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The Effects of Biochar on Microbial Community Composition in and Beneath Biological Soil Crusts in a *Pinus massoniana* Lamb. Plantation

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Abstract: Biological soil crusts (BSCs) hold promise for reducing soil erosion in subtropical forest plantations, and microorganisms profoundly affect the formation and development of BSCs. The effects of biochar as a soil conditioner on the diversity and structure of soil microbial communities in BSCs are largely unknown. Therefore, our aim was to determine how biochar might improve microbial community composition and BSC function. Herein, a field experiment was conducted in a *P. massoniana* plantation; the addition of biochar was the treatment, and no biochar addition was the control (CK). Soil microbial communities associated with moss BSCs (in and beneath BSCs) with and without the addition of biochar were analyzed by Illumina sequencing technology. The results showed that Acidobacteria (28.35%), Proteobacteria (22.53%), Actinobacteria (17.41%), and Chloroflexi (16.74%) were the dominant bacterial phyla, whereas Basidiomycota (70.00%) and Ascomycota (22.76%) were the dominant fungal phyla in BSCs. The soil bacterial and fungal OTU number and richness in BSCs were higher than those beneath BSCs. The relative abundances of Acidobacteria, Chloroflexi, and Basidiomycota were higher in BSCs than beneath BSCs, whereas the relative abundances of Actinobacteria, Firmicutes, Ascomycota, and Chytridiomycota showed the opposite trend. Beneath BSCs, biochar addition increased the soil bacterial OTU number and richness (ACE index and Chao1) but decreased the soil fungal OTU number and richness. Biochar had little effect on soil microbial community structures in BSCs; however, beneath BSCs, it significantly increased the relative abundances of Acidobacteria, Chloroflexi, and Basidiomycota and significantly decreased the relative abundances of Actinobacteria, Firmicutes, Ascomycota, and Chytridiomycota. Biochar-induced changes in soil microbial communities were related to soil environmental factors, especially urease activity, organic matter content, pH, total nitrogen content, and sucrase activity. We demonstrated the different effects of biochar on soil microbial communities in and beneath the BSCs of subtropical forest plantations; these findings provided new insights into soil stabilization with BSCs below the forest canopy in subtropical regions.

Keywords: biological soil crusts; microbial community structure; biochar; soil environmental factor

1. Introduction

Biological soil crusts (BSCs) result from an intimate association between soil particles and differing proportions of photoautotrophic (e.g., cyanobacteria, algae, lichens, and bryophytes) and heterotrophic (e.g., bacteria, fungi, and archaea) organisms that live within, or immediately on top of, the uppermost millimeters of soil. Soil particles are aggregated through the presence and activity of these often extremotolerant biota that desiccate regularly, and the resultant living crust covers the surface of the ground as a coherent layer [1–3].

BSCs occupy up to 12% of the planet's terrestrial surface [4] and cover 70% of the dryland surface [5]. They play an important role in the ecosystem, and their critical ecological functions have been well documented by numerous researchers; these functions include the enhancement of soil resistance to wind and water erosion [2,6], a decrement in nutrient loss caused by surface runoff [7], the exchange and fixation of carbon and nitrogen [6,8], a decrease in soil surface albedo [9], the regulation of hydrological processes [10], and the establishment of vascular plants [11]. Hence, BSCs can be described as the living skin of the earth [12]. Previous studies on BSCs mainly focused on their composition, succession, and seasonal variation, as well as their effects on soil erosion, physicochemical properties, hydrological process, and the carbon and nitrogen cycle [3,7,10,13–16]. More and more studies have been conducted on the microbial community and function of BSCs [17,18], with a majority of these studies on BSCs performed in temperate regions [5,13,15]. Recent research found that BSCs are able to coexist in a mesic subtropical forest environment, where they developed from initially light cyanobacteria- and algae-dominated crusts to later-stage moss-dominated crusts [19,20]. The development of BSCs was found to be affected by many micro-climatic and micro-environmental factors, such as light conditions, nutrient conditions, tree traits, soil attributes, and terrain attributes [19–21]. As a result, BSCs are sporadically distributed in the soil surface of forest plantations in subtropical regions. However, research on BSCs in such areas remains scarce.

Microorganisms are an important part of BSCs [3]. Among them, bacteria are the most abundant microorganisms. They play a key role in nutrient cycling, carbon and nitrogen fixation, and the decomposition of organic matter (OM), whereas fungi are involved in the decomposition of OM as well as the matter and energy cycle [17,18,22]. The community structure and diversity of these microorganisms profoundly affect the formation and development of BSCs. Moreover, microorganisms play an important role in the formation and evolution of soil fertility [23]. The interaction between soil microorganisms and minerals can alter the mineral surface properties and microbial activity, thus affecting soil fertility and other environmental variations [24]. The OM from various sources and forms must be decomposed and mineralized by microorganisms before re-entering the soil biogeochemical cycle [25,26]. In addition, microorganisms can also directly activate fixed phosphorus, potassium, and other nutrients by secreting organic acids and functional enzyme components [27]. Thus, microorganisms in BSCs can also influence soil ecosystem functions and capabilities beneath BSCs [28]. However, microorganisms are highly sensitive and susceptible to external environmental factors, such as vegetation cover type, soil characteristics, biogeography, and other disturbances [9,25,28].

Biochar is a black, carbon-rich solid product obtained by heating biomass at 300 °C–700 °C under limited oxygen conditions [29]. The significant influence of biochar on the enhancement of soil fertility is widely accepted. While the effects of biochar on soil physicochemical properties, N₂O and CH₄ emissions, nutrient retention, and anthropogenic chemical adsorption have been well studied [30–33], its impact on the biological functions of soil is relatively poorly understood [34,35]. Biochar's highly porous surface, pore structure, and strong adsorption capacity can provide the space and nutrients necessary for the survival and reproduction of soil microorganisms [35]. To date, only a few studies have focused on the potential effects of biochar on the soil microbial community structure and diversity in BSCs. The biochar-coupled remediation of BSCs could be a sustainable and feasible approach to maintaining soil stability [36].

Pinus massoniana is one of the most important tree species for forest resources and ecological services in southern China. However, soil properties were negatively affected by monoculture plantations, and soil erosion commonly occurs in *P. massoniana* plantations [37,38]. The phenomenon of large-scale soil erosion below the canopy in *P. massoniana* plantations still exists, and the problem of water and soil erosion is still serious [39]; however, great efforts have been made in the development of forestry ecological engineering for the prevention of red soil erosion [40]. BSCs are the most important drivers of soil erosion control in subtropical forest plantations [41]. Biochar may affect the soil microbial

communities associated with BSCs, thus further influencing the ecological function of BSCs in controlling soil erosion. In the present study, a field experiment was conducted to examine the effects of biochar on soil microorganisms in and beneath BSCs in a *P. massoniana* plantation in subtropical China. The Illumina sequencing technology was used to analyze the shift in the soil microbial community. The objectives of this study were to (i) compare the soil microbial community's structure and diversity in and beneath BSCs and (ii) examine the shift in soil microbial community composition in and beneath BSCs after biochar addition. The results obtained provided new insights into the microbial regulation of BSCs in subtropical forest plantations.

2. Material and Methods

2.1. Study Site

The present study was conducted in the Soil and Water Conservation Demonstration Garden of Jinggang Mountain in Ji'an City (Jiangxi Province, China), which is also known as the Taihe County Tiger Mountain Watershed (26°50'–26°51' N, 114°52'–114°54' E), with an altitude of 80–200 m. The study area comprises many gentle hills, with a slope of about 5°, and is located in a subtropical warm and wet transition zone with a subtropical monsoon climate. The mean annual precipitation is 1363 mm, the mean temperature is 18.6 °C (extreme temperatures are 40.4 °C and −6 °C), and the frost-free period lasts 288 d. The soil is red soil developed from quaternary red clay, with a thickness of 3–40 cm; it can be classified as degraded red soil because of intense erosion. The field experiment was performed in a plantation of *Pinus massoniana* that was planted in 1984, with plant spacing of 1 m × 1 m. Serious water and soil erosion caused by high rainfall can be detected below the canopy in the plantation. In addition, moss-dominated BSCs are distributed sporadically in the soil surface of the *P. massoniana* plantation.

2.2. Experimental Design and Soil Sampling

Two treatments, namely biochar addition (Biochar) and no biochar addition (CK), were designed in the *P. massoniana* plantation. Each treatment comprised three plots of 25 m × 25 m in size. A total of 12 kg of biochar was added to each plot for the Biochar treatment. The biochar was purchased from Yichun Fengcheng Ningneng Biomass Power Generation Limited Company, Yichun City, China. The raw material of the biochar was rice straw, and the biochar was produced by a continuous vertical biomass furnace at 450 °C. The basic properties of the biochar were as follows: pH, 10.4; OM content, 467.0 g·kg^{−1}; total nitrogen (TN), 5.90 g·kg^{−1}; total phosphorus (TP), 1.50 g·kg^{−1}; and total potassium (TK), 29.50 g·kg^{−1}. Each plot was ditched to a depth of 5 cm and filled back with soil and biochar in June 2018. After 2 years, the rates of BSC cover in these plots ranged from 3% to 7%. Three samples of BSC with sizes of 30 cm × 30 cm each were chosen randomly in each plot, and soil samples from the three BSC samples were collected and mixed as one sample in a valve bag. Simultaneously, soil samples were also collected from beneath the three BSC samples; they were mixed as one sample in another valve bag. A total of 12 samples were collected and transported to the laboratory in a car refrigerator at 4 °C. Each sample was divided into two parts; one part was air-dried at room temperature for 2 weeks and stored in a valve bag at 4 °C for the analysis of soil physicochemical properties and enzyme activities, and the other part was sieved through a 0.25 mm mesh sieve and stored in a valve bag at −80 °C for microbial DNA extraction.

2.3. Determination of Soil Physicochemical Properties and Enzyme Activities

Soil pH was measured using a digital pH meter (PHS-3D, Shanghai Leici Instrument Limited Company, Shanghai, China) in a 1:5 (w/v) soil–water suspension. OM content was evaluated by potassium dichromate colorimetry [42] with a spectrophotometer (AA900T, Perkin Elmer, Norwalk, CA, USA). The TN content was determined using an elemental analyzer (Vario MACRO cube, Elementar Trading Shanghai, Shanghai, China). The TP concentration was ascertained by molybdenum blue colorimetry [43], and TK content was

determined by flame photometry (Model 425 Flame Photometer, Sherwood, Chicago, IL, USA). Urease (UE) activity was evaluated by an indophenol blue colorimetric method [44]. Acid phosphatase (ACP) activity was measured using a p-nitrophenylphosphate disodium method [45]. Sucrase (SC) activity was determined by a 3,5-dinitrosalicylic acid method, and polyphenol oxidase (PPO) activity was ascertained by a catechol colorimetric method [46].

2.4. Soil DNA Extraction, PCR Amplification, and High-Throughput Sequencing

Total DNA was extracted from: (i) three composite soil samples from BSCs that received CK treatment, (ii) three composite soil samples from beneath BSCs that received CK treatment, (iii) three composite soil samples from BSCs that received biochar treatment, and (iv) three composite soil samples from beneath BSCs that received biochar treatment. A total of 0.50 g of fresh soil was subjected to DNA extraction using a metagenomic DNA extraction kit (GENErery) following the manufacturer's instructions. The extracted DNA was stored in a refrigerator at -20°C , and the DNA concentration was determined by UV spectrophotometer. The amplicons were obtained after amplification, with the primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) for the V3-V4 region of the bacterial 16S rRNA gene, and the primers ITS1F (CTTGGTCATT-TAGAGGAAGTAA) and ITS2 (GCTGCGTTCCTTCATCGATGC) for the internal transcribed spacer (ITS) region of the fungal ITS gene [47]. The amplicons were sequenced in an Illumina platform (EDC-810, Beijing Bai Mai Biotechnology, Co. Ltd., Beijing, China).

The original data were filtered by a Trimmomatic tool, and the primer sequences were identified and removed by Cutadapt software according to parameters allowing a maximum error ratio of 20% and a minimum coverage of 80%. Then, the reads of each sample were spliced using FLASH v1.2.11 software, according to a minimum overlap length of 10 bp. The circular consensus sequencing (CCS) sequences of different samples were identified by Lima (V1.7.0) software using barcode sequences; the chimeras were removed, and the high-quality CCS sequences were obtained. Subsequently, USEARCH was used to cluster the sequences at a 97% similarity level, and the operational taxonomic units (OTUs) were filtered, with a 0.005% sequence number as the threshold. The feature sequences were compared using the classify-consensus-blast function in QIIME2 and the GreenGene database (<http://greengenes.secondgenome.com/>, accessed on 12 October 2020), and sequences that could not be accurately compared were classified by a classifier (classify-sklearn). Based on the similarity between the sequences, the sequences were classified into OTUs for species annotation so as to evaluate the relative abundance of bacteria and fungi at the phylum, class, order, family, genus, and species levels in each sample. According to the OTUs, microbial richness indices (ACE and Chao1 indices) and diversity indices (Simpson and Shannon indices) were calculated using the alpha index analysis software QIIME2 (<https://qiime2.org/>, accessed on 15 October 2020).

2.5. Statistical Analysis

Duncan's multiple comparison ($p < 0.05$) was performed on soil physicochemical properties, enzyme activities, and diversity indices for different treatments using the 'multcomp' package of the R language software (R 3.6.2). A Pearson's correlation analysis was conducted to examine the association between the soil environmental factors and microbial diversity using the 'ggplot' package of R 3.6.2. The results are expressed as "mean \pm standard error" ($n = 3$). Venn plots of bacterial and fungal OTUs, as well as the relative abundance plots of the species at the phylum level with a relative abundance higher than 1%, were generated by the 'VennDiagram' package of R 3.6.2. The relative abundances of the dominant species at the phylum level in different treatments were compared using one-way analysis of variance (ANOVA) and plotted using R 3.6.2. The bacterial and fungal OTUs numbers were normalized by Lg (OTUs + 1), and redundancy analysis (RDA) of the microbial community and soil environmental factors was conducted using Canoco 5.0. The soil environmental factors were analyzed via a Monte Carlo test based on an axis length of <4 in the DCA results, and the test for significance was 999 permutations.

3. Results and Analysis

3.1. Soil Physicochemical Properties and Enzyme Activities

In the CK, the soil pH, OM content, TN content, and UE activity in the BSCs were significantly higher than those beneath the BSCs ($p < 0.05$) (Table 1). Biochar addition had different effects on the soil physicochemical properties and enzyme activities in and beneath the BSCs. Biochar treatment presented higher values of soil pH (14.47% and 6.55%, $p < 0.05$), OM content (99.77% and 40.12, $p < 0.05$), TN content (52.29% and 74.32%, $p < 0.05$), and TK content (19.97% and 15.51%, $p < 0.05$) in and beneath the BSCs, respectively, when compared with those noted in the CK. Significantly higher values of soil UE activity (21.59%, $p > 0.05$) and SC activity (105.15%, $p < 0.05$) in the BSCs were also observed with biochar treatment, whereas the higher values of the soil SC and UE activity beneath the BSCs with biochar treatment did not reach a significant level compared with CK treatment.

Table 1. Soil physicochemical properties and enzyme activities in and beneath the BSCs with biochar addition.

Position	Treatment	pH	OM (g·kg ⁻¹)	TN (g·kg ⁻¹)	TP (g·kg ⁻¹)	TK (g·kg ⁻¹)	UE (U·g ⁻¹)	ACP (U·g ⁻¹)	SC (U·g ⁻¹)	PPO (U·g ⁻¹)
In BSCs	CK	3.73 c	13.16 b	1.53 b	0.317 a	6.26 bc	499.04 a	47660.07 a	31.31 b	40.76 a
	Biochar	4.27 a	26.29 a	2.33 a	0.207 b	7.51 a	607.77 a	46500.84 a	64.20 a	39.51 a
Beneath BSCs	CK	3.97 b	6.48 d	0.74 c	0.287 ab	5.80 c	178.82 b	49020.77 a	17.88 b	35.90 ab
	Biochar	4.23 a	9.08 c	1.29 b	0.280 ab	6.70 b	193.03 b	41211.91 b	22.26 b	24.06 b

Note: OM, Organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; UE, urease; ACP, acid phosphatase; SC, sucrase; PPO, polyphenol oxidase; different lowercase letters represent significant differences among treatments ($p < 0.05$).

3.2. Soil Microbial Diversity

A 16 rRNA database of 215,206 quality sequences and an ITS database of 268,396 quality sequences were produced from the soil samples. The coverage percentages of both databases were over 94%, indicating the results of sequencing were reliable. The bacterial OTUs detected were 684, 734, 722, and 748 in the four groups (In BSCs-CK, In BSCs-Biochar, Beneath BSCs-CK, and Beneath BSCs-Biochar), whereas the fungal OTUs were 517, 488, 369, and 239, respectively (Figure 1). The numbers of common bacterial and fungal OTUs among the four groups were 674 and 183. The number of bacteria identified in the BSCs was lower than those beneath the BSCs, whereas the number of fungi showed the opposite trend (Table 2). Biochar addition increased the number of bacteria (class, order, family, genus, and species), but decreased the number of fungi, both in and beneath the BSCs, compared to the CK treatment.

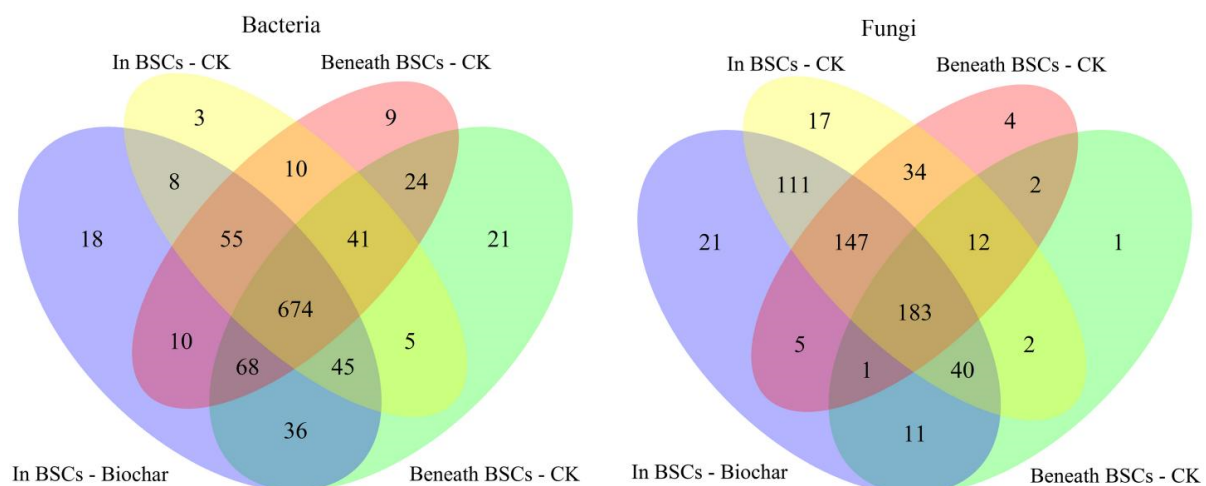


Figure 1. Venn diagrams showing the number of OTUs that were shared in and beneath BSCs with biochar addition.

Table 2. Overview of soil microbial community in and beneath BSCs with biochar addition.

Microbes	Position	Treatment	Kingdom	Phylum	Class	Order	Family	Genus	Species
Bacteria	In BSCs	CK	1	17	40	88	127	190	204
		Biochar	1	17	42	94	135	194	205
	Beneath BSCs	CK	1	17	43	93	137	201	217
		Biochar	1	18	44	97	139	212	226
Fungi	In BSCs	CK	1	7	21	52	78	107	85
		Biochar	1	7	20	53	75	102	80
	Beneath BSCs	CK	1	6	20	45	64	82	65
		Biochar	1	6	16	40	54	59	44

Both in the CK and Biochar treatments, the richness, diversity, and OTU number of soil bacteria were higher than those of soil fungi (Table 3). In the CK treatment, the soil bacterial richness (ACE and Chao1 indices) and OTU number in BSCs were significantly higher than those beneath BSCs ($p < 0.05$) (Table 3), and this trend was also detected in the fungal Chao1 index and the OTU number (Table 3). Biochar addition showed a positive effect on soil bacterial diversity and a negative effect on soil fungal diversity; in addition, the influence of biochar on soil bacterial and fungal diversity indices, richness indices, and OTU numbers was more pronounced in the soil beneath BSCs than in the soil of BSCs. The soil bacterial richness indices (ACE and Chao1 indices) and the OTU number beneath BSCs were 32.21%, 36.63%, and 49.40% higher under biochar treatment, respectively, when compared with those in the CK treatment. However, the soil fungal richness indices (ACE and Chao1 indices) and the OTU number beneath BSCs were 20.40%, 33.17%, and 37.88% lower under biochar treatment, respectively, when compared with those in the CK treatment. Biochar had no effect on soil bacterial or fungal indices, i.e., the Simpson or Shannon indices ($p > 0.05$) (Table 3).

Table 3. Soil microbial diversity in and beneath BSCs under biochar addition.

Microbes	Position	Treatment	OTU Number	ACE	Chao1	Simpson	Shannon
Bacteria	In BSCs	CK	677 ± 78 a	751.2 ± 58.9 a	752.0 ± 63.3 a	0.985 ± 0.002 a	7.471 ± 0.163 a
		Biochar	657 ± 114 a	753.6 ± 78.7 a	764.8 ± 85.1 a	0.984 ± 0.003 a	7.432 ± 0.144 a
	Beneath BSCs	CK	343 ± 25 b	414.2 ± 27 b	409.9 ± 24.8 b	0.976 ± 0.005 a	6.675 ± 0.209 a
		Biochar	512 ± 163 a	547.7 ± 149 ab	560.0 ± 146.7 ab	0.976 ± 0.008 a	6.913 ± 0.438 a
Fungi	In BSCs	CK	409 ± 56 a	453.0 ± 47.7 a	463.3 ± 52.4 a	0.834 ± 0.043 a	4.541 ± 0.520 a
		Biochar	385 ± 24 a	441.0 ± 14.8 a	442.0 ± 22.1 ab	0.756 ± 0.041 a	4.074 ± 0.222 a
	Beneath BSCs	CK	230 ± 38 b	346.2 ± 43.2 ab	323.8 ± 38.3 bc	0.899 ± 0.030 a	4.856 ± 0.347 a
		Biochar	143 ± 7 b	275.6 ± 44.4 b	216.4 ± 23.9 c	0.788 ± 0.111 a	4.072 ± 0.806 a

Note: different lowercase letters represent significant differences among treatments ($p < 0.05$).

3.3. Soil Microbial Composition

A total of 21 bacterial phyla were detected, among which the bacterial phyla with a relative abundance higher than 1% were Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes, Planctomycetes, WPS-2, Deinococcus-Thermus, Patescibacteria, Bacteroidetes, and Verrucomicrobia (Figure 2A). In particular, Proteobacteria (22.39%), Acidobacteria (21.89%), Actinobacteria (17.58%), and Chloroflexi (16.22%) were the dominant phyla, accounting for more than 78.00% of the total bacterial population. In general, the relative abundance of Acidobacteria in the soil of BSCs (28.35%) was higher than in the soil beneath BSCs (15.43%). In the CK treatment, the relative abundances of Acidobacteria and Chloroflexi were 158.40% and 28.27% higher, respectively, in the soil of BSCs compared with those in the soil beneath BSCs; in contrast, the relative abundance of Actinobacteria was 24.55% lower in the soil of BSCs compared with that in the soil beneath BSCs. Biochar had little effect on the relative abundance of the dominant bacterial phyla in the soil of BSCs. However, in the soil beneath BSCs, the relative abundances of Acidobacteria and Chloroflexi were 57.17% and 48.77% higher, respectively, while the relative abundances

of Actinobacteria and Firmicutes were 43.18% and 30.97% lower in biochar treatment, respectively, when compared with those in the CK treatment.

A total of seven fungal phyla were detected, including Basidiomycota, Ascomycota, Chytridiomycota, Mortierellomycota, Rozellomycota, Mucoromycota, and Basidiobolomycota. The fungal phyla with a relative abundance higher than 1% were Basidiomycota, Ascomycota, and Chytridiomycota (Figure 2B). Among them, Basidiomycota (64.19%) and Ascomycota (25.95%) were the dominant fungal phyla, accounting for more than 90.00% of the total fungal population. The relative abundances of Basidiomycota and Ascomycota were 70.00% and 22.76% in the soil of BSCs and 58.38% and 29.15% in the soil beneath BSCs, respectively. In the CK treatment, the relative abundance of Basidiomycota was 47.51% higher in the soil of BSCs than that in the soil beneath BSCs, whereas the relative abundances of Ascomycota and Chytridiomycota were 62.42% and 57.82% lower, respectively, in the soil of BSCs than those in the soil beneath BSCs. Biochar addition had little effect on the relative abundance of fungal phyla in the soil of BSCs. However, in the soil beneath BSCs, the relative abundance of Basidiomycota was 52.31% higher, while the relative abundances of Ascomycota and Chytridiomycota were 62.42% and 57.82% lower in biochar treatment, respectively, when compared with those in the CK treatment.

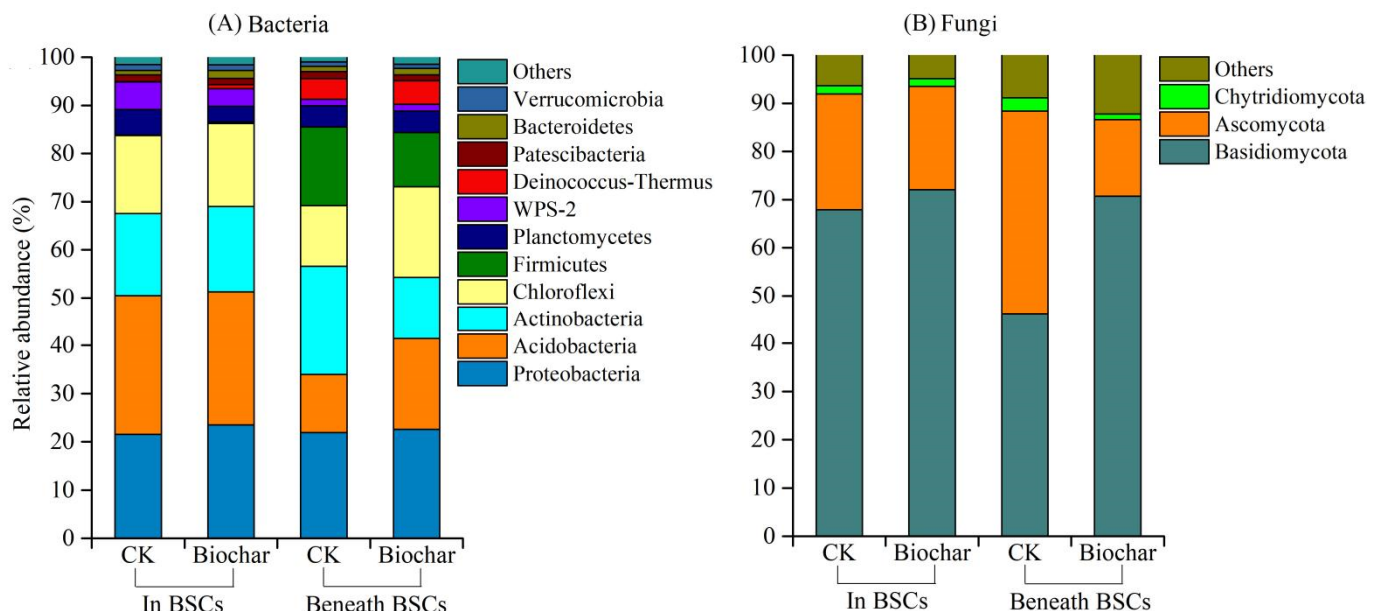


Figure 2. Relative abundances of dominant microbial phyla in and beneath BSCs with biochar addition.

3.4. Relationship between Soil Microorganisms and Soil Environmental Factors

Correlation analysis showed that the bacterial diversity indices (Simpson and Shannon indices) were not significantly correlated with the soil physicochemical properties or enzyme activities. The bacterial richness was significantly correlated with soil OM content, TN content, UE activity, and SC activity (Figure 3A). The ACE and Chao1 indices of soil bacteria were significantly positively correlated with soil TN content ($r = 0.631$ and $r = 0.643$, $p < 0.05$), UE activity ($r = 0.586$ and $r = 0.613$, $p < 0.05$), OM content ($r = 0.586$ and $r = 0.595$, $p < 0.05$), and SC activity ($r = 0.581$ and $r = 0.588$, $p < 0.05$). The fungal OTU number was significantly positively correlated with soil UE activity ($r = 0.828$, $p < 0.01$), TN content ($r = 0.641$, $p < 0.05$), PPO activity ($r = 0.635$, $p < 0.05$), OM content ($r = 0.624$, $p < 0.05$), and SC activity ($r = 0.583$, $p < 0.05$) (Figure 3B). The fungal richness was affected by soil UE activity and PPO activity. The fungal ACE index was significantly positively correlated with soil UE activity ($r = 0.753$, $p < 0.01$), and the fungal Chao1 index was significantly positively correlated with soil UE activity ($r = 0.805$, $p < 0.01$) and PPO activity ($r = 0.594$,

$p < 0.05$). The fungal Simpson and Shannon indices were significantly positively correlated with soil ACP activity ($r = 0.578$ and $r = 0.579$, $p < 0.05$).

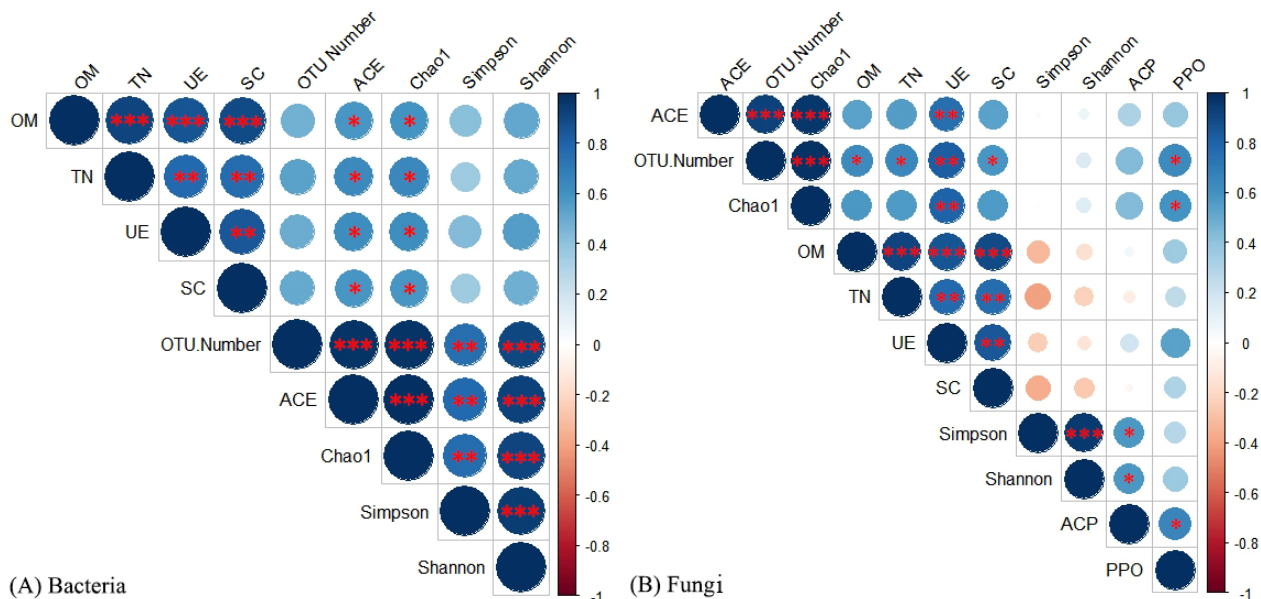


Figure 3. Pearson's correlation of microbial α -diversity and soil environmental factors. Note: blue and red indicate positive and negative correlations; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The RDA was applied to analyze the relationships between soil microbial community distributions and soil environmental factors (Figure 4). On the whole, the axes (as constrained by the measured soil environmental variables) explained 49.23% and 65.40% of the variances in the soil bacterial and fungal communities, respectively. The first two axes explained 43.37% and 5.15% of the composition of the soil bacterial community (Figure 4A) and 62.94% and 2.28% of the composition of the soil fungal community, respectively (Figure 4B). According to the Monte Carlo permutation test, the first two canonical axes were highly significant for the composition of the soil bacterial community ($F = 2.60$; $p = 0.022$) and for the composition of the soil fungal community ($F = 3.30$; $p = 0.04$). The microbial community in the soil of BSCs was significantly different from that in the soil beneath BSCs. While biochar application had only a slight influence on the soil microbial community in BSCs, it significantly changed the composition of the soil microbial community beneath BSCs. The key soil environmental factors that influenced the composition of the soil bacterial community were soil UE activity, OM content, and TN content, whereas those that affected the soil fungal community were soil UE activity, pH, OM content, and SC activity (Table 4). Among these factors, soil UE activity was the key soil environmental factor that drove bacterial and fungal communities ($p < 0.01$). UE activity and OM content were positively correlated with the occurrence of Bacteroidetes and Basidiomycota and negatively correlated with the occurrence of Deinococcus–Thermus and Firmicutes (Figure 5). TN content was positively correlated with the occurrence of WPS-2, Acidobacteria, Proteobacteria, and Actinobacteria. In addition, pH was negatively correlated with the occurrence of Ascomycota and Chytridiomycota.

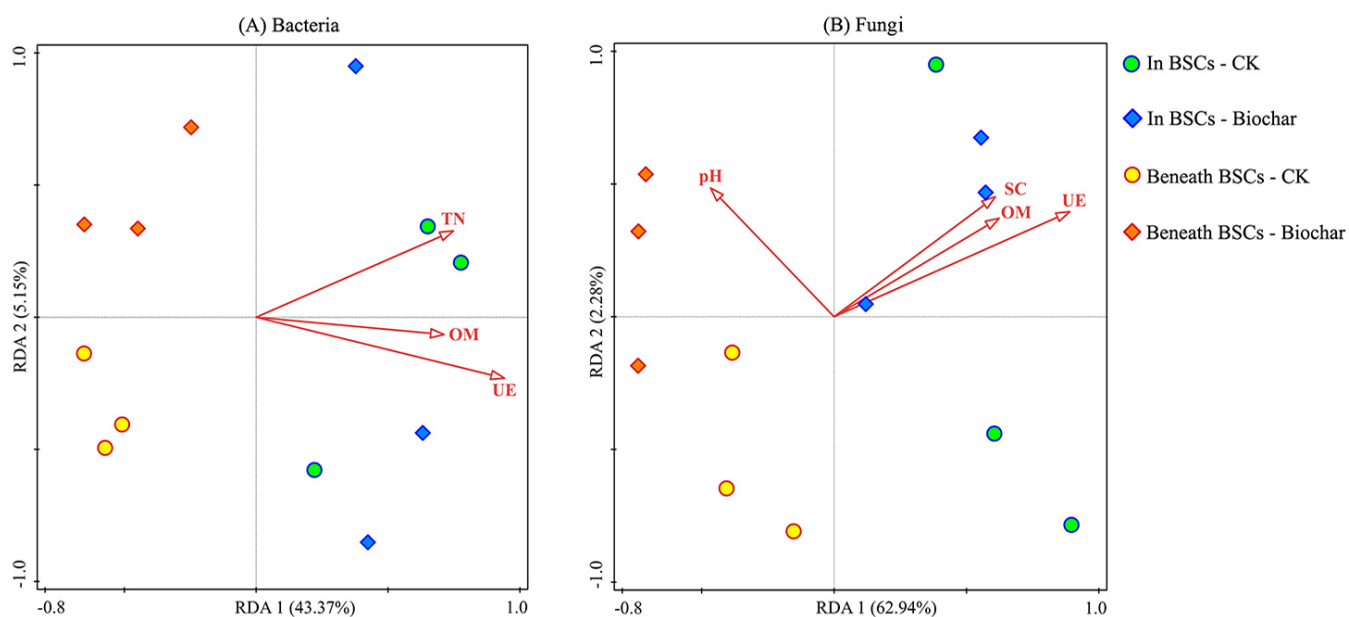


Figure 4. RDA of the microbial community in relation to soil environmental factors.

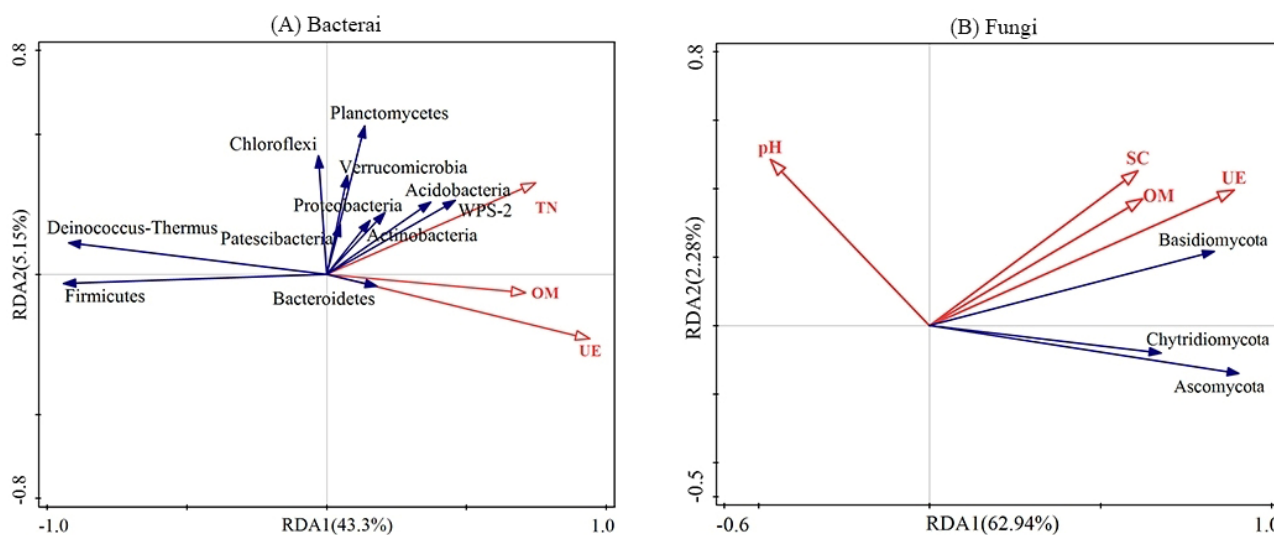


Figure 5. The relationship between the microbial community and soil environmental factors. Note: OM, Organic matter; TN, total nitrogen; UE, urease; SC, sucrose.

Table 4. Result of the Monte Carlo permutation test for the influence of the soil environmental factors on microbial community structure.

Microbes	Name	Explains %	Contribution %	pseudo-F	P
Bacteria	UE	38.8	78.7	6.3	0.006
	TN	3.5	7.2	0.6	0.602
	OM	6.9	14.1	1.1	0.314
Fungi	UE	50.2	76.8	10.1	0.008
	pH	11.4	17.4	2.7	0.102
	OM	2.1	3.2	0.5	0.686
	SC	1.8	2.7	0.4	0.734

4. Discussion

According to Guo et al., it is generally assumed that the annual soil erosion rates in forests in the south of China are higher than $1.89 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, which is a significant

rate [39]. BSCs play a critical role in soil stabilization and the biogeochemical cycle [48,49] and often occur in mesic environments after the restoration of degraded land [19,50]. Hence, BSCs may be a promising approach to controlling soil erosion in subtropical forest plantations. Microorganisms generally create the basic matrix of BSCs, facilitating colonization by bryophytes, lichens, and microfauna [3], which in turn enrich microbial diversity in BSC soil. Previous studies were conducted mainly in arid and semi-arid temperate regions [51,52], suggesting that the microbial communities in BSC soils are related to the soil type, BSC developmental stage, and BSC type [53–55]. To date, there are only a few studies on microbial communities in BSC soils in mesic subtropical regions. This study explored the effect of biochar on the composition of the microbial communities associated with BSCs in a *P. massoniana* plantation in subtropical China.

We found that the BSC type at our study site was moss-dominated crust. Generally, cyanobacterial crusts are indicators of early-stage crusts and are widespread under drier conditions [56,57], whereas moss crusts are later-stage crusts and mostly occur under moister conditions [58,59]. Hence, it is no wonder that the BSC type at our study site was moss-dominated crust. The results showed that Acidobacteria, Proteobacteria, Actinobacteria, and Chloroflexi were the dominant bacterial phyla, similar to those observed in the soil of BSCs on the Loess Plateau [60]. The fungal phyla Basidiomycota was more dominant than Ascomycota in our study (Figure 3), but Ascomycota was the predominant fungal phyla in the soil of BSCs on the Loess Plateau [60]. This difference may be attributable to the effect of vegetation type on the abundance of Basidiomycota; the samples of BSCs on the Loess Plateau were collected from a shrubby forest composed of *Artemisia sphaerocephala*, *Caragana korshinskii*, and *Salix psammophila*, whereas those examined in the present study were collected from a plantation of *P. massoniana*. It is well known that *P. massoniana* is a tree species that coexists with ectomycorrhizal species, which is beneficial to the growth and reproduction of ectomycorrhizal fungi. Although ectomycorrhizal fungi include Basidiomycota, Zygomycetes, and Ascomycota, most belong to the phylum Basidiomycota. As a result, the relative abundance of Basidiomycota was higher than that of Ascomycota in the present study, which was in contrast to the results found on the Loess Plateau.

BSCs can effectively improve nutrient accumulation and promote favorable soil conditions [61,62], thus significantly altering the structure of the soil microbial community, resulting in significant differences in the soil microbial community in and beneath BSCs [52,54,63]. Liu et al. found that the relative abundance of Acidobacteria was higher in BSCs [54]. Similarly, our results showed that the relative abundances of Acidobacteria and Basidiomycota in BSC soil were much higher than those in the soil beneath BSCs; in contrast, Firmicutes showed the opposite trend, which was attributed to the higher TN content, OM content, and UE activity in the soil of BSCs. Moreover, the relative abundance of Ascomycota in the soil of BSCs was much lower than in the soil beneath BSCs, which may be attributable to the niche competition of Basidiomycota in the soil of BSCs. The higher relative abundances of Acidobacteria, Chloroflexi, and Basidiomycota in the soil of BSCs indicated that these microorganisms play an important role in the formation and function of BSCs.

Numerous studies have shown that biochar can improve soil pH, nutrient content, and enzyme activity [64–67]. Similarly, the results of the present study also demonstrated that biochar increased soil pH, OM content, TN content, TK content, UE activity, SC activity, and UE activity. Research on the effects of biochar on soil microorganisms has shown that biochar can alter the diversity of the microbial community in soil [67,68], which may be regulated by many factors, including the biochar raw material and preparation process, as well as the soil type [68]. Moreover, biochar may also enhance the formation of BSCs [36]. The present study was the first to investigate the effects of biochar on the structure and diversity of the soil microbial community in and beneath the BSC of a forest plantation. We found that biochar had a positive effect on bacterial diversity (OTU number and richness) and a negative effect on fungal diversity in the soil beneath BSCs. This finding is consistent with the results reported in previous studies [69,70]. The main reason for the increase in soil bacterial diversity beneath BSCs following biochar amendment may be the increase in soil

nutrients (OM and TN content) or soil porosity caused by biochar [62]; bacteria in soil prefer micro-pores where the availability of organic substrates, water, and nutrients is relatively high [71–73]. The diversity of these communities is dependent on the biochemical nature of the environment and organic carbon [74]. The decrease in soil fungal diversity with biochar addition may be owing to the specific resources available for soil microorganisms in biochar, the high content of minerals (K^+ , Ca^{2+} , Mg^{2+} , etc.) in biochar, or the highly stable organic compounds in biochar that could inhibit the growth of some fungi [70]. Moreover, biochar, as a soil improver, increased acidic soil pH, which was beneficial to soil bacteria but not to soil fungi, which usually prefer low pH [75]. However, the effects of biochar on the microorganisms in the soil of BSCs are also easily affected by environmental factors, such as surface runoff, raindrop splash, animal activity, and human-induced disturbances. Therefore, we cannot conclusively state that the bacterial and fungal diversity in the soil of BSCs was affected by biochar, despite the fact that the nutrient content of the BSC soil was higher with biochar treatment.

Enzymes play important roles in soils, such as mediating biochemical transformations involving organic residue decomposition and nutrient cycling [76]. For example, UE is widely distributed in soils and plays an important role in the soil nitrogen cycle [77], whereas PPO and SC perform crucial functions in the soil carbon cycle [78,79]. Soil enzyme activity is closely related to microorganisms. The relationships between enzymes and soil microbial diversity in BSCs need to be further explored, even though a large body of literature exists on the relationship between enzymes and microorganisms in soil [80–82]. In the present study, correlation analysis showed that the OTU number and diversity of bacteria were related to UE and SC activities, whereas those of fungi were associated with UE, ACP, and PPO activities in the soil beneath BSCs; these findings are consistent with a previous study conducted in a degraded karst ecosystem [62].

A soil microbial community can be significantly affected by biochar [35,63,83,84]. The present study found that biochar had little effect on the structure of the soil microbial community in BSCs, but this finding may be attributed to the effects caused by other environmental factors, such as surface runoff, raindrop splash, animal activity, and human-induced disturbances. However, biochar extensively affected the structure of the soil microbial community beneath BSCs by increasing the relative abundances of Acidobacteria, Chloroflexi, and Basidiomycota and decreasing the relative abundances of Actinobacteria, Firmicutes, Ascomycota, and Chytridiomycota. The effects of biochar on the soil microbial community are related to the biochar type, biochar amount applied, soil conditions, and reaction time, resulting in diverse influences on different types of microorganisms [35,68,85]. In our study, the variances in the composition of soil bacterial and fungal communities caused by biochar were related to soil environmental factors, such as UE activity ($p < 0.05$), organic matter content, pH, total nitrogen content, and SC activity. These positive effects of biochar on the microbial community (microbial composition and bacteria diversity) and soil nutrients were beneficial to the formation and stabilization of BSCs; BSCs develop from cyanobacteria- to lichen- or moss-dominated crusts with improvements in soil nutrient content, soil texture, and microbial communities, especially bacterial communities, because bacteria represent the highest proportion of the microbial biomass in BSCs and play important roles in the BSC successional process [86–89].

The addition of biochar can alter the structure of the soil microbial community and decrease or increase the relative abundance of certain microorganisms by affecting soil properties [35]; for example, biochar can enhance soil water content, resulting in a decrease in the relative abundance of desiccated microorganisms such as Gemmatimonadetes [83]. Acidobacteria can degrade plant residues and single carbon compounds. Many studies have shown that the relative abundance of Acidobacteria decreased, owing to the increase in soil pH, with biochar addition [64,84]. However, the present study observed contrasting results beneath BSCs that may be related to the amount of biochar applied. Cheng et al. reported that the relative abundance of Acidobacteria decreased after a 10 t hm^{-2} biochar application [85]. The amount of biochar applied in our study (12 t hm^{-2}) was close to

10 t hm⁻². Hence, the change in Acidobacteria after biochar addition was consistent with the findings of Cheng et al. [85], which was related to the increase in TN content caused by biochar. Chloroflexi can produce energy through photosynthesis and presents facultative anaerobic characteristics [90,91]. The reason for the increase in the relative abundance of Chloroflexi in the soil beneath BSCs following biochar addition may be the beneficial effect of biochar on BSC development and the reduction in the oxidation level in the soil beneath BSCs with biochar amendment. In addition, biochar can improve soil porosity and facilitate light entry into the soil beneath BSCs, which may also be the reason for the increase in the relative abundance of Chloroflexi. Actinobacteria include many oligotrophic bacteria that can decompose cellulose and lignin [92]. Biochar addition increased the contents of soil OM, TN, and other nutrients, leading to a decrease in the relative abundance of oligotrophic bacteria, thus causing a reduction in the abundance of Actinobacteria.

In our study, Basidiomycota and Ascomycota were the most ubiquitous fungi in the soil. Biochar addition led to a higher relative abundance of Basidiomycota but a lower relative abundance of Ascomycota beneath BSCs, which was consistent with the results of adding rice straw biochar to a tobacco plantation [93]. Basidiomycota is the second largest fungal phylum, with a wide variety of fungi, including those that are beneficial or harmful to plants and those that are positively correlated with highly stable organic components (cellulose, lignin, etc.) [94]. Biochar addition increased the relative abundance of Basidiomycota in the soil beneath BSCs, owing to the presence of a large number of stable aromatic organic compounds in biochar, resulting in a higher soil OM content, which promoted the growth and reproduction of Basidiomycota. Ascomycota is the largest fungal phylum, comprising fungi with the ability to degrade OM, and is the most abundant in soil with high OM content [95,96]. The effect of biochar on Ascomycota is related to the amount of biochar applied [97], and the addition of 20–60 t hm⁻² biochar has been reported to significantly increase Ascomycota abundance [98], whereas the 12 t hm⁻² biochar amendment applied in the present study significantly reduced the abundance of Ascomycota.

5. Conclusions

The biochar-coupled remediation of BSCs could be a sustainable and feasible approach to maintaining soil stability and controlling soil erosion. Our study focused on the effects of biochar on the soil microbial community associated with moss-dominated BSCs in a *P. massoniana* plantation because of the key roles played by microorganisms in the formation of BSCs. Higher relative abundances of Acidobacteria, Chloroflexi, and Basidiomycota were noted in the soil of BSCs when compared with those beneath BSCs, which indicated that Acidobacteria, Chloroflexi, and Basidiomycota play important roles in the formation and function of BSCs. We found that biochar altered the composition of the microbial community in the soil beneath BSCs by increasing the relative abundances of Acidobacteria, Chloroflexi, and Basidiomycota, which was beneficial to the development of BSCs. We further found that biochar increased bacterial diversity but decreased fungal diversity in the soil beneath BSCs. Biochar influenced the soil microbial community associated with BSCs, and this effect was partly attributed to soil environmental factors. Our study suggested that biochar addition was an effective measure for regulating the soil microbial community beneath moss-dominated BSCs, thus promoting the development of BSCs in *P. massoniana* plantations. These findings provided significant guidance for the biochar-coupled remediation of BSCs with the goal of decreasing soil erosion below the canopy of forest plantations in subtropical regions.

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