26′34.20″ E	2815	65	102	7.88	12.6	73.53	0.36	0.26	1.09
Plot E	36°52′24.00″ N 102°26′36.00″ E	2847	70	89	6.46	13.5	41.28	0.17	0.62	1.80
Plot F	36°52′24.00″ N 102°26′31.80″ E	2795	64	90	8.14	10.6	88.43	0.50	0.34	0.78

DBH: Diameter at breast height.

2.2. Experimental Design, Plant and Soil Sampling

To minimize the influence of plant species on the soil microbial community, we selected six plots (each 20 m × 50 m, and labelled Plot A, Plot B, Plot C, Plot D, Plot E, and Plot F; Table 1) in the *P. crassifolia* forest, with a distance of 500 m to 2000 m between any two plots. This distance ensured that each plot was independent to avoid pseudo-replication. We surveyed all woody stems with a diameter greater than 1 cm at breast height (DBH) and calculated the above-ground biomass of the tree layer. Five quadrats (1 m × 1 m) were selected randomly in each plot to measure the understory (shrub, herb, and litter) biomass. In July, five soil samples (5 cm diameter) to a depth of 50 cm were collected randomly from each quadrat along a soil depth gradient (0~10 cm, 10~20 cm, 20~30 cm, and 30~50 cm). The surface forest soils were easily affected by the external disturbance, and we sampled the soil each 10 cm up to 30 cm soil layer. The deeper soils were affected very little by external disturbance, and therefore we sampled the 20 cm layer. Additionally, the soil biotic and nutrient contents were also relatively stable in the deep soil layer. The samples were sieved through a 2 mm mesh to remove rocks, roots, and debris. Part of each soil sample was stored at −80 °C for DNA extraction, and the rest of the sample was air-dried for analysis of physical and chemical properties.

2.3. Soil Bacteria and Fungi Analysis

Total DNA was extracted from each soil sample (0.25 g) using the MoBio PowerSoil DNA Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and quality of DNA were determined using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The PCR amplification, Illumina MiSeq sequencing and processing of sequenced data are detailed in the supplements [30–32]. In summary, the number of reads of each sample at the whole soil depths was in a band of 29,417–124,146, which is sufficient for our analysis (Figure S1).

2.4. Data Analyses

Potential microbial biomarkers along soil depths were obtained using the linear discriminant analysis (LDA) effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/, accessed on 20 March 2023) [30], as described by Zhou et al. [33].

The Kyoto Encyclopaedia of Genes and Genomes (KEGG), using 16S rRNA gene sequences by the Tax4fun package in R 3.5.1 [34], determined the potential functions of the bacteria. The functional guilds of fungi were determined using 'FUNGuildR' package [35].

To control the random effects of the six plots, we initially analyzed the effects of plots on soil physical and chemical properties and microbial α diversity (operational taxonomic units—OTUs and Chao1, PD, and Shannon–Wiener indices) using linear mixed-effects models (Table S1). Then, the fixed effects of soil depth on soil physicochemical properties and microbial α diversity were analyzed by one-way ANOVA with LSD tests, accepting $p < 0.05$ as the level of significance. The data were transformed to meet the assumptions of normal distribution and homogeneity for ANOVA. The β diversities of the bacterial and fungal communities along the soil's depth were determined by constrained principal co-ordinates analysis (CPCoA) based on Bray–Curtis distances.

Redundancy analysis (RDA) tested the relationships among soil properties and soil microbial communities according to the first axis lengths of detrended correspondence analysis (DCA). Soil properties that affected the soil microbial communities were selected to build the RDA model using the 'step' function in the stats R package. The canonical analysis of principal coordinates (CAP) constrained soil depths and sample plots, and selected soil properties using the 'capscale' function in the vegan R package [10] to determine key factors driving soil bacterial and fungal communities.

According to the CAP results, a structural equation model (SEM) was generated to determine the pathways of the effects of soil depth on soil bacteria and fungi diversities and functions using Amos version 23.0 (Amos Development, Spring House, Armonk, NY, USA). In the SEM analysis, the soil microbial diversity and functions were presented by the first principal components (PC1) for bacterial diversity, fungal diversity, bacterial function, and fungal function, which explained 77.3%, 69.9%, 71.8%, and 43.4% of the total variance, respectively. Because only 43.4% of the variance of fungal function PC1 was explained, a second principal component (fungal function PC2) was added in the SEM analysis, which increased the explanation to 74.4%. The maximum likelihood estimation method was applied to the SEM, and the goodness of fit of the models was determined by chi-square (χ^2), the Akaike information criterion (AIC), and the root mean square error of approximation (RMSEA).

3. Results

3.1. Soil Microbial Composition

Bacterial phyla Verrucomicrobia, Acidobacteria, and Proteobacteria dominated all soil depths with relative abundances of 63.3%, 64.7%, 59.0%, and 55.3% at the 0~10 cm, 10~20 cm, 20~30 cm, and 30~50 cm soil layers, respectively (Figure 1a, Table S1). The phyla Proteobacteria, Planctomycetes, and Bacteroidetes decreased, while Crenarchaeota increased with an increase in soil depth (Figure 1a, Table S1).

Fungi were dominated by the phyla Basidiomycota and Ascomycota (Figure 1b). Basidiomycota was the most abundant fungi across all soil depths, with average relative abundances of 32.3%, 37.8%, 31.5%, and 36.3% at the 0~10 cm, 10~20 cm, 20~30 cm, and 30~50 cm soil depths, respectively (Figure 1b, Table S1). Ascomycota displayed a decreasing trend with soil depths, but overall, there was no difference in the relative abundances of Basidiomycota and Ascomycota among soil depths (Figure 1b, Table S1). The relative abundance of Zygomycota was less than 0.1%, and decreased from 0.092% in 0~10 cm to 0.016% in 30~50 cm (Figure 1b, Table S1).

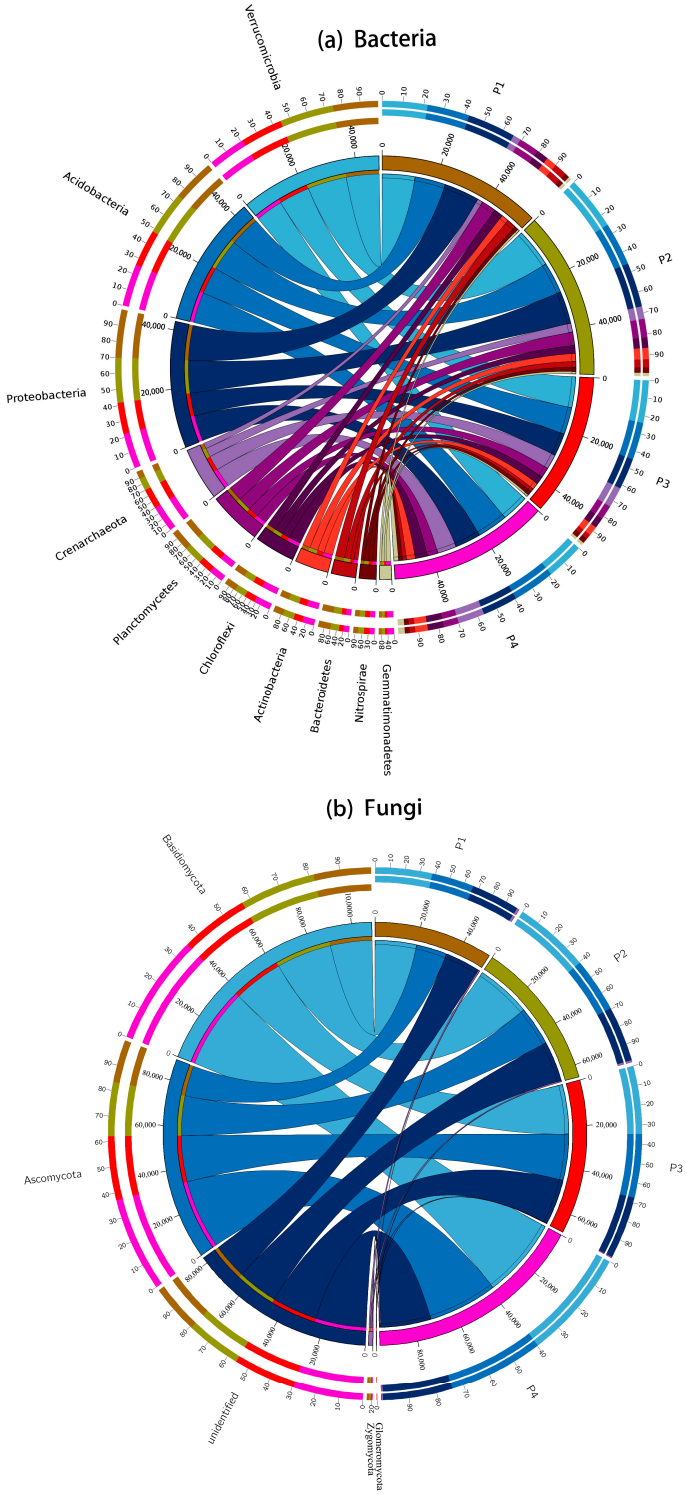

Figure 1. Phyla composition of the (**a**) bacteria and (**b**) fungi for different soil depths of the natural *Picea crassifolia* forest in the Qinghai–Tibet Plateau. P1, P2, P3, and P4 represent the soil depths of 0–10 cm, 10–20 cm, 20–30 cm, and 30–50 cm, respectively.

Linear discriminant analysis (LDA) effect size (LEfSe) analyses revealed that 16 bacterial clades were biomarkers of the four soil layers (Figure 2a). Ten of these biomarkers were in the 0~10 cm soil layer, and included the families Hyphomonadaceae, Acetobacteraceae, Erythrobacteraceae, and Nannocystaceae; genera *Caulobacter* and *Dokdonella*; family Actinosynnemataceae; genus *Adhaeribacter*; genus *Rhodocytophaga*; and order Gemmati-

monadales. In addition, only two, one, and three clades were enriched in the 10~20 cm, 20~30 cm, and 30~50 cm soil layers, respectively. The genera *Chondromyces* and *125ds10* (family Proteobacteria) were the biomarkers of the 10~20 cm soil layer, the order SB-34 was the biomarker of the 20~30 cm soil layer, and the order E2, the genus *Sedimentibacter* and the family Tissierellaceae (phylum Firmicutes) were the biomarkers of the 30~50 cm soil layer.

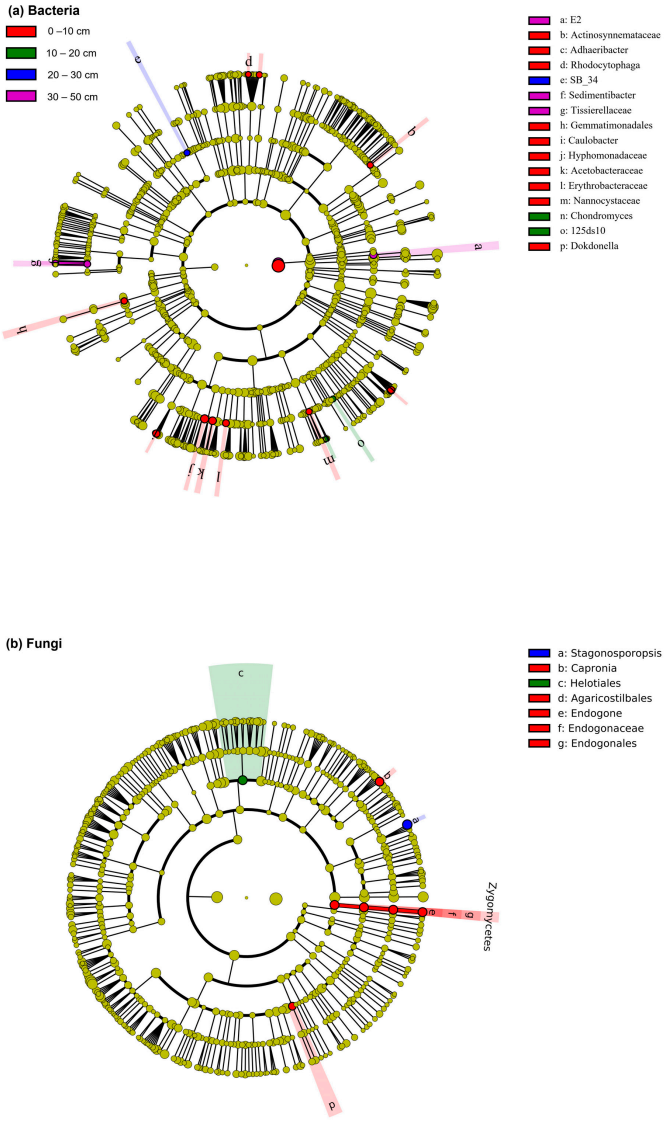

Figure 2. LEfSe analysis of the abundance of (**a**) bacteria and (**b**) fungi at different soil depths in the natural *Picea crassifolia* forest.

As for the fungal community (Figure 2b), seven clades were biomarkers of the four soil layers. The genus *Capronia*, orders Agaricostilbales, and Zygomycota, family Endogonaceae, genus *Endogone*, family Mucoraceae, and genus *Mucor* were the biomarkers of the 0~10 cm soil layer; order Helotiales was the biomarker of the 20~30 cm soil layer; and the genus *Stagonosporopsis* was the biomarker of the 30~50 cm soil layer.

3.2. Soil Microbial Community Diversity

Soil bacterial α diversity, including the number of OTUs and Chao1, PD, and Shannon–Wiener indices, decreased significantly with soil depths. Soil fungal α diversity also decreased with soil depths, but the indices of PD and Shannon–Wiener were not significant (Tables S1 and 2).

Table 2. Soil microbial α diversity indices at different soil depths.

Microbial Taxa	Soil Depth	OTUs [1]	Chao1	PD	Shannon–Wiener
Bacteria	0–10 cm	2703 ± 86 ^{a}	4806 ± 158 ^{a}	160.2 ± 5.18 ^{a}	9.04 ± 0.24 ^{a}
	10–20 cm	2543 ± 69 ^{b}	4602 ± 137 ^{ab}	151.6 ± 4.89 ^{b}	8.73 ± 0.15 ^{b}
	20–30 cm	2451 ± 92 ^{b}	4502 ± 170 ^{b}	149.3 ± 4.62 ^{b}	8.61 ± 0.19 ^{bc}
	30–50 cm	2247 ± 139 ^{c}	4223 ± 208 ^{c}	139.8 ± 5.89 ^{c}	8.35 ± 0.28 ^{c}
Fungi	0–10 cm	965 ± 68 ^{a}	1577 ± 108 ^{a}	215.9 ± 19.22 ^{a}	6.17 ± 0.27 ^{a}
	10–20 cm	907 ± 107 ^{ab}	1494 ± 120 ^{ab}	212.6 ± 29.13 ^{a}	5.90 ± 0.86 ^{a}
	20–30 cm	851 ± 99 ^{ab}	1392 ± 145 ^{bc}	224.5 ± 43.64 ^{a}	5.65 ± 0.84 ^{a}
	30–50 cm	797 ± 74 ^{b}	1330 ± 76 ^{c}	197.6 ± 35.19 ^{a}	5.31 ± 0.94 ^{a}

[1] OTUs means the number of operational taxonomic units. Means with different lowercase letters among soil depths amd among microbial taxa differ from each other ($p < 0.05$).

Based on Bray–Curtis distances and the CPCoA analysis, β diversity of the soil bacterial communities differed ($p = 0.001$) among depths (Figure 3). This analysis explained 19.6% of the variance (Figure 3a), while the effect of soil depth attributable to the sampling site was not significant (Figure 3b, $p = 0.13$). Moreover, the soil bacterial communities in the 0~10 cm and 10~20 cm layers were divergent in a similar manner from the community in the 30~50 cm layer (separation by the second component, Figure 3a).

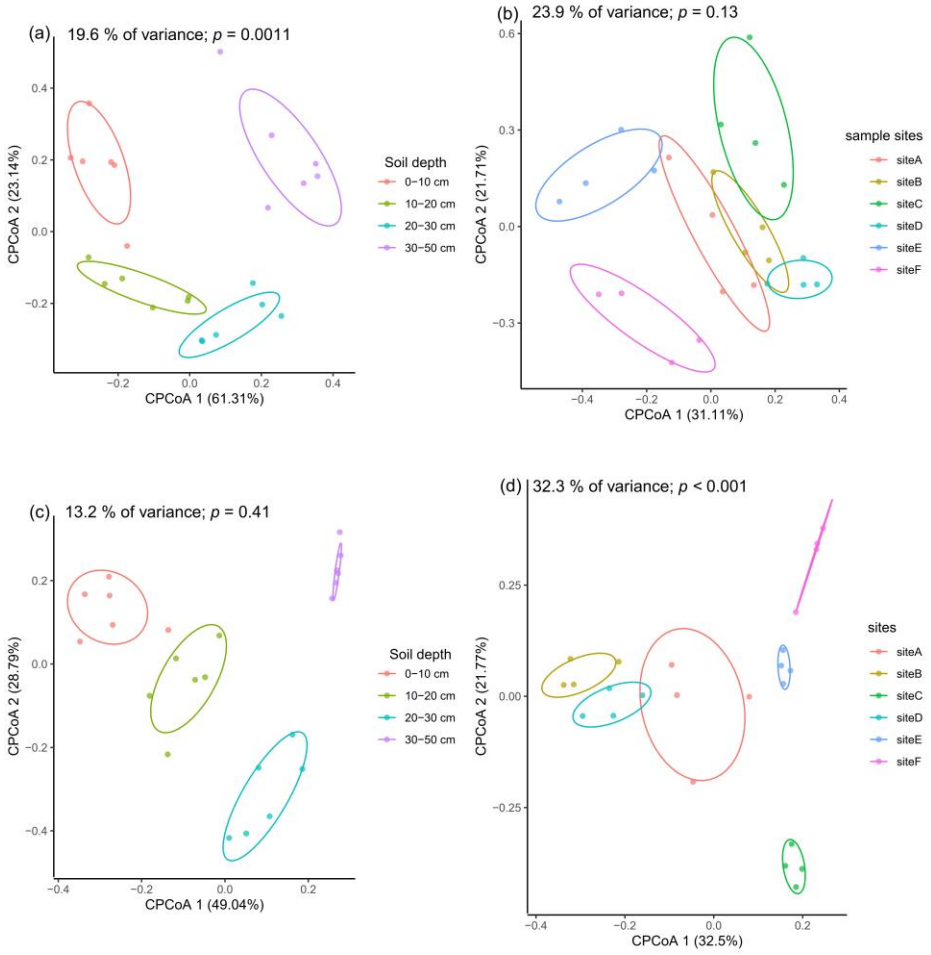

Figure 3. Constrained PCoA of Bray–Curtis distances in soil bacteria along a soil depth gradient (**a**) and sampling site (**b**), and fungi along a soil depth gradient (**c**) and sampling sites (**d**) in the natural *Picea crassifolia* forest in the Qinghai–Tibetan Plateau.

The CPCoA analysis on soil fungal communities along soil depths explained only 13.2% of the variance, and was not significant (Figure 3c, $p = 0.41$); however, when plots were included, this analysis explained 32.3% of the variance (Figure 3d, $p < 0.001$).

3.3. Functional Potentials of Soil Microbial Communities

The functions of the bacterial communities were predicted using the Tax4Fun approach (Figure 4a). The predicted functions at all soil depths were dominated by metabolism-related pathways, especially of carbohydrates (average 13.2%), nitrogen such as amino acids (average 12.0%), and nucleotides (average 4.83%). Other dominant KEGG categories included membrane transport (average 13.0%) and signal transduction (average 8.34%). Among these categories, pathways involved in the metabolism of co-factors and vitamins (average 6.66%) and transcription increased with soil depth (Figure 4a,b). In contrast, pathways of cellular community-eukaryotes and signaling molecules decreased with soil depth (Figure 4d,e).

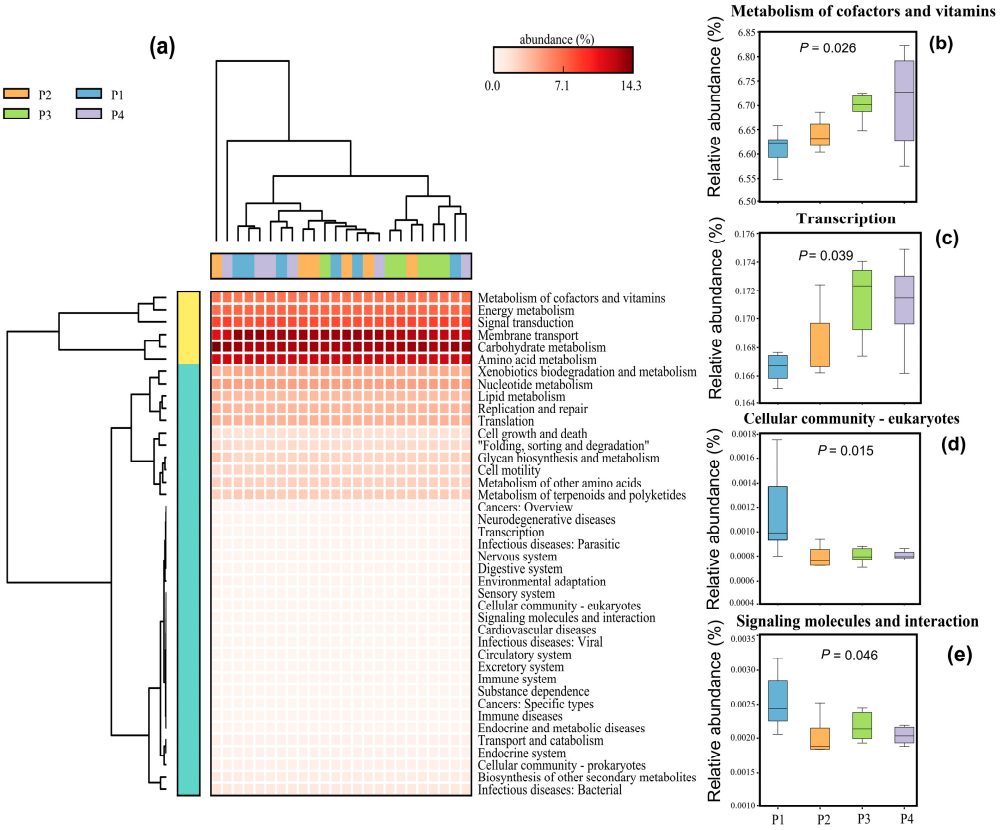

Figure 4. Relative abundance of the predicted KEGG categories along a soil depth gradient in the natural *Picea crassifolia* forest in the Qinghai–Tibetan Plateau; all functions (**a**), metabolism of cofactors and vitamins (**b**), transcription (**c**), cellular community—eukaryotes (**d**) and signaling molecules and interaction (**e**). P1, P2, P3, and P4 represent the soil depths at 0–10 cm, 10–20 cm, 20–30 cm, and 30–50 cm, respectively.

There was no significant difference in the endophytes, arbuscular mycorrhiza, ectomycorrhiza, and undefined saprotrophs along the soil depth gradient (Figure 5a; Table S1). The fungal guilds differed markedly among the six plots in the 0–50 cm soil layer (Figure 5b). In the identified fungal guilds, endophytes accounted for more than 40% of the guilds in plots A, B, and D, while the arbuscular mycorrhizal guilds dominated in the other three plots. There was no ectomycorrhizal guild in plots B, C, and D (Figure 5b).

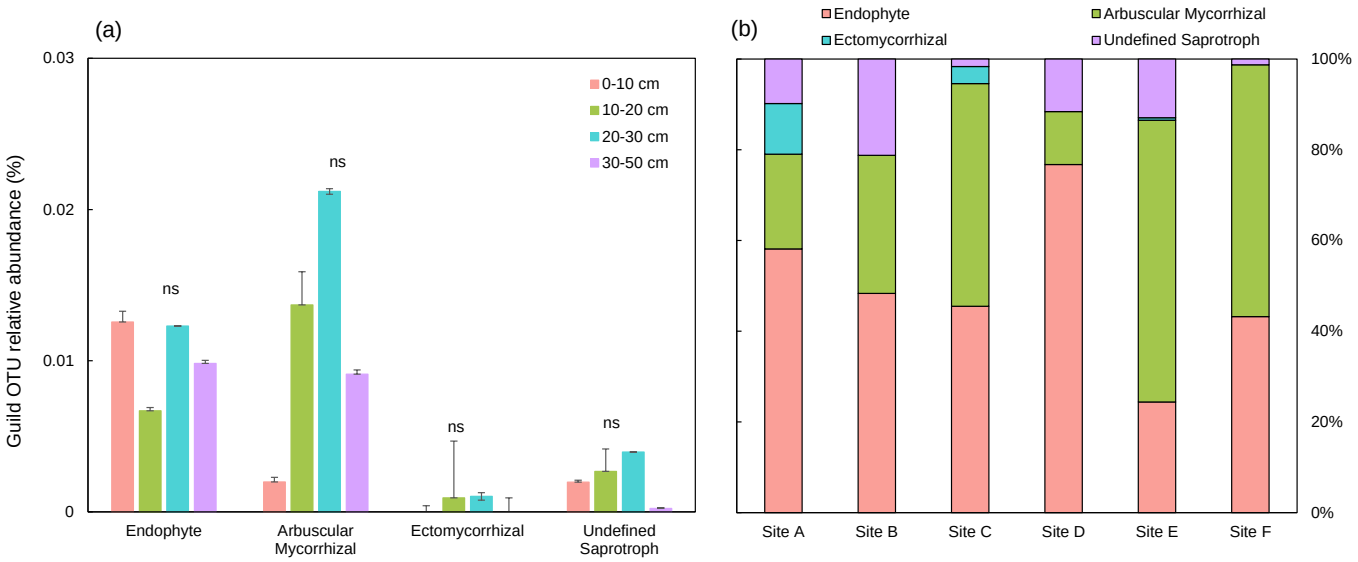

Figure 5. The proportion of operational taxonomic units (OTUs) assigned to guilds along a soil depth gradient (**a**) and among six sampling sites (**b**) in the natural *Picea crassifolia* forest in the Qinghai–Tibetan Plateau.

3.4. Factors Related to Soil Microbial Communities

Based on the canonical analysis of principal coordinates (CAP), soil depth was the most dominant factor driving soil bacterial communities ($p = 0.001$), explaining 23.7% of the variation, followed by plot ($p = 0.016$) (Table 3). Soil organic carbon (SOC) was also significant ($p = 0.019$) and explained 7.28% of the variation. For soil fungal communities, plot was significant ($p = 0.001$) and explained 40.2% of the variation, followed by soil depth ($p = 0.015$), which explained 13.9% of the variation (Table 3).

Table 3. The β diversity in soil bacterial and fungal community composition among soil depths and among plots based on the CAP analysis.

Factor	Bacterial Communities					Fungal Communities				
	df	**SS** [1]	***F***	***p***	**Explained Variation**	**df**	**SS** [2]	***F***	***p***	**Explained Variation**
Soil depth	3	0.298	2.574	0.001	23.7%	3	0.896	1.544	0.015	13.9%
Sample plot	5	0.308	1.595	0.016	24.4%	5	2.599	2.685	0.001	40.2%
Soil organic carbon	1	0.092	2.374	0.019	7.28%	1	0.250	1.291	0.148	
Soil pH	1	0.060	1.560	0.132		1	0.180	0.925	0.578	
Residual	13	0.502				15	2.516			

[1] Sum of squares, [2] Sum of squares.

3.5. The Pathways Determining Soil Microbial Diversity and Functions

Based on the CAP results, soil pH and soil organic carbon (SOC) were the important soil properties driving soil microbial communities and dominant functions. Therefore, the two soil properties were included in the SEM analysis, which revealed the different pathways of soil depths on soil bacterial and fungal diversity and functions through soil pH and SOC (Figure 6).

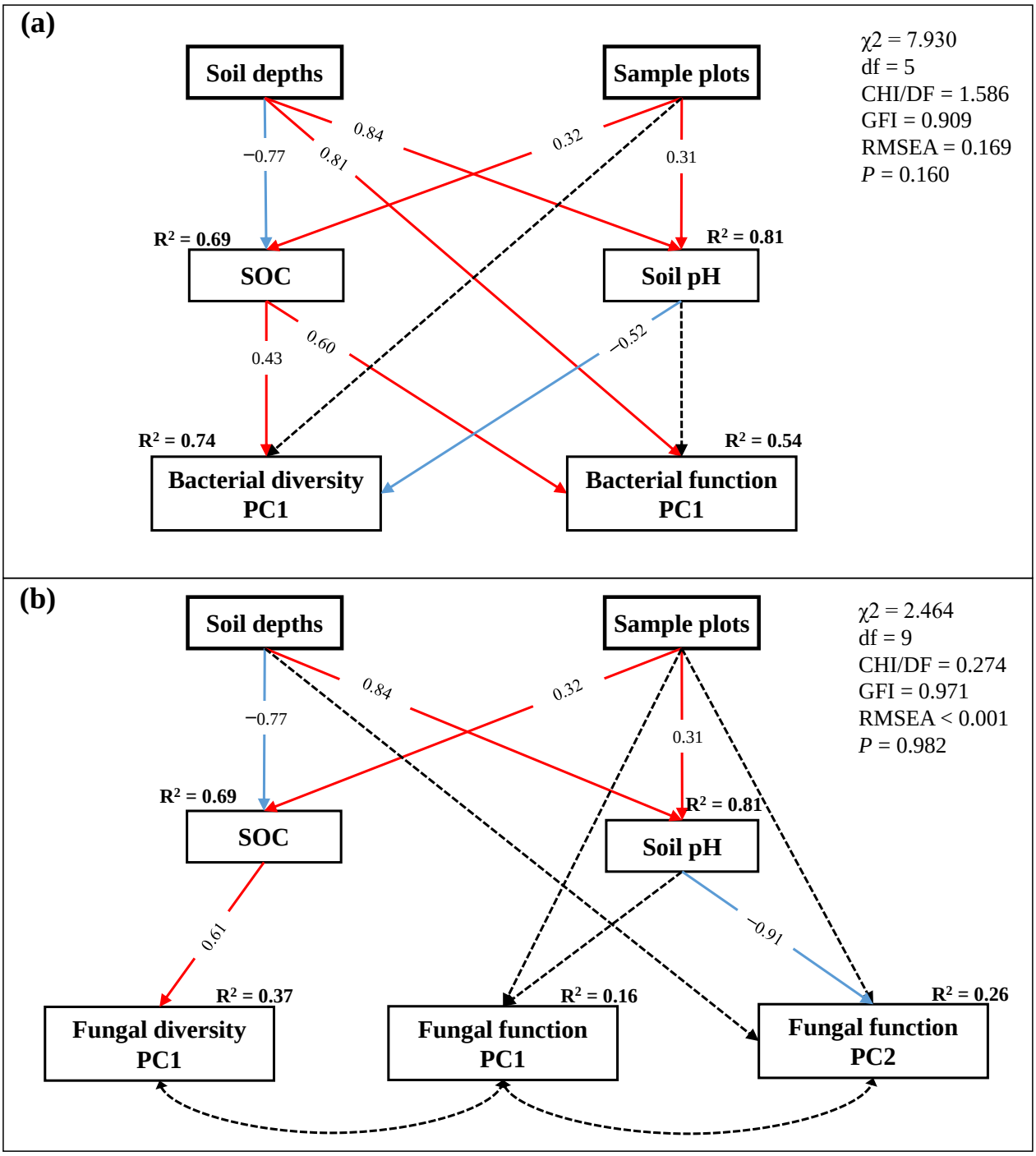

Figure 6. The pathways of the effects of soil depth on soil bacterial community diversity and function (**a**), and fungal community diversity and function (**b**) through the selected soil properties based on structure equation modelling (SEM). Single-headed arrows indicate the direction of causation, and double-headed arrows represent covariance between related variables. The red and blue lines indicate significantly positive and negative pathways, respectively ($p < 0.05$). The black dashed lines represent non-significant pathways ($p > 0.05$). The standardized path coefficients and the proportion of variance explained (R^2) are presented adjacent to the arrows and alongside each response variable in the model, respectively.

Soil depth increased soil pH directly but decreased SOC, and, thus, had the most negative effect on soil bacterial diversity (r = −0.767), followed by soil pH (r = −0.518). The SOC had the greatest positive effect on soil bacterial diversity (r = 0.429). Soil function was

affected directly by soil depth (r = 0.806), followed by SOC (r = 0.601) and soil pH (r = 0.278). The total effects of plot on soil bacterial diversity and function were less than soil depths, soil pH, and SOC (Figure 6a; Table 4). Overall, the four variables explained 74% and 54% of the total variance in soil bacterial diversity and function, respectively (Figure 6a).

Table 4. The pathways of soil depth on soil microbial diversity and function based on the structural equation model (SEM) analysis.

Variables		Bacterial Diversity PC1	Bacterial Function PC1	Fungal Diversity PC1	Fungal Function PC1	Fungal Function PC2
Soil depth	Direct effects		0.806			0.473
	Total effects	−0.767	0.579	−0.469	0.301	−0.292
Sampling plot	Direct effects	−0.195			−0.333	0.404
	Total effects	−0.219	0.277	0.194	−0.222	0.123
Soil pH	Direct effects	−0.518	0.278		0.357	−0.906
	Total effects	−0.518	0.278		0.357	−0.906
Soil organic carbon	Direct effects	0.429	0.601	0.609		
	Total effects	0.429	0.601	0.609		

Soil depth affected soil fungal diversity and the four functions, including fungal function PC1 and PC2. The SOC was the most dominant factor (r = 0.609) affecting soil fungal diversity, followed by soil depth (r = −0.469), while soil pH was the most dominant factor affecting soil fungal function PC1 (r = 0.357) and fungal PC2 (r = −0.906), followed by soil depth (r = 0.301 and −0.292, respectively). The effect of plot on soil fungal function was direct but not significant ($p > 0.05$), and its total effects were mainly via soil pH. Although the effect of sample plot was non-significant, it is worth noting that the total effects of plot and soil depth on soil fungal diversity and functions were opposite (Figure 6b; Table 4).

4. Discussion

4.1. Soil Bacterial Community with Soil Depths

This study demonstrated that three dominant soil bacteria phyla—Proteobacteria, Acidobacteria, and Verrucomicrobia—accounted for greater than 50% of the relative abundance in the four soil depths, owing to their wide range of adaptability. The dominance of Proteobacteria and Acidobacteria is common in forest soils [4,15]. Acidobacteria has a wide phylogenetic diversity, spanning 26 subdivisions [36]. Its genomic, physiological, and metabolic versatilities allow for flexibility in a fluctuating soil environment, and, therefore, it is ubiquitous [37]. Verrucomicrobia is facultative anaerobic, saccharolytic, and free-living [38], and generally exhibits a unimodal pattern, peaking in soil depths of 10~20 cm [39] or 20~40 cm [8]. In the current study, Verrucomicrobia peaked at 20~30 cm, consistent with the previous studies.

Of the three dominant bacterial phyla, the relative abundances of Proteobacteria and Verrucomicrobia indicated significant depth-related patterns. Proteobacteria is a copiotrophic bacteria and, thus, is more abundant in the upper soil layer with greater SOC and N contents (Table S2), which is consistent with previous reports [8,40,41]. The relative abundances of Bacteroidetes and Planctomycetes are also depth-related [11], increasing with depth, which is characterized by greater availability of C and N [27,42].

Verrucomicrobia is an oligotrophic bacterium, tolerant of low-nutrient soils and able to use recalcitrant C sources [8]. The relative abundance of Verrucomicrobia displayed a unimodal pattern with soil depth, which might be due to their preference for microaerobic rather than fully aerobic or anaerobic environments [39]. SB-34, an anaerobic class [43] belonging to Chloroflexi, peaked in subsoils, which is consistent with other reports [44,45].

Sedimentibacter and Tissierellaceae, belonging to Firmicutes which are the predominant C cyclers in the deep soil rhizosphere [46], were abundant in the 30~50 cm soil depth. They are classified as Clostridia, which use fermentative metabolism [43]. Crenarchaeota

were the most abundant (14%) bacteria in the deepest soil depth, which is consistent with previous studies [8,47,48]. These bacteria drive autotrophic nitrification in deep soil [49].

In the present study, specific soil bacterial taxa were enriched in different soil depths, and their relative abundances were not synchronous along soil depths. In addition, the asynchronous responses of specific bacterial taxa between two adjacent layers affected soil bacterial β diversity, which was a consequence of soil depth rather than sampling plot according to the CPCoA analysis.

Changes in specific soil bacterial taxa are usually related to their specific ecological functions. In the current study, the functions of metabolism-related pathways, especially C and N metabolism, dominated the surface soils and decreased with soil depths, which is consistent with other reports [50–52]. This demonstrates that bacteria in surface soils have higher rates of C usage than bacteria in deeper soils, up to an order of magnitude higher [53]. This presumably results from greater amounts of soil organic C in the surface than deeper soils due to plant litter [54] (Table 1), which supports the soil copiotrophic bacteria in the surface soils. Several studies indicated that N-cycling functional gene abundances and biomass decreased with soil depth [55,56]. By contrast, metabolism of co-factors and vitamins, and transcription increased in deeper soils, as predicted by metagenomic analysis, which may help specific soil bacteria improve the efficiency of nutrient utilization in a harsh environment [57].

In summary, the soil bacterial community and functions were affected mainly by soil depth but were not affected by plots. Soil pH affected the soil bacterial community, as has been reported in previous studies [19,58,59]. In general, soil bacteria cannot tolerate a wide pH range [20], and deviations of 1.5 pH units from the in situ pH of bacterial communities can reduce their activity by 50% [60]. In addition, SOC, N, and P were reported to be key drivers in determining the soil community composition and diversity [61], and soil available nutrients, especially C substrates [8], were reported to be important in structuring the soil bacterial community along a soil depth gradient [48]. Thus, the SOC shaped the soil bacterial community rather than the total N and total P in the CAP analysis. In the current study, the main drivers of soil bacterial community composition—soil pH (which increased), soil organic content (which decreased with soil depth), and soil bacterial functions (mainly metabolism)—were affected directly by SOC.

4.2. Soil Fungal Characteristics with Soil Depths

It was reported that soil fungal biomass and the number of OTUs [15] were affected negatively along soil depths, but that soil fungal composition [10] was not related to soil depths, which was also observed in the present study. The hyphae formed by some soil fungi expand across soil depths [62]; thus, the number of fungi correlated with soil depth would decrease. Furthermore, some soil fungi, such as Zygomycota (Mortierella) and Saccharomycetes (Kazachstania), are arbuscular mycorrhizal fungi (AMF) and could be correlated with plants rather than soil depths.

A decreasing trend in Ascomycota with soil depth was also observed. Most Ascomycota were saprotrophic and, thus, located in the surface soils [63], which could explain this observation. Moreover, the relative abundance of soil fungi, such as Zygomycota, displayed depth-related patterns, which could be explained, at least in part, to the decrease in relative abundances in the families Entomophthoraceae and Mucoraceae in deeper soils.

The number of OTUs and the Chao 1 index in soil fungi decreased with soil depth, which is consistent with previous studies on forest soils [64,65]; however, α diversity indices, such as PD and Shannon–Wiener, were not affected by soil depth [66] (Table 2). Soil fungi tend to live in larger pores than bacteria, and this could explain this observation [67]. In the present study, soil bulk density increased, albeit not significantly, with soil depth, which might also explain the insignificant soil fungal diversity patterns with soil depth. In addition, soil fungi can decompose larger substrates than bacteria, contributing to a greater diversity in the subsoils [7,68]. Based on the CPCoA analysis, soil fungal β diversity was not affected by soil depth, but was affected by sampling plot. This finding was supported

by other studies in which site was the dominant driver of changes in the soil fungal community [56,69].

Soil fungal community composition and its relative abundance dominate soil ecological functions. Furthermore, plot history and management strategy should also be considered [29,59]. In the present study, soil fungi had four dominant ecological functional guilds which were not affected by soil depths but were distinct among the six plots. According to the fungal ecological function analysis, AMF dominated the forest soils, followed by the endophyte, ectomycorrhizal (ECM), and undefined saprotroph fungi. AMF usually appear in plant roots and promote N and P absorption [70], and the hyphae resist environmental stress, such as warming [71]. In the current study, AMF increased with soil depth, which could be linked to the decreased soil nutrient content. Endophytic fungi in the forest ecosystem usually promote nutrient cycling of wood and litter, which is generally plentiful in the surface soil layer (Figure 5b). Furthermore, the host specificity of ectomycorrhizal fungi [72] demonstrated that the effect of *Picea crassifolia* is important, but that soil depth is not. The strong correlation between undefined saprotroph soil fungi and hosts is common in *Picea crassifolia* forests [73], indicating that plot affects undefined saprotrophs more so than soil depth. Overall, soil fungal functions are generally affected by plant type and plot.

Along with soil depth, soil edaphic factors (e.g., soil pH, SOC, contents of soil total N and soil total P, and their stoichiometric characteristics) created a strong ecological filter for the soil microbial community. In addition, sampling plots, that is, the dispersal limitation of geographic distance, were also important in structuring the soil microbial community. In the current study, the filter and dispersal limitations were the drivers of the soil bacterial and fungal communities, but their relative contributions varied.

Soil depth and plot affected soil fungal patterns, but their effects differed. Soil depth affected the number of soil fungal OTUs and the Chao 1 index through soil organic content [16]. Soil fungi require less N but more C than soil bacteria to maintain an optimal C to N ratio [74]. Therefore, soil fungal OTUs and Chao 1 were correlated positively with soil organic content but not with soil pH in the SEM analysis. Plot, to a certain extent, affected mainly soil fungal β diversity and ecological functions, which was consistent with the results of Li et al. [66] and Yuan et al. [41], who reported that soil fungi were more closely associated with geographic distance than soil properties [75]. Soil-forming processes also affected soil fungi [12,29], especially in the deeper soil layers [16].

5. Conclusions

We demonstrated that: (1) the vertical patterns of soil bacterial and fungal communities differed along soil depths. The relative abundances of soil bacteria, such as Proteobacteria, Planctomycetes, and Bacteroidetes, decreased, and those of archaeal and anaerobic bacteria increased along soil depths. The number of OTUs and the Chao 1 index of soil fungi decreased with soil depth, whereas soil fungal β diversity and ecological functions did not; and (2) SOC and pH structured the soil bacterial community patterns and functions along soil depths. Soil fungal β diversity and functions were related mainly to sample plots, namely, the dispersal limitation of geographic distance, rather than soil depth. The current study differentiated the vertical patterns between soil bacterial and fungal communities and demonstrated the importance of SOC and geographic distance in shaping soil microbial communities.

Specific soil bacterial and fungal taxa corresponded to different potential functions with soil depths. For example, some soil bacterial taxa that preferred deep soil layers exhibited greater metabolism of co-factors and vitamins, and transcription, which helped to improve the efficiency of nutrient utilization. Consequently, in further studies, the soil microbial composition and potential functions of specific soil microbial taxa should be examined concomitantly to improve the maintenance and management of the ecosystem functions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14051016/s1, Table S1: The ANOVA analysis for soil physicochemical properties and microbial community characteristics along soil depths; Table S2: The characteristics of soils and roots along the soil depths. Figure S1: The rarefaction curves of soil bacteria (a) and fungi (b).

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