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Multiple Factors Jointly Lead to the Lower Soil Microbial Carbon Use Efficiency of *Abies fanjingshanensis* in a Typical Subtropical Forest in Southwest China

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Abstract: *Abies fanjingshanensis* trees are the only remaining Abies species in a type of subtropical forest of southwest China and are in imminent danger. Previous studies suggested that the massive death of Abies was caused by the unbalanced chemometrics and nutrients in the soil. To the best of our knowledge, for the first time, we evaluated the microbial carbon use efficiency (CUE) in the rhizospheric topsoil and subsoil of *A. fanjingshanensis*, at high elevation, middle elevation, and low elevation as well as investigated their physicochemical indices, soil enzyme activities, bacteria, fungi, and microbial biomass. The results showed that the physicochemical parameters (TP, SOC, AK, AP, MC, TN, NO_3-N, NH_4-N and cation exchange capacity) of the topsoil were higher than those of the subsoil. Acidobacteria, Proteobacteria, Planctomycetes, and Actinobacteria were the dominant phyla in the two soil layers. Candidatus_Koribacter was the main indicator species in the rhizospheric topsoil and subsoil. The positive correlation in the bacterial co-occurrence networks implied that cooperation was dominant between the bacteria in four soil types, and the same phenomenon was found in the co-occurrence networks of fungi. A structural equation model confirmed that pH was the most important factor affecting microbial CUE in the topsoil and subsoil. We inferred that the microorganisms in the acidic soil environment were forced to consume more energy to maintain cellular pH, while less energy was used for growth. The increased solubility of some toxic metals in the acidic soil affected the microbes, resulting in a lower microbial CUE in the *A. fanjingshanensis* rhizospheric soil. Our results highlight that pH values in soil mainly affected microbial CUE, and a lower microbial CUE may be another important factor in the death of large numbers of *A. fanjingshanensis*. Several measures must be carried out to improve the microbial CUE in the rhizospheric soil of *A. fanjingshanensis* by the department of forest management, such as adding the appropriate biochar and nitrogenous fertilizer.

Keywords: *Abies fanjingshanensis*; microbial carbon use efficiency; physicochemical indices; bacteria; fungus

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1. Introduction

Soil microorganisms are important components of the soil and decomposers in the forest ecosystem [1,2], as they actively participate in the material circulation and energy flow and play a vital role in maintaining the structure and function of the ecosystem [3]. A variety of microorganisms inhabit the soil, and each has different physiological activities.

The metabolic functions of soil microorganisms are diverse, as they metabolize almost all organic substances that are biosynthesized [4]. Organic substances can also be mineralized into carbon dioxide and inorganic compounds, such as nitrogen, sulfur, phosphorus, and other elements, or transformed into another organic substance [5]. Soil microbial diversity indirectly reflects soil physicochemical properties [6]. Furthermore, the abundance and change in microorganisms reflect their adaptation to the environment [6]. Altitude also affects the forest soil environment and further affects the community structure and diversity of soil microorganisms [7]. Therefore, studying the relationship between altitude and soil microbial diversity reflects the changes in forest soil ecology and the microenvironmental climate.

Microbial carbon use efficiency (CUE) in soil microorganisms refers to the ratio of microbial growth to carbon absorption [8], reflecting the soil organic carbon (SOC) metabolism influenced by the microbial communities [9]. A high microbial CUE generally indicates high growth efficiency of the soil microorganisms, which is beneficial for accumulating and stabilizing microbial-sourced carbon in the soil [9]. In contrast, a low microbial CUE is beneficial for respiration and reduces soil carbon storage, indicating a decrease in soil carbon sequestration potential [10]. Xiong et al. [11] suggested that microbial CUE increased during the summer with increasing elevation in Wuyishan National Park, Fujian Province, China, while an opposite trend was observed during winter. Lv et al. [12] studied the changes and impact mechanism of soil microbial CUE in ancient woodland at different altitudes (980 to 1765 m) on Daiyun Mountain. The results showed that the microbial CUE varied from 0.1 to 0.4 and increased with elevation. Microbial CUE was negatively correlated with temperature, indicating that as altitude increased, the temperature decreased, which was a key factor promoting the increase in soil microbial CUE. Zhang et al. [13] selected six forest rhizosphere soils at different altitudes on Mount Taibai in the Qinling Mountains and measured their physicochemical properties, extracellular enzyme activities, microbial community, and vegetation characteristics. The results showed that the microbial CUE in the rhizosphere soil trended upward with increasing altitude. CUE increased by 4.36% from the lowest altitude of 0.505 to the highest altitude of 0.527 but decreased at elevations of 1603 and 2405 m. The variation in microbial CUE in the rhizosphere soil within the altitude gradient was affected by several environmental factors, and the influence of the soil matrix (such as soluble organic carbon and ammonium nitrogen content) dominated. Although several studies have been conducted on the CUE of forest soil, few studies have investigated perennial low-temperature areas in subtropical forest soil to study forest soil microorganisms. The soil microenvironment in these regions has gradually changed with global warming, leading to endangered plants growing on the soil. Therefore, further investigation of the soil microorganisms and CUE in these areas and revealing the relationship between soil microorganisms in subtropical regions and forest ecology in the region was of great significance for protecting local plants.

Fanjing Mountain is a complete and independent subtropical forest ecosystem that includes the globally unique Rhinopithecus brelichi and *Abies fanjingshanensis* [14]. However, A. *fanjingshanensis* has massively died in recent years. Several studies have been conducted on the cause of death of *A. fanjingshanensis* [15,16]. Liu et al. [14] demonstrated that total-C, total-N, hydrolyzed-N, and available-P contents of the forest soils were higher at higher altitudes, with median values for *A. fanjingshanensis* forest soil > Taxus chinensis var. mairei soil > Davidia involucrata soil. The C:N, C:P, and N:P ratios of the soil in *A. fanjingshanensis* stands were the largest and significantly higher than those of soils in T. chinensis var. mairei or D. involucrata stands. Li et al. [17] reported that the organic matter and alkaline nitrogen content in the 0–20 cm soil layer are significantly correlated with altitude. The organic matter content increased first and then decreased with altitude. The correlation between various indicators of soil in the 20–40 cm layer and altitude was relatively lower. However, although those studies considered the effect of elevation on soil physicochemical parameters, several factors caused the death of *A. fanjingshanensis*, according to an on-the-spot investigation. Furthermore, whether the death of *A. fanjingshanensis* was related to microbial CUE of the rhizosphere soil is unknown.

To fill these knowledge gaps, this study collected forest soil samples in high (HE), middle (ME), and low elevation (LE) areas of Fanjing Mountain and revealed the relationship between the physicochemical parameters, microorganisms, and CUE in the soil at different elevations. We hypothesized that (1) the difference in the microbial communities and their diversity would be relatively higher along with the changing elevation; (2) the CUE rate would play a relatively important role in the different elevations; (3) the soils of other tree species besides *A. fanjingshanensis* have more complex co-occurrence networks and higher network stability. Thus, this study aimed to (1) determine the physicochemical parameters of the *A. fanjingshanensis* soil and several altitudes; (2) evaluate the CUE and soil enzyme activities in different altitude soils; (3) investigate the diversity, dominant phyla, indicator species, and co-occurrence networks of bacteria and fungi in the soil, and (4) reveal the most important factor affecting the microbial CUE in rhizospheric soil.

2. Materials and Methods

2.1. Study Area

Fanjing Mountain (27°49′50″ to 28°1′30″ N, 108°45′55″ to 108°48′30″ E) is located in the Tongren area of northeastern Guizhou Province, southwest China and is a national nature reserve. It is the main peak of the Wuling Mountains, with the highest peak at an altitude of 2572 m. The nominated area for the World Natural Heritage site of Mount Fanjing is 402.75 km^2, with a buffer zone area of 372.39 km^2. Fanjing Mountain is one of the earliest areas in southern China to become land, with a long history of geologic evolution. Fanjing Mountain is mainly composed of metamorphic rock, surrounded by vast karst landforms with unique geological, ecological, biological, and landscape characteristics. The Fanjing Mountain ecosystem preserves many ancient relics and rare and endangered species and is the only habitat and distribution area for *Rhinopithecus brelichi* and *A. fanjingshanensis.* It is also the most important protected area for the Shuiqinggang forest in Asia. The typical dome-shaped mountain ecosystem forms spectacular subalpine mountains and hilly landforms. Fanjing Mountain is located in the middle of the mid-subtropical zone and the transitional slope zone between the Yunnan-Guizhou Plateau and the hills in western Hunan, with a height difference of 2000 m [14]. As influenced by the East Asian Monsoon and the large difference in mountain height, the mountain has a humid climate in a large area, and the three-dimensional climate in a small area is significantly different. In addition, there is little interference by human activity, which allows Fanjing Mountain to contain the richest biodiversity at the same latitude on earth and is an important species gene pool.

2.2. Soil Sample Collection and Chemical Analysis

Four soil samples, including those of *A. fanjingshanensis* (ABI, 2304 m), high elevation (HE, 2338 m), middle elevation (ME, 1427 m), and low elevation (LE, 634 m), were collected in June 2021. Three sampling points were randomly selected at the core area of each sample (20 m × 20 m). During sampling, the dead branches and fallen leaves were removed from the surface, and a 3.5 cm diameter soil drill was used to collect the topsoil (0–20 cm) and the subsoil (20–40 cm). The soil in the same layer was mixed, and about 500 g of mixed soil was placed in an ice bag and transported to the laboratory. The crushed stone and visible roots were removed, and the soil sample was divided into two parts after passing through a 2 mm sieve. One part was placed in 4 °C storage to determine soil available nutrients, microbial biomass C (MBC), and microbial biomass N (MBN). The other part of the soil was naturally air-dried, passed through 100 mesh, and was used to determine the physicochemical parameters.

Total carbon (C) and N in the soil were measured using a C and N element analyzer (Elemental Vario EL III, Elemental, Germany). A 5.0 g portion of fresh soil was extracted using a 2 mol/L KCI solution with a water-to-soil ratio of 4:1 to determine NH_4^+–N and NO_3^-–N. The supernatant was measured using a continuous flow analyzer (SAN++, Skalar, the Netherlands) [17]. Cation exchange capacity (CEC) in soil was analyzed by the Ammonium chloride-ammonium acetate exchange method. The mechanical composition in

soil was determined by a Laser particle analyzer (Mastersizer 3000, Malvern, The United Kingdom). Total phosphorous (TP) in the soil was determined using the $HClO_4$–H_2SO_4 method, and the sample was digested and decomposed, filtered (0.45 μm), and measured using the continuous flow analyzer [18]. Available phosphorus (AP) was extracted with Mehlich III, and the supernatant was measured using the continuous flow analyzer [19]. After MBC and MBN were determined by the fumigation–K_2SO_4 method, the content of total organic carbon in the filtrate was determined with a total organic carbon analyzer (TOC-VCPH/CPN, Shimadzu Instruments Co., Ltd., Japan) [20]. TN content was measured using a continuous flow analyzer, and the difference between the fumigated and non-fumigated soil samples was divided by the coefficients Kc = 0.45 and Ky = 0.54 to obtain the MBC and MBN content in the soil. The soil pH was tested by potentiometry using a soil-to-water ratio of 1:2.5 [21]. Three parallel samples were run for each sample. Moisture content (MC) was determined using the drying method (105 °C). The methods to determine β-glucosidase (βG), cellulosebiohydrolase (CBH), n-acetyl glucosaminidase (NAG), and acid phosphatase (AP-Tase) were taken from Xiong et al. [11].

2.3. Soil Enzymes Activities and Microbial Carbon Use Efficiency

βG, CBH, NAG, and AP-Tase were utilized to calculate microbial CUE. According to Sinsabaugh et al. [22], we calculated the ratios of C, N, and P by determining enzyme activity. Microbial CUE was calculated based on the following C:N stoichiometric equations [11]:

$$CUE_{C:N} = CUE_{max}[S_{C:N}/(S_{C:N} + K_N)] \quad (1)$$

$$S_{C:N} = (1/EEA_{C:N})(B_{C:N}/L_{C:N})] \quad (2)$$

where the meaning of $S_{C:N}$, the half-saturation constant K_N, CUE_{max} (0.6), $EEAC{:}N$ and $L_{C:N}$ are referred with Sinsabaugh et al. [22] and Xiong et al. [11].

2.4. DNA Extraction and Polymerase Chain Reaction (PCR) Amplification

lluminaMiSeq sequencing was performed on all of the soil samples. Total microbial DNA was extracted according to the E.Z.N.A. Soil DNA kit instructions (Omega Bio tek, Norcross, GA, USA) that included buffer SLX mlus, buffer SP2, HTR reagent, buffer XP2, DNA wash buffer, elution buffer, Hibind DNA micro elute column, 2 mL collection tubes. The special operated process can scan the official website (https://www.omegabiotek.com) (Accessed on 22 August 2023). A 1% agarose gel electrophoresis method was used to detect DNA extraction quality, and the NanoDrop2000 spectrophotometer was used to determine DNA concentration and purity. Bacterial primers were used, including 338F (5′-ACTCCTACCCCACCAG-3′) and 806R (5′-GACTACHVCCCTWTCTAAT-3′), while the fungal primers were ITSIF (5′-CTTCATTTAGAGAGATAA-3′) and ITIS2R (5′-GCTGCTTCTTCATCCATGC-3′). The amplification procedure was pre-denaturation at 95 °C for 3 min, 27 cycles (denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s), followed by stable extension at 72 °C for 10 min, and storage at 4 °C (PCR instrument: ABIGeneAmp 9700 Type). The PCR reaction system included 4 μL of 5× TransStart FastPfu buffer, 2 μL of 2.5 mmol/L dNTPs, 0.8 μL of the upstream primer (μmol/L), 0.8 μL of the downstream primer (5 pmol/L), 0.4 μL of transStart FastPfu DNA polymerase, 10 ng of template DNA, complement to 20 μL.

After mixing the PCR products from the same sample, 2% agarose gel electrophoresis was used to recover the PCR products. The AxyPrep DNA Gel Extraction Kit (Axygen-Biosciences, Union City, CA, USA) was utilized to purify the recovered products, and 2% agarose gel electrophoresis was used to detect the products. The Quantum Fluorometer (Promega, Madison, WI, USA) was used to detect and quantify the recovered products. The NEXTFLEX Rapid DNA-Seq Kit was employed to prepare the library. The Illumina Miseq PE300 platform was used for sequencing (Beijing Baimike Biotechnology Co., Ltd., Beijing, China). Fastp software was used for quality control of the original sequence [23], and FLASH software was employed for splicing [24]. OTU clustering was performed

on sequences based on 97% similarity with the chimeras removed using UPARSE software [25,26]. RDP classifiers were utilized to annotate the species classification for each sequence by comparing the bacteria to the Silva 16S rRNA database and fungi to the UNITE ITS database, with a matching threshold of 70%.

2.5. Statistical Analysis

The data were statistically analyzed using Excel 2019 (Microsoft Inc., Redmond, WA, USA), SPSS (SPSS Inc., Chicago, IL, USA), and R language (R 3.0.2). Redundancy analysis (RDA) was performed to reveal the relationship between microorganism (phylum level) and physicochemical indexes using the "Ape", "vegan", "psych", and "reshape2" packages in the R language. Single-factor analysis of variance and the least significant difference test were used to detect differences in the selected indicators. A structural equation model (SEM) was used to evaluate potential hypotheses and was analyzed using IBM SPSS Amos 24.

3. Results

3.1. The Physicochemical Indexes in Topsoil and Subsoil in ABI, HE, ME and LE

Most of the physicochemical parameters were not significantly different in the ABI rhizospheric topsoil or the subsoil (Table 1). The topsoil and subsoil pH values in the ABI rhizospheric topsoil were slightly higher than those at the same elevation but lower than those in the soil at the ME. SOC, TP, TN, AP, and C:N were significantly different between the ABI rhizospheric soil and the other elevations. The SOC and TP contents in ABI were significantly higher than those in the soil at HE, ME, and LE. The AP concentration increased in the topsoil and subsoil with an increase in elevation. TN content was significantly higher in the ABI rhizospheric topsoil than that at the other elevations. The C:N ratio in the ABI rhizospheric topsoil was lower than that in the soil at the other elevations, while the opposite was true in the lower soil layers. Taken together, except for pH, Cu, K, and TP, the selected physicochemical parameters were of higher magnitude in topsoil than in subsoil, and the ABI rhizospheric soil had greater contents than those at the same elevation (HE) and different elevations (ME and LE). CEC contents in the topsoil were higher than that in the subsoil, of which its CEC values in the topsoil of ME are highest. According to the international grading standards, the soil in this study was regarded as light clay.

Table 1. The physicochemical parameters in topsoil and subsoil of ABI, HE, ME and LE.

Indexes	0–20 cm				20–40 cm			
	ABI	HE	ME	LE	ABI	HE	ME	LE
pH	4.05 ± 0.34 ^{a}	4.04 ± 0.25 ^{a}	4.65 ± 0.23 ^{a}	4.03 ± 0.74 ^{a}	4.07 ± 0.52 ^{a}	4.31 ± 0.28 ^{a}	4.77 ± 0.29 ^{a}	4.69 ± 0.09 ^{a}
Ca	1.67 ± 0.47 ^{a}	0.42 ± 0.23 ^{a}	0.75 ± 0.68 ^{a}	1.07 ±631 ^{a}	1.98 ± 1.78 ^{a}	0.58 ± 0.12 ^{a}	0.72 ± 0.81 ^{a}	0.57 ± 0.18 ^{a}
Cu	26 ± 6 ^{a}	29 ± 24 ^{a}	23 ± 2 ^{a}	13 ± 2 ^{a}	28 ± 11 ^{a}	50 ± 54 ^{a}	26 ± 4.37 ^{a}	13 ± 1 ^{a}
Fe	28 ± 10 ^{a}	15 ± 4 ^{a}	22 ± 5 ^{a}	22 ± 3 ^{a}	31 ± 13	16 ± 1 ^{a}	27 ± 2 ^{a}	29 ± 5 ^{a}
K	2.26± 0.63 ^{b}	8.86 ± 0.65 ^{a}	2.79 ± 1.52 ^{b}	4.50 ± 0.29 ^{b}	2.20 ±0.13 ^{b}	10.58 ±0.34 ^{a}	3.35 ± 1.87 ^{b}	5.89 ± 1.55 ^{ab}
Zn	1.82 ± 0.89 ^{a}	1.56 ± 0.47 ^{a}	0.43 ± 0.19 ^{a}	0.50 ± 0.22 ^{a}	0.37 ± 0.01 ^{ab}	0.19 ± 0.01 ^{a}	0.41 ± 0.02 ^{ab}	0.32 ± 0.02 ^{b}
TP	0.63± 0.02 ^{a}	0.59 ± 0.01 ^{b}	0.64 ± 0.02 ^{ac}	0.41 ± 0.01 ^{d}	0.68 ± 0.15 ^{s}	0.82 ± 0.81 ^{b}	0.58 ± 0.18 ^{ac}	0.11 ± 0.03 ^{d}
SOC	235± 88 ^{a}	141 ± 11 ^{b}	196 ± 33 ^{c}	170 ± 37 ^{d}	160 ± 79 ^{a}	59 ± 24 ^{b}	51 ± 7 ^{b}	57 ± 24 ^{b}
AK	121 ± 39 ^{a}	101 ± 11 ^{a}	102 ± 28 ^{a}	128 ± 16 ^{a}	83 ± 27 ^{a}	49 ± 12 ^{a}	53 ± 10 ^{a}	41 ± 9 ^{a}
AP	109 ± 26 ^{a}	90 ± 22 ^{b}	63 ± 12 ^{c}	63 ± 10 ^{c}	67 ± 18 ^{a}	58 ± 28 ^{a}	38 ± 9 ^{b}	27 ± 3 ^{b}
MC	161 ± 2 ^{a}	100 ± 2 ^{b}	58 ± 2 ^{c}	38 ± 3 ^{d}	91 ± 3 ^{a}	48 ± 1 ^{c}	65 ± 27 ^{b}	35 ± 2 ^{d}
TN	9.99 ± 1.70 ^{a}	5.28 ± 0.42 ^{b}	3.52 ± 0.68 ^{b}	3.39 ± 0.82 ^{b}	5.04 ± 0.68 ^{a}	1.89 ± 0.52 ^{b}	2.29 ± 0.64 ^{b}	2.31 ± 0.41 ^{b}
C:N	24 ± 8 ^{a}	27 ± 4 ^{a}	56 ± 6 ^{b}	56 ± 23 ^{b}	31 ± 11 ^{a}	38 ± 27 ^{a}	25 ± 9 ^{a}	25 ±11 ^{a}
CEC	25 ± 2 ^{a}	10 ± 1 ^{b}	28 ± 2 ^{a}	11 ± 1^{b}	18 ± 1 ^{a}	6 ± 0 ^{b}	15 ±1 ^{a}	8 ± 0 ^{b}
0.25–1.00 mm	17 ± 3 ^{a}	25 ± 2 ^{b}	7 ± 0 ^{c}	13 ± 1 ^{a}	16 ± 1 ^{a}	16 ± 1 ^{a}	0.88 ± 0.09 ^{b}	17 ± 1 ^{a}
0.05–0.25 mm	15± 2 ^{a}	10 ± 0 ^{b}	26 ± 2 ^{c}	17 ± 1 ^{a}	10 ± 1 ^{a}	4 ± 0 ^{b}	19 ± 0 ^{c}	6 ± 0 ^{b}
0.01–0.05 mm	20 ± 2 ^{a}	19 ± 1 ^{a}	21 ± 3 ^{a}	15 ± 0 ^{a}	16 ± 1 ^{a}	21± 1 ^{a}	27 ± 1 ^{b}	22 ± 0 ^{a}
0.005–0.01 mm	7 ± 0 ^{a}	17 ± 2 ^{b}	16 ± 1 ^{b}	18 ± 1 ^{b}	21 ± 2 ^{a}	11 ± 1 ^{b}	11 ± 1 ^{b}	15 ± 1 ^{b}
0.001–0.005 mm	18 ± 1 ^{a}	14 ± 1 ^{a}	15 ± 1 ^{a}	22 ± 1 ^{a}	22 ± 3 ^{a}	22 ± 1 ^{a}	18 ± 1 ^{a}	22 ± 2 ^{a}
<0.001 mm	22 ± 2 ^{a}	15 ± 1 ^{b}	15 ± 1 ^{b}	15 ± 1^{b}	15 ± 1 ^{a}	26 ± 2 ^{b}	24 ± 0 ^{b}	18 ± 1 ^{a}

Note: The unit of Ca is g/kg, Cu is mg/kg, Fe is g/g; K is g/kg, Zn is mg/g, TP is g/kg, SOC is g/kg, AK is mg/kg, AP is mg/kg, MC is %, TN is g/kg, CEC is c mol/kg, and mechanical composition (0.25–1.00 mm, 0.05–0.25 mm, 0.01–0.05 mm, 0.005–0.01 mm, 0.001–0.005 mm and <0.001 mm) is %; Mean ± SD, n = 3. Different superscript letters in each row represent significant differences between different treatments (ANOVA, $p < 0.05$).

3.2. Microbial Biomass in Topsoil and Subsoil in ABI, HE, ME and LE

Figure 1 shows that the NO_3–N, NH_4–N, MBC, MBN, and MBP concentrations in the ABI rhizospheric topsoil were significantly higher than those at the HE, ME, and LE. The NH_3-N content in the LE topsoil was relatively lower than that of the other elevations. The MBC: MBN ratios in the ABI and HE topsoil were similar but significantly greater than that at ME and LE. Almost identical patterns were observed in the subsoil. In contrast, the MBC: MBN ratio in the ABI and LE subsoil was significantly higher than that of the HE and ME values. The higher the Chao1 and Ace values, the greater the number of operational taxonomic units contained in the community and the greater the community richness.

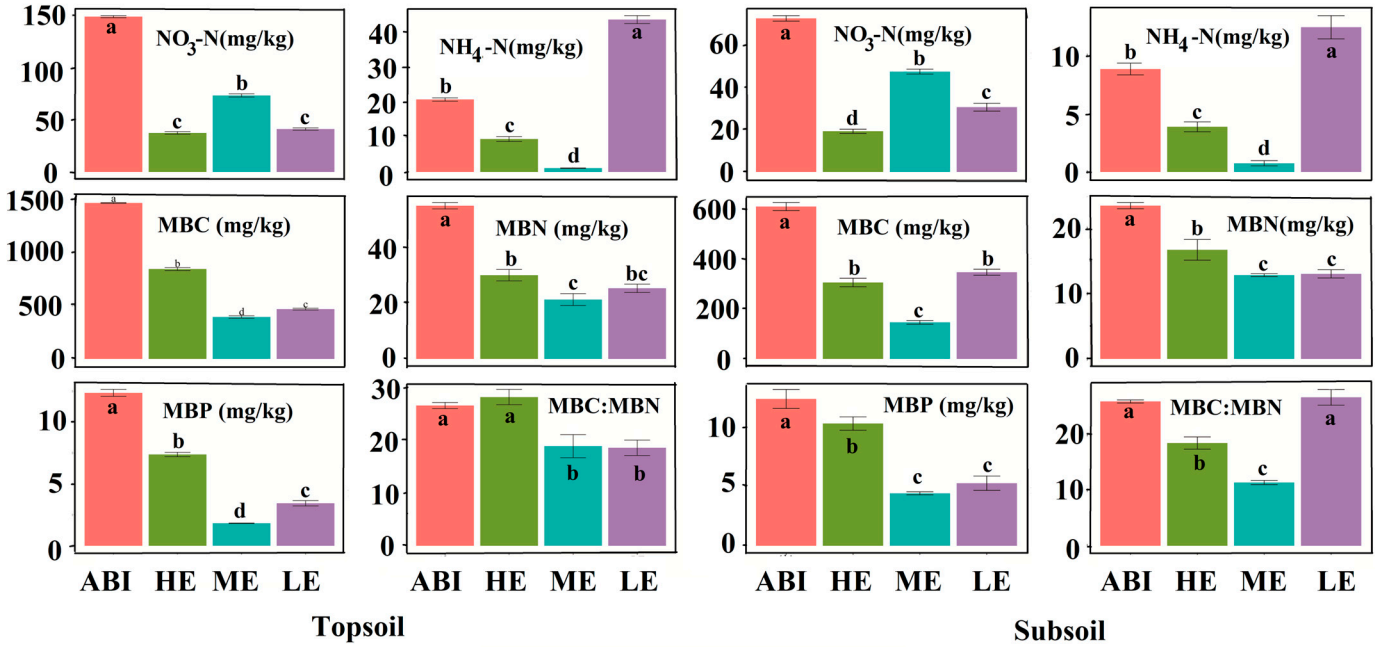

Figure 1. NO_3-N, NH_4-N, MBC, MBN, MBP and MBC:MBP concentrations in rhizospheric topsoil and subsoil. Note: Mean ± SD, n = 3. Different superscript letters in each row represent significant differences between different treatments (ANOVA, $p < 0.05$).

3.3. CUE and Soil Enzyme Activities

The differences in the CUE at different elevations in the soil are shown in Figure 2. The CUE values of ABI topsoil were significantly lower than those of the LE, ME, and HE soils ($p < 0.05$). Although the CUE value increased in the subsoil, the value was still lower in comparison with the other elevations. The contents of C-enzymes in ABI topsoil and subsoil tended to be greater than that in the soil at the other elevations. The content of N-enzymes in the two soil layers was the maximum at ME. The content of P-enzymes in the ME soil was significantly higher than that at the other elevations ($p < 0.05$). Although the C-enzyme content was higher in ABI soil, lower CUE values were detected in rhizosphere soil, and the ME rhizosphere soil had a higher CUE value.

3.4. α-Diversity and Community Composition of Bacteria and Fungus in Soil in ABI, HE, ME and LE

The higher the Chao1 and Ace values, the higher the number of OTUs contained in the community and the greater the community richness. The richness of the bacterial and fungal communities in the topsoil of ABI, HE, ME, and LE were similar, but the richness of the ME community was slightly higher (Table 2). The richness of the topsoil community was higher than that of the subsoils. A higher Simpson index indicated low community diversity, which was negatively correlated with other diversity indices. A small difference in fungal community diversity was detected in the topsoil and subsoil. The higher the Shannon value, the richer the community diversity. The community diversity of the ABI fungus was lower than that of the HE, ME, and LE. The results of whole_tree PD_ exhibited

higher bacterial and fungal community diversity in topsoil than that in the subsoils. The coverage values in the soil layers and the four elevations were approximate. Overall, the bacterial and fungal community diversity in the ME soil was higher than that in the ABI and other elevations, and there was richer community diversity in the topsoil.

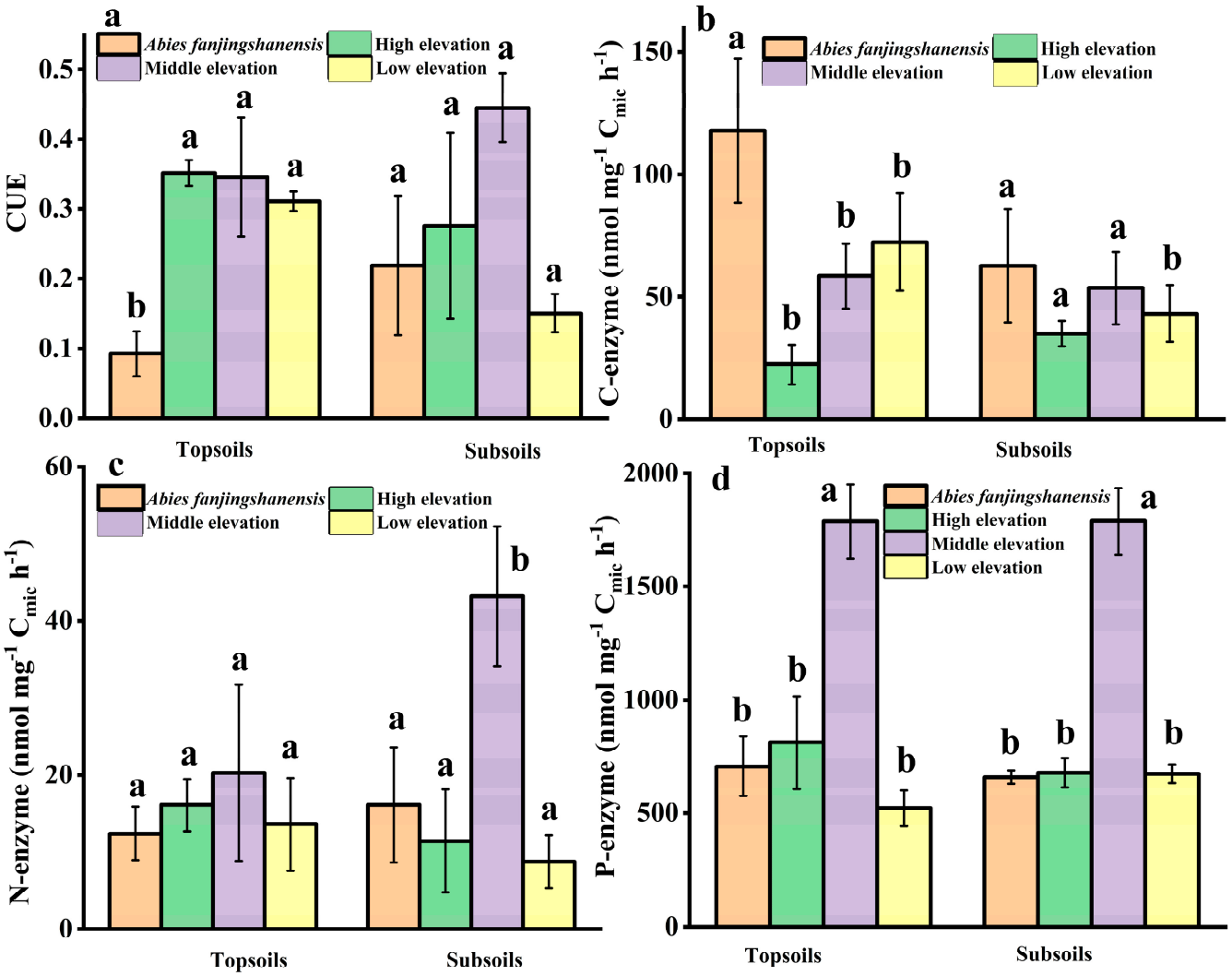

Figure 2. The CUE (**a**), C−enzymes (**b**), N−enzymes (**c**) and P−enzymes (**d**) contents in topsoil and subsoil in ABI, HE, ME and LE. Note: Mean ± SD, n = 3. Different superscript letters in each row represent significant differences between different treatments (ANOVA, $p < 0.05$).

Table 2. The α-diversity of microbia and fungi in topsoil and subsoil of ABI, HE, ME and LE.

		ABI		HE		ME		LE	
		Bacterials	**Fungus**	**Bacterials**	**Fungus**	**Bacterials**	**Fungus**	**Bacterials**	**Fungus**
ACE	Topsoil	1309 ± 98 ^{a}	603 ± 25 ^{a}	1257 ± 35 ^{b}	661 ± 83 ^{b}	1319 ± 24 ^{a}	639 ± 36 ^{a}	1289 ± 29 ^{b}	553 ± 53 ^{b}
	Subsoil	1286 ± 107 ^{a}	597 ± 23 ^{a}	1150 ± 131^{a}	577 ± 87 ^{b}	1346 ± 28 ^{b}	598± 31 ^{a}	1252 ± 58 ^{a}	534 ± 96 ^{c}
Chao1	Topsoil	1325 ± 101 ^{a}	625 ± 12 ^{a}	1287 ± 43 ^{a}	638 ± 27 ^{a}	1340 ± 41 ^{a}	647 ± 37 ^{b}	1310 ± 37 ^{a}	556 ± 59 ^{c}
	Subsoil	1296 ± 111 ^{a}	620 ± 27 ^{a}	1085 ± 251 ^{b}	562 ± 39 ^{b}	1367 ± 37 ^{a}	625 ± 43 ^{a}	1280 ± 40 ^{a}	555 ± 109 ^{b}
Simpson	Topsoil	0.99 ± 0.00 ^{a}	0.83 ± 0.06 ^{a}	0.98 ± 0.01 ^{a}	0.93 ± 0.03	0.99 ± 0.00 ^{a}	0.93 ± 0.03 ^{a}	0.99 ± 0.00 ^{a}	0.89 ± 0.08 ^{a}
	Subsoil	0.99 ± 0.00 ^{a}	0.83 ± 0.05 ^{a}	0.98 ± 0.00 ^{a}	0.95 ± 0.01 ^{a}	0.99 ± 0.00 ^{a}	0.88 ± 0.05 ^{a}	0.99 ± 0.00 ^{a}	0.83 ± 0.13 ^{a}
Shannon	Topsoil	8.29 ± 0.22 ^{a}	3.85 ± 0.64 ^{a}	7.89 ± 0.29 ^{a}	5.18 ± 1.00 ^{b}	8.44 ± 0.24 ^{a}	5.17 ± 0.73 ^{b}	8.62 ± 0.03 ^{b}	5.13 ± 0.79 ^{b}
	Subsoil	8.19 ± 0.22 ^{a}	3.83 ± 0.47a	7.43 ± 0.47 ^{b}	5.95 ± 0.61 ^{b}	8.24 ± 0.02 ^{a}	4.49 ± 0.51 ^{c}	8.19 ± 0.03 ^{a}	4.63 ± 1.53 ^{c}
PD_whole_tree	Topsoil	63 ± 4 ^{a}	86 ± 7 ^{a}	60 ± 2 ^{a}	90 ± 1 ^{a}	62 ± 1 ^{a}	91 ± 7 ^{a}	61 ± 2 ^{a}	83 ± 3 ^{a}
	Subsoil	61 ± 5 ^{a}	82 ± 4 ^{a}	52 ± 10 ^{a}	77 ± 5 ^{a}	63 ± 0 ^{a}	85± 1 ^{a}	60 ± 2 ^{a}	82 ± 10 ^{a}
Coverage	Topsoil	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}
	Subsoil	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}	0.99 ± 0.00 ^{a}

Mean ± SD, n = 3. Different superscript letters in each row represent significant differences between different treatments (ANOVA, $p < 0.05$).

In this study, 23 bacterial phyla and 10 fungal phyla were detected in the topsoil and subsoil, respectively (Figure 3). Among them, Acidobacteria, Proteobacteria, Planctomycetes, and Actinobacteria were the dominant phyla in the two soil layers; the proportion of Acidobacteria and Proteobacteria gradually increased in topsoil, except for the ABI, and decreased with increasing elevation. There was a larger discrepancy in the fungal proportions. Although Basidiomycota and Ascomycota were the dominant phyla in the two soil layers, the proportion of Basidiomycota decreased slowly with increasing soil depth and elevation, respectively. The top 50 bacterial genera with the highest relative abundance were selected for the linear discriminant analysis effect size analysis, and only species with scores >3.5 were displayed (Table 3). Acidobacteria, Acidobacteriia, Acidobacteriales, Candidatus_Koribacter and Koribacteraceae were the indicator species in topsoil compared with the soils at the other elevations. Candidatus_Koribacter, Koribacter, Roseiarcus, Beijerinckiaceae, and Roseiarcus were the indicator species in the subsoil. Clavulinaceae, Tuber, Tuberaceae, and Tuber_zhongdianense were the fungal indicator species in the topsoil. Agaricales, Archaeorhizomycetales, Archaeorhizomycates, Archaeorhizomyces and Archaeorhizomycetaceae were the indicator species in the subsoil.

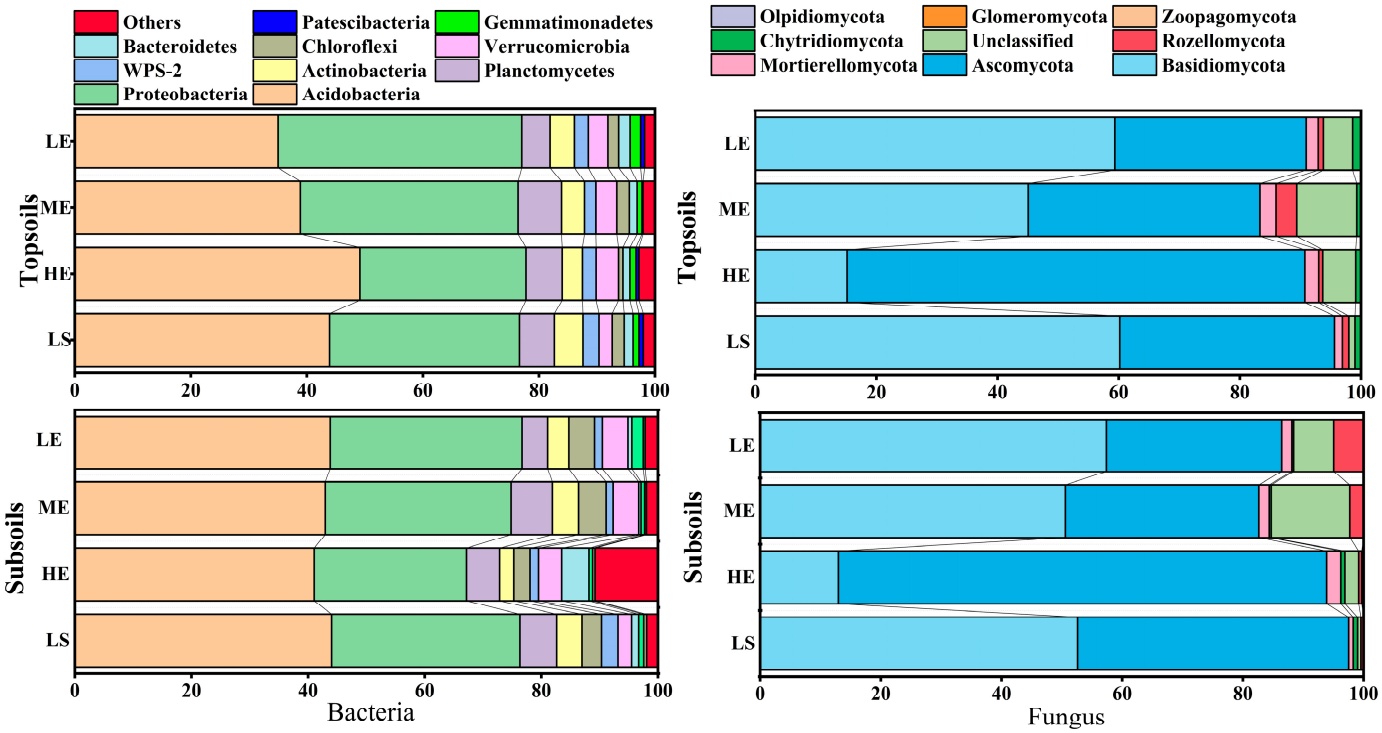

Figure 3. The proportion of relative abundance of bacteria and fungus under phylum level.

3.5. Factors Driving the Seasonal Variation in Soil Microbial Community Composition

Figure 4 shows the relationship between microorganisms and the soil physicochemical parameters. A significant positive correlation was observed between bacteria/fungus and the K content in the soil. Additionally, TP and MC contents were positively correlated with bacteria and fungus in the topsoil, respectively. TN in the topsoil was strongly positively correlated with microbial C:N and MC. Bacteria in the subsoil were positively correlated with the microbial C:N ratio. Fungi were positively correlated with Cu, TP, SOC, AK, MC, TP, and the microbial C:N ratio. SOC content was strongly positively correlated with AK, MC, TN, and the C:N ratio. Overall, the relationships between microorganisms and the subsoil physicochemical parameters were closer than those in the topsoil. Similarly, stronger correlations with the physicochemical parameters were found in the subsoil.

Figure 5 shows the relationships between bacteria (phylum level) and the soil physicochemical parameters. pH had a positive effect on Chloroflexi and a negative effect on

Bacteroidetes and Patescibacteria. The C:N ratio was positively and negatively correlated with Patescibacteria and Bacteroidetes, respectively. The physicochemical parameters mainly affected Ascomycota, Zoopagomycota, Mortierellomycota, Rozellomycota, and Basidiomycota. pH was positively correlated with Rozellomycota and Basidiomycota but negatively correlated with Ascomycota. Fe, TP, AP, Zn, and K affected fungi (phylum level).

Figure 6a–d displays the co-occurrence networks of bacteria in the ABI, HE, ME, and LE soils. The modularity indexes were 0.51, 0.56, 0.50, and 0.46 in ABI, HE, ME, and LE, reaching a degree of modularity, as their values were >0.44. Both the number of nodes and the edges of the microbial networks were slightly different in the bacteria and fungi in the ABI, HE, ME, and LE soils. A positive correlation in the microbial network reflected a cooperative relationship between species, while a negative correlation was competitive. The cooperative microbial network was dominant for bacteria and fungi. A tiny difference in the degree and complexity of the co-occurrence networks was found in the four rhizosphere soil samples (Figure 7a). The centrality of LE was higher in rhizosphere soil than that in the other soil elevations. The importance of a node depended on the number of neighboring nodes and the importance of the neighboring nodes (Figure 7b). A connected neighboring node was more important. This was evidence that the important nodes of the microbial co-occurrence network in the rhizosphere soil were richer in LE, enhancing network stability.

Table 3. LEfSe analysis of bacterial and fungus communities in topsoil and subsoil in ABI, HE, ME and LE (Scores > 3.5).

		ABI	HE	ME	LE
Bacterial	Topsoil	-	p_Acidobacteria c_Acidobacteriia o_Acidobacteriales g_Candidatus_Koribacter f_Koribacteraceae	f_Xanthobacteraceae	c_Gammaproteobacteria p_Proteobacteria o_Betaproteobacteriales
	Subsoil	-	g_Candidatus_Koribacter f_Koribacteraceae f_Beijerinckiaceae g_Roseiarcus	f_Burkholderiaceae	g_Candidatus_Solibacter o_Gammaproteobacteria_ Incertae_Sedis
Fungus	Topsoil	f_Clavulinaceae g_Tuber f_Tuberaceae s_Tuber zhongdianense	o_Archaeorhizomycetales c_Archaeorhizomycetes g_Archaeorhizomyces f_Archaeorhizomycetaceae g_Meliniomyces f_Helotiaceae	s_Lactarius salmonicolor s_Lactarius horakii f_Agaricaceae f_Entolomataceae	f_Cylindrosympodiaceae o_Venturiales s_Sympodiella_quercina g_Sympodiella o_Xylariales
	Subsoil	o_Agaricales o_Archaeorhizomycetales c_Archaeorhizomycetes g_Archaeorhizomyces f_Archaeorhizomycetaceae	O_Hypocreales f_Erysiphaceae s_Erysiphe paeoniae g_Erysiphe o_Erysiphales o_Eurotiales f_Nectriaceae f_Aspergillaceae f_Aspergillaceae s_Paecilomyces_penicillatus g_Paecilomyces g_Fusariumg Aspergillus s_Aspergillus_flavus f_Trichocomaceae g_Talaromyces	f_Entolomataceae	

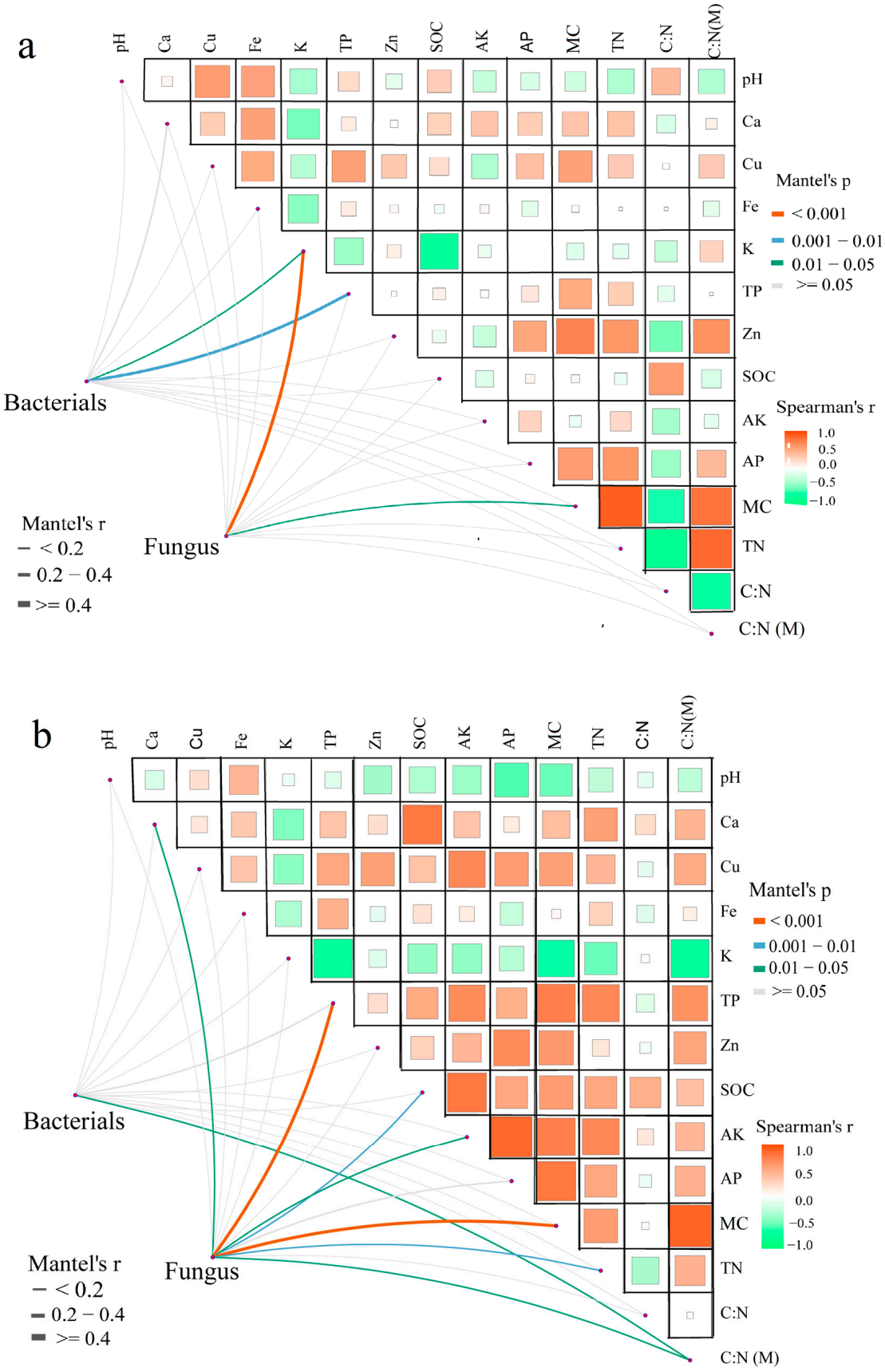

Figure 4. Relationship between bacteria/fungus and physicochemical indexes in topsoil (**a**) and subsoil (**b**).

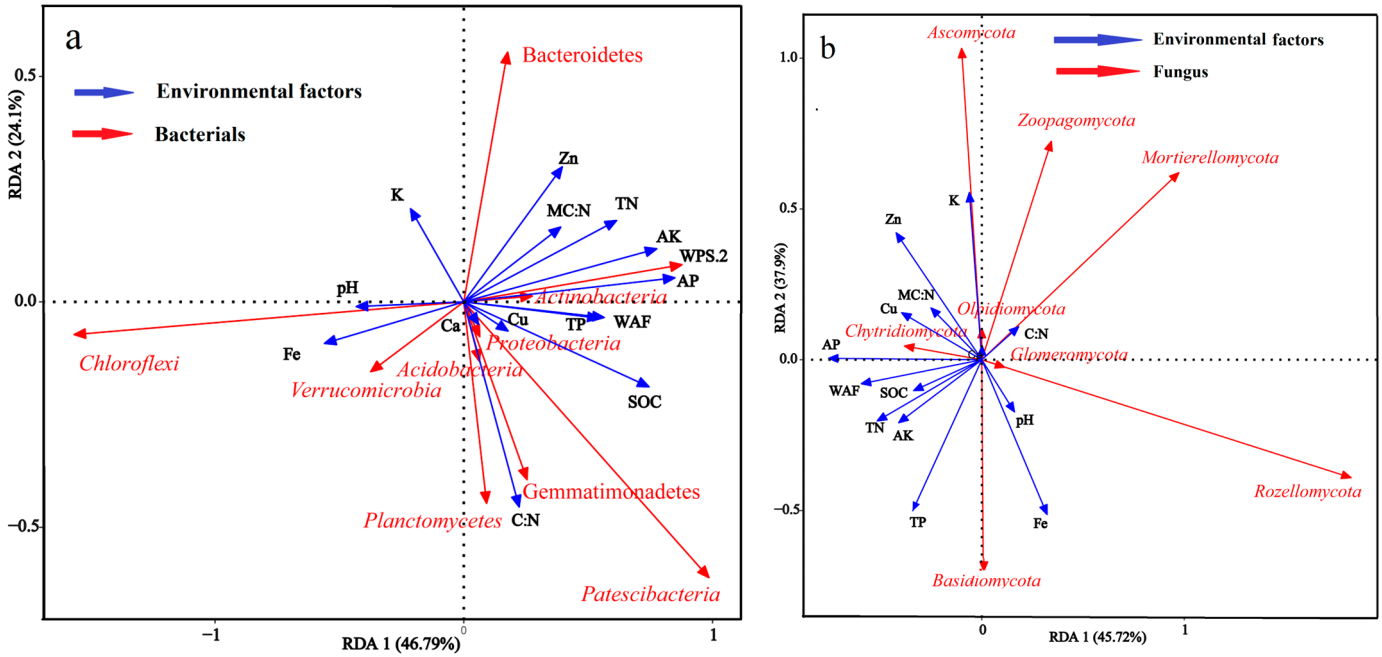

Figure 5. Relationship between bacteria (**a**) and fungus (**b**) (phylum level) and physicochemical parameters in soil using Redundancy analysis.

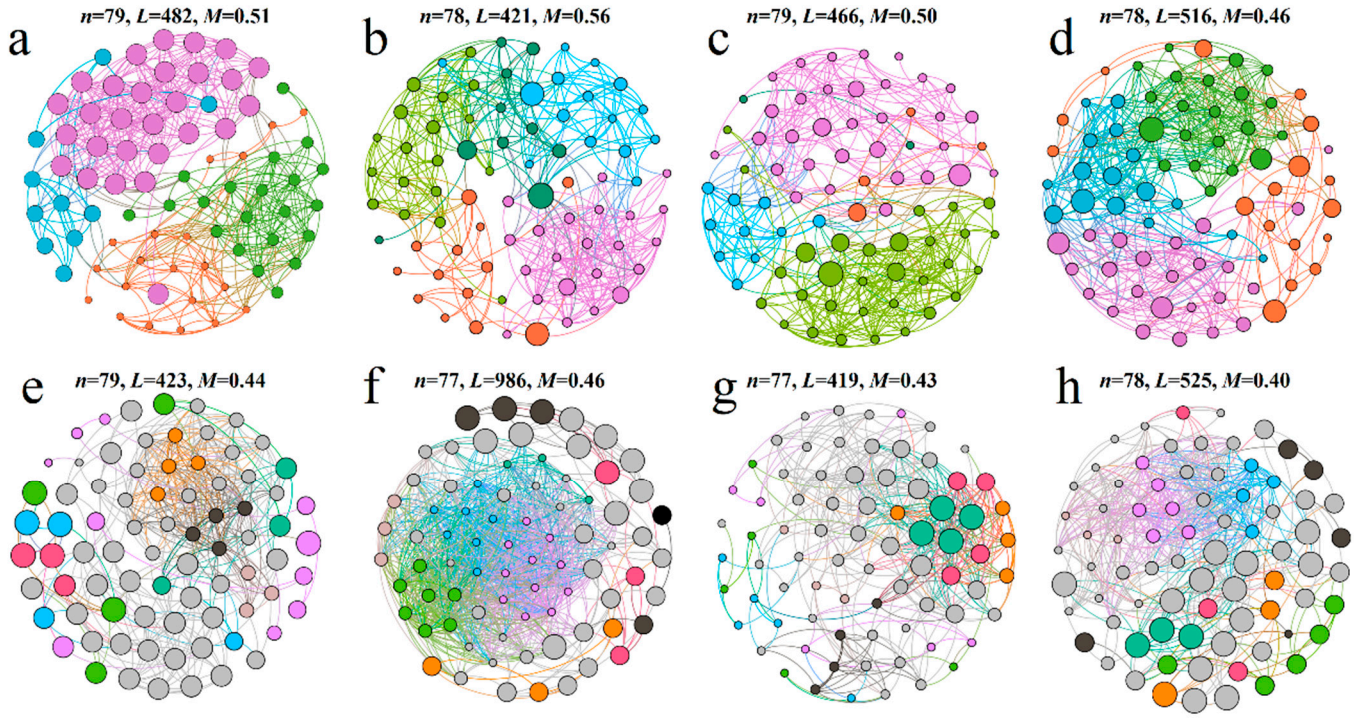

Figure 6. Co-occurrence networks of bacteria in ABI (**a**), HE (**b**), ME (**c**) and LE (**d**), and fungus in ABI (**e**), HE (**f**), ME (**g**) and LE (**h**) in soil. Small modules with <5 nodes were displayed in gray, and large modules with ≥5 nodes were in other colors.

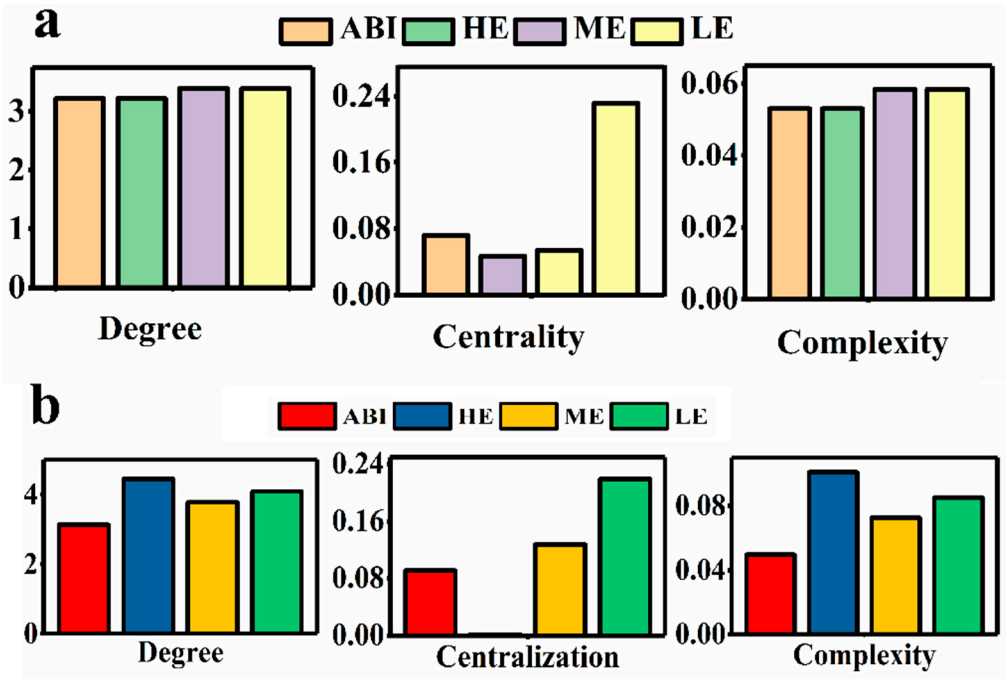

Figure 7. Degree, centrality, and complexity of co-occurrence networks of bacteria (**a**) and fungus (**b**) in ABI, HE, ME and LE.

4. Discussion

Overall, the physicochemical parameters in topsoil were of higher magnitude than those in the subsoil, and their contents in rhizospheric soil in ABI were greater than that in soils in HE, ME and LE. Although ABI grows in stone crevices, there are massive fallen leaves in surface soil. Fungi produce highly acidic humic acid when organic matter is decomposed, which lowers soil pH and reduces fertility [27], which explains the lower pH in the soil of ABI. However, soil pH is higher at mid-altitude due to natural (low temperature and a slower mineralization rate) and anthropogenic factors (mainly tourism activities) [28]. Although the temperature gradually decreases as the altitude increases, the plant species that make up the vegetation at high altitudes are relatively isolated, and most grow in stone crevices, causing the soil pH to gradually decrease [29]. Our result found that the contents of most of the soil physicochemical parameters, particularly SOC, increased first at LE and ME and then decreased in the HE. Many studies have shown that the carbon and nitrogen contents in soil increase with the increase in altitude, and simultaneously, soil microbial activity weakens. The weakened soil microbial activity will make the decomposition of litter slow down and deposit in the soil, significantly increasing the soil carbon and nitrogen content with altitude [30]; our studies also found this phenomenon. There was an annual average temperature of 7.3 °C, and the lowest temperature was −2.3 °C in the ABI growing environment. The lower soil temperature retarded the rate of chemical reactions and microbial activities, which was beneficial for the accumulation of organic matter. The higher moisture content probably restrained the ABI root system from absorbing soil nutrients [31]. Therefore, NO_3-N, MBC, MBP, and MBN contents in ABI topsoil and subsoil were significantly greater than those of the other elevation soils. Generally, with increasing soil pH value, the variable negative charge of soil colloid increased, and the cation exchange capacity increased [31]. This coincided with our result that pH values and CEC contents in the soil in ME were higher than that in the other elevation soils. The space between the particles of clay soil was small, the ventilation was poor, the soil temperature rose slowly, the water content was high, and the organic matter was easy to accumulate [32]. These peculiarities also resulted in lower microbial activity.

Microbial α-diversity in the soil tended to vary at different elevations. In the ABI rhizosphere soil, Acidobacteria, Proteobacteria, and Planctomycetes were the dominant phyla of

bacteria in the two soil layers, while the dominant fungal phyla were Basidiomycota and Ascomycota. Chen et al. [33] reported that Basidiomycota and Ascomycota are the dominant groups in a study of soil fungal communities in different forest types in the Xiaoxing'an Mountains, which was consistent with our results. Basidiomycetes and Ascomycetes are important soil decomposers, among which Ascomycota are mostly saprophytes, which play a key role in the degradation of complex organic matter [34], while basidiomycetes have an enhanced ability to decompose lignocellulose in plant residues [35]. Candidatus_Koribacter is the main indicator species in the rhizospheric topsoil and subsoil, which is a methanotroph in phylum Acidobacteria [36]. Tuberaceae is commonly found in coniferous and broad-leaved mixed forests, forming specialized mycorrhizal symbioses with various host plants. TP and MC contents were positively correlated with bacteria and fungi in the topsoil, respectively. Topsoil TN was strongly positively correlated with the microbial C:N ratio and MC. The relationships between microorganisms and the physicochemical parameters in the subsoil were more highly correlated than those in the topsoil. The amount of soil moisture has a significant impact on the growth and activity of soil microorganisms. Fungi are more sensitive than bacteria, indicating that under different soil moisture conditions, different communities of soil microorganisms have different adaptations and regulatory mechanisms [37]. It is generally believed that fungi have an advantage over bacteria in soil lacking water because bacteria are more adaptable to the environment and have a higher tolerance [38], whereas fungi have a single-celled structure, which is more flexible and not limited by water [39]. The important nodes in the microbial co-occurrence network of the rhizosphere soil at LE were richer, which enhanced network stability. The positive correlation in microbial networks is dominant. Chen et al. [40] suggested that the diversity of the microbial communities, the complexity of the co-occurrence networks, and the multifunctionality of ecosystems significantly decrease with increasing altitude. Positive correlations in these studies suggest cooperative behaviors, i.e., mutualistic interactions, syntrophic interactions, cross-feeding, and commensalism between co-existing members as well as taxa occupying similar guilds or niches [41].

pH was the most important factor affecting carbon use efficiency in topsoil (Figure 8). The pH values in topsoil and subsoil in ABI were relatively lower than those in the soils of the other elevations. In this study, there are two possible mechanisms resulting in the lower microbial CUE in soil in ABI. Firstly, an acidic environment forces microorganisms to consume more energy to maintain cell pH while less energy is used for growth. Secondly, under a low soil pH, the solubility of toxic metals such as Al^{3+} increases, and cells are subjected to stress [42], resulting in a decrease in soil CUE. In addition, the SOC contents in the topsoil were higher than that in the subsoil. Higher CUE values accelerated the decomposition of SOC, leading to the loss of SOC [43], while a lower CUE reduced the decomposition of soil carbon by microorganisms, which was beneficial for the accumulation of soil carbon. Fungal communities facilitate the decomposition of complex compounds and decompose litter, thereby promoting the stability of soil microbial biomass carbon and organic matter accumulation mediated by fungi [44]. The diversity of the fungus in the ABI rhizosphere soil and at LE was relatively lower, which is probably another reason leading to the lower CUE value. The turnover time of forest soil microbial biomass increases with increased soil depth. The lower microbial carbon uptake rate in deep soil may be partially compensated for by the longer microbial biomass carbon turnover time [45]. Soil microbial CUE affects ecosystem processes, such as soil carbon fixation, turnover, mineralization, and greenhouse gas emissions, as well as biogeochemistry feedback to climate change. Soil microbial CUE plays an important role in regulating soil microbial-mediated carbon and nutrient transformation and is also a key regulatory factor for soil microbial biomass turnover and soil carbon sequestration [46]. However, some still limitations of the current study should be discussed. It will be necessary to strengthen studies of soil microbial CUE in forest ecosystems, particularly in different forest vegetation types and different growth and development stages [44].

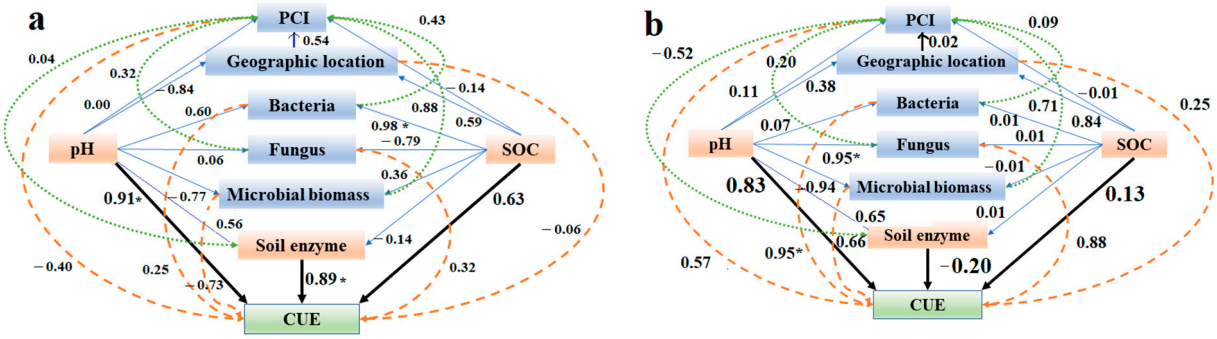

Figure 8. Structural equation model of microbial CUE in topsoil (**a**) (x^2 = 7.05, df = 7, p = 0.29, CFI = 0.92 and RMSEA= 0.00) and subsoil (**b**) (x^2 = 9.15, df = 8, p = 0.33, CFI = 0.94 and RMSEA = 0.00) ("*" represents that p value is lower than 0.05).

5. Conclusions

In this study, cooperation of the bacterial and fungal microbial networks was dominant, and there is a lower difference in the degree and complexity of the co-occurrence network in the four rhizosphere soil types. The structural equation model demonstrated that pH was the most important factor for carbon use efficiency in topsoil and subsoil. We inferred that the microorganisms in acidic soil environments were forced to consume more energy to maintain pH, while less energy was used for growth. Nevertheless, increasing the solubility of some toxic metals in acidic soil also coerced the microbial cells, resulting in a lower microbial CUE in ABI rhizospheric soil. Previous studies suggested that the massive death of ABI was mainly caused by the unbalanced chemometrics and nutrients in the soil. However, our results highlight that pH values in soil mainly affected microbial CUE, and a lower microbial carbon use efficiency may be another important factor as to why the ABI died in large numbers. Our suggestion is that, in the rhizospheric soil of ABI, the suitable application of biochar can enhance the pH value, and appropriately adding the exogenous nitrogenous fertilizer will increase the microbial CUE. The change in microbial CUE in soil is a long-term process which is affected by multiple factors. Therefore, future work will incorporate more influencing factors and conduct short-term and long-term studies to reveal the impact of different influencing factors on microbial CUE at multiple time scales and their interaction mechanism.

Author Contributions: Conceptualization, X.W. and Z.Z.; methodology, X.W. and Z.Z.; software, J.Z.; validation, Y.L., W.L., G.M. and X.H.; formal analysis, J.Z.; investigation, Y.L.; resources, W.L.; data curation, G.M.; writing—original draft preparation, X.W. and Z.Z.; writing—review and editing, X.W.; visualization, Z.Z.; supervision, Z.Z.; project administration, X.W. and Y.L.; funding acquisition, X.W. and Y.L. All authors have read and agreed to the published version of the manuscript.

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