

## Article

# Effects of Different Native Plants on Soil Remediation and Microbial Diversity in Jiulong Iron Tailings Area, Jiangxi

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**Abstract:** Phytoremediation is an important solution to heavy metal pollution in soil. However, the impact of plants on microbial communities in contaminated soil also requires attention. Community-level physiological profiling (CLPP) based on the Biolog™ EcoPlate and high-throughput sequencing were used to study the soil microbial community in this article. The rhizosphere and bulk soil samples of six native species were collected from the iron mine tailings on Jiulong Mountain, Jiangxi Province. According to the average well color development (AWCD), all plants improved the activity and diversity of the contaminated soil microbial community to varying degrees. *Cunninghamia lanceolata* is considered to have good effects and led to the appearance of *Cunninghamia lanceolata* > *Zelkova schneideriana* > *Toona ciliata* > *Alnus cremastogyne* > *Cyclobalanopsis myrsinifolia* > *Pinus elliottii*. The Shannon–Wiener diversity index and principal component analysis (PCA) show that the evenness and dominance of soil microbial communities of several plants are structurally similar to those of uncontaminated soil (UNS). The results of high-throughput sequencing indicated that the bacterial community diversity of *C. lanceolata*, *A. cremastogyne*, and *P. elliottii* is similar to UNS, while fungal community diversity is different from UNS. *C. lanceolata* has a better effect on soil nutrients, *C. myrsinifolia* and *P. elliottii* may have a better effect on decreasing the Cu content. The objective of this study was to assess the influence of native plants on microbial communities in soils and the soil remediation capacity. *Mortierellomycota* was the key species for native plants to regulate Cu and microbial community functions. Native plants have decisive influence on microbial community diversity.

**Keywords:** iron mine tailing; heavy metal; phytoremediation; Biolog™ EcoPlate; soil microbial community



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## 1. Introduction

As a large number of toxic and hazardous substances enter the natural environment, serious environmental pollution is being produced in the process of excavation and mining of metals, which will greatly increase human health and safety risks [1,2]. The method of ecological restoration of contaminated soil by plants is friendly to the environment and has good economic benefits, so it has attracted much attention. Studies have shown that the long-term effects of certain plants can reduce the content of pollutants in water and soil contaminated by metals and metalloids [3,4]. The effect is mainly due to a variety of activities, including the removal, detoxification, and immobilization of various pollutants to reduce toxic levels and toxicity by plants in the process of accumulating biomass, resulting in the diversity and metabolic function of the microbial community in the rhizosphere soil. Some researchers believe that the phytoremediation function is derived from the synthetic protein products controlled by its specific genes, such as metallothionein and phytochelatin. Some also believe that certain bacteria and fungi in the soil microbial community can enhance the phytoremediation function by regulating the detoxification and metabolites of

plants [5–8]. Researchers found that the rhizobacteria of colonizing plants in mine tailing soil was a key point for plant growth and phytoremediation [9].

The significance of soil remediation lies in the return of soil to a condition of ecological stability together with the establishment of plant communities it supports or supported to conditions prior to disturbance. However, most of the plants that originally grew on metal tailings have been removed during the mining process. Therefore, this study focused on changes in soil properties and microbial diversity compared with uncontaminated soil, which is necessary to assess the remediation effect of different plants on tailings. The application of soil microorganisms has been widely reported in the phytoremediation process, such as the bacterial genera *Acidovorax*, *Alcaligenes*, *Bacillus*, *Mycobacterium*, *Paenibacillus*, *Pseudomonas*, and *Rhodococcus*. It has also been found that *Bacillus* sp. has a biosorption function for toxic metals by PS-6 [10].

It is certain that if there is plant growth on the abandoned metal tailings, it will indeed have an impact on soil nutrient content and microbial community metabolic diversity. Phytoremediation and phytostabilization lead to changes in the microbial composition of contaminated soils, with pH and nutrient status improving to healthy soils [11,12]. At present, more than 45 families are considered to have the potential to absorb heavy metals, mainly herbaceous plants [13]. The bark and trunk parts of arbor species have poor ability to adsorb heavy metals and have received less attention; however, they have great potential for heavy metal phytoremediation due to the large biomass. Although there are plant species such as *Acacia saligna* that have been shown to have heavy metal tolerance and phytoremediation capabilities [14], alien species have potential risks of biological invasion and should be carefully considered [15]. Regrettably, there is no unified conclusion on whether native arbor plants can perform phytoremediation by driving changes in the metabolic diversity of microbial communities and how to select suitable species to solve the pollution from tailings in Jiangxi. Indigenous plant species that can tolerate the low pH and heavy metal soil conditions of tailings environments should be emphasized to fill gaps in research and application foundations. In this study, the following six native tree species that have certain tolerance and resistance and can grow well in weakly acidic soil conditions were selected as the research objects. *Cunninghamia lanceolata* and *Pinus elliottii* are coniferous evergreen species in southern China. *Zelkova schneideriana* and *Cyclobalanopsis myrsinifolia* are common broad-leaved trees. *Toona ciliate* and *Alnus cremastogyne* are relatively precious plant species of great significance to ecological diversity [16–18]. They are widely distributed native tree species that can achieve a fast-growing high yield and have high economic value under artificially cultivated conditions. If these species can play a better role in the remediation of heavy metal-contaminated soil, this can effectively promote research on the increasingly serious issue of soil pollution.

Biolog™ EcoPlate is a convenient and efficient microbial community research method that can obtain a large amount of data for analysis. It can effectively reflect the utilization intensity of different carbon source substrates by the microbial community and reflect the diversity of the metabolic function to a certain extent [19–21]. The community-level physiological profiling (CLPP) method is based on the single carbon source utilization mode of Biolog™ EcoPlate [22], which can compare different microbial communities and is suitable for comparison between contaminated and uncontaminated soil microbial communities [23,24]. With the development of molecular biology, high-throughput sequencing technology is widely regarded as an important means to evaluate the diversity and dynamics of soil microbial communities, and it has outstanding advantages over traditional culture-based methods [25]. Considering that the high-throughput method still requires a relatively high cost, its convenience and practicability in the possible large-scale tailings soil survey in the future were lower than that of using Biolog™ EcoPlate. Our research hopes to verify the applicability of the Biolog™ EcoPlate to the study of soil microbial communities under the conditions of heavy metal pollution by metal tailings, explore the remediation effects of six native plants on contaminated soil and on microbial communities,

and further discuss the impact of phytoremediation capabilities and effects possible reasons for differences.

## 2. Materials and Methods

### 2.1. Site Description

The experimental area is located on the Jiulong Forestry Farm, Jiulong Mountain, Jiangxi Province, China, at 27°36′31.71″ N, 114°52′53.67″ E, and the altitude is 431 m. It belongs to a humid mid-subtropical monsoon climate, with an average annual temperature of 17.5 °C and precipitation of 1590.9 mm. The parent rock is mainly shale, and the soil is a Jiangxi red loam. *C. lanceolata*, *P. elliotii*, *Z. schneideriana*, *C. myrsinifolia*, *T. ciliate*, and *A. cremastogyne* were planted in adjacent areas at a density of 2 m × 2 m. Each stand containing plants of a particular species was divided into three repetition blocks of 20 m × 20 m.

### 2.2. Soil Sampling

All soil samples were collected at a depth of 0–20 cm in April 2019. Each bulk soil sample was a mixture of 5 soil cores (1 from each corner and 1 from the center of the block) sampled from each block; similarly, each rhizosphere soil sample was the rhizosphere soil of 5 plants randomly selected in the block—shake off the loose soil around the roots, and then collect the remaining soil attached to the roots. Uncontaminated soil was sampled at a distance of 2.5 km from iron tailings. There were a total of 39 soil samples in 3 replicates of the rhizosphere soil, bulk soil, and uncontaminated soil. All samples were immediately sieved (2 mm), stored at 4 °C, and transported to the laboratory. They were either stored at –80 °C before microbial community analysis or air-dried and ground before chemical property analyses.

### 2.3. Analyses of Soil Properties

Using soil water suspension with a ratio of 1:2.5 *w/v* to measure soil pH and electric conductivity (Ec), soil organic carbon (SOC) was determined by the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub> oxidation method. Total nitrogen (TN) was measured using a 2300 Kjeltex Analyzer Unit (FOSS, Höganäs, Sweden). The contents of total phosphorus (TP), total potassium (TK), available nitrogen (AP), available potassium (AK), as well as heavy metal elements were measured by inductively coupled plasma emission spectrometry (Spectro Analytical Instruments, Spectro Arcos ICP, Kleve, Germany).

### 2.4. Experiments with Biolog™ EcoPlate and High-Throughput Sequencing

A Biolog™ EcoPlate (Biolog Inc, Hayward, CA, USA) containing 31 carbon source substrates were used to analyze the metabolic characteristics of soil microbial communities. The Eco plate inoculum was prepared according to the method described by Classen et al. [26]. We added fresh soil (equivalent to 30 g dry soil) into a sterilized 270 mL Erlenmeyer flask containing 0.85% NaCl solution, shook at 180 rpm for 30 min, took 3 mL supernatant and added to 27 mL concentration of 0.85% NaCl solution. After mixing, we took 3 mL of supernatant and added it to 27 mL of 0.85% NaCl solution. We added 150 µL of the diluted solution with a concentration of 10.3 g·mL<sup>-1</sup> to each well of the ecological plate, and then placed it in a constant temperature incubator at 28 °C. The above operations were all carried out in a sterile environment. On the MicroLog™ microplate reader (version 4.20.05; Biolog Inc, Hayward, CA, USA), read the 590 nm and the wavelength value of 750 nm (turbidity).

Average well color development (AWCD) was used to characterize the metabolic activity of soil microbial communities [27]:

$$AWCD = \frac{\sum(C - R)}{n} \quad (1)$$

C represents the value at 590 nm minus the value at 750 nm of each carbon source hole; R represents the optical density value of the control hole; *n* is 31 in this study.

The following indexes were always used to assess the level of functional diversity of microbial communities:

Shannon–Wiener diversity index ( $H'$ ) [28]:

$$H' = - \sum P_i \ln P_i \quad (2)$$

Pielou evenness index ( $J$ ) [29]:

$$J = \frac{H'}{\ln S} \quad (3)$$

Simpson dominance index ( $D$ ) [30]:

$$D = 1 - \sum P_i^2 \quad (4)$$

McIntosh diversity index ( $U$ ) [31]:

$$U = \sqrt{\left(\sum ni^2\right)} \quad (5)$$

McIntosh evenness index ( $E$ ):

$$E = \frac{N - U}{N - \frac{N}{\sqrt{S}}} \quad (6)$$

where  $P_i$  represents the ratio of the (C–R) value in the  $i$ -th non-control well to the sum of all non-control wells (C–R) values,  $S$  represents the number of types of carbon source substrates used by the microbial community, and  $ni$  represents the  $i$ -th value of the carbon source hole (C–R).  $N$  represents the sum of the (C–R) values of 31 carbon sources

We used high-throughput sequencing technology (Illumina HiSeq 2500, BioMarker Technologies Corporation, Beijing, China) [32] to compare the microbial communities of soil samples of *C. lanceolata*, *T. ciliata*, *A. cremastogyne* and uncontaminated. Soil DNA was extracted from the frozen soil samples by using the Power Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. The ITS1 forward primer (5'-CTTGGTCATTTAGAGGAAGTAA-3') and the ITS2 reverse primer (5'-GCTGCGTTCTTCATCGATGC-3') were used for amplifying the fungal ITS1 barcode region, while the 338 forward primer (5'-ACTCCTACGGGAGGCAGCA-3') and the 806 reverse primer (5'-GGACTACHVGGGTWTCTAAT-3') were used for amplifying the bacterial V3–V4 barcode region. The polymerase chain reaction (PCR) parameters were as follows: denaturation at 95 °C for 5 min; 25 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 40 s; and final extension at 72 °C for 7 min. Amplicons were purified using a GeneJET Gel Extraction Kit (Thermo Scientific, Waltham, MA, USA) and quantified using a Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA). The raw paired-end reads were joined with FLASH (v.1.2.11) (CCB, Auf, Trabizon, Turkey) [33] and analyzed using QIIME (v.1.8.0) (Knight Lab, Boulder, CO, USA) [34]. To study the microbial diversity information of the samples, clean tags were clustered at a 97% sequence similarity level using USEARCH in QIIME [35]. Different operational taxonomic units (OTUs) were obtained [36]; then, based on SILVA (bacteria) and UNITE (fungal) taxonomy databases, the OTUs were classified and annotated and used to compute and plot rarefaction curves with the help of R (R v.4.1.2) (Ihaka, R., etc., Auckland, New Zealand)

## 2.5. Statistical Analyses

An analysis of variance (ANOVA) was conducted using SPSS 26.0 (IBM Co., New York, NY, USA) and was used to evaluate the differences in the soil properties and heavy metal content between different samples. Once a significant effect was indicated ( $p < 0.05$ ), a pairwise comparison between all samples was performed using Tukey's HSD test ( $\alpha = 0.05$ ). Data from Biolog™ EcoPlate after 72 h incubation was used to calculate the diversity index, perform the principal component analysis (PCA), and produce the figures by SPSS 26.0

(Nie, N. H., etc., Palo Alto, CA, USA) and Origin 2018 (OriginLab, Northampton, MA, USA). The cluster analysis of the microbial genus level was obtained by R (R v.4.1.3) using the unweighted pair-group method with arithmetic means (UPGMA). Redundancy analysis (RDA) or canonical correlation analysis (CCA) was performed while taking into account the relationships of the microbial community, treatments, and environmental factors. The RDA/CCA analysis mentioned above was performed using the “vegan” package in R (R v.4.1.3). A Spearman correlation analysis was performed using the “corrplot” package in R (R v.4.1.3).

### 3. Results

#### 3.1. Chemical Characteristics of the Study Soils

The main sources of heavy metal pollution are exchangeable metal ions and some metal complexes in soil. Soil with heavy metal content exceeding the risk screening value may pose risks to vegetation growth and the soil ecological environment (GB 15618-2018). According to related research, the soils of mine tailing were polluted with different loads of Cu, Zn, and Pb. High heavy metal loads may lead to soil acidification [37,38]. The highest values of these metals can exceed  $1000 \text{ mg}\cdot\text{kg}^{-1}$ , which is more than five times higher than the national safety standard. In this study, soil pH ranged from 4.6 to 6.3. The pH of the rhizosphere and bulk soil were significantly higher than those of the uncontaminated area (Table 1). The values of Cu were below 2 times risk screening values, and the values of Zn and Pb were below 1.5 times risk screening values.

The values of SOC and TN in rhizosphere and bulk soils were relatively low and ranged from  $2.41 \text{ g}\cdot\text{kg}^{-1}$  to  $7.88 \text{ g}\cdot\text{kg}^{-1}$  and from  $0.41 \text{ g}\cdot\text{kg}^{-1}$  to  $0.83 \text{ g}\cdot\text{kg}^{-1}$ , and were lower than one-third and one-half of those of UNS. At this point, *C. lanceolata* had the relatively best soil nutrient status, while *P. elliotii* was relatively poor. The values of TK, AK, TP, and AN in rhizosphere and bulk soils were ranged from  $18.46 \text{ g}\cdot\text{kg}^{-1}$  to  $24.87 \text{ g}\cdot\text{kg}^{-1}$ ,  $10.01 \text{ mg}\cdot\text{kg}^{-1}$  to  $25.86 \text{ mg}\cdot\text{kg}^{-1}$ ,  $0.31 \text{ g}\cdot\text{kg}^{-1}$  to  $0.69 \text{ g}\cdot\text{kg}^{-1}$ , and  $56.00 \text{ mg}\cdot\text{kg}^{-1}$  to  $383.25 \text{ mg}\cdot\text{kg}^{-1}$ .

#### 3.2. Average Well Color Development

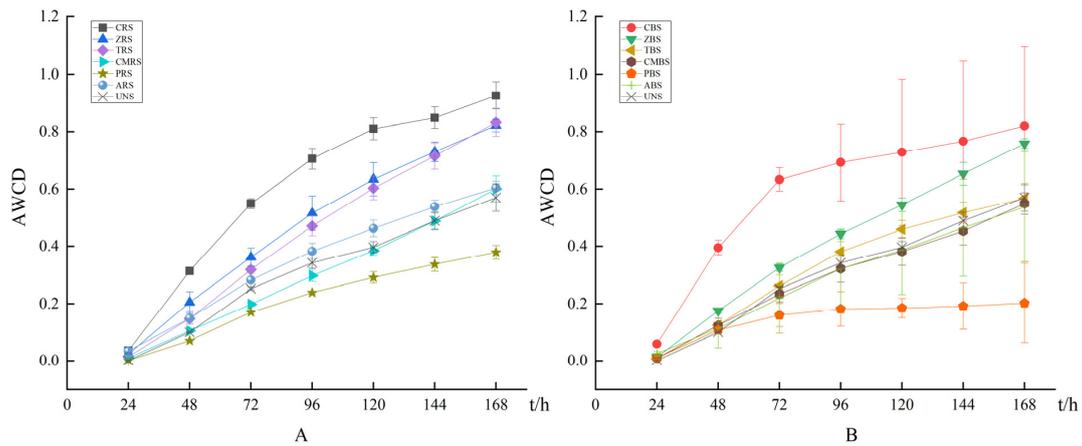
With the increase of incubation time in 0–168 h, the slope of the rhizosphere soil curve reached its maximum value at 72 h (Figure 1), when the rhizosphere soil microbial community had the highest utilization rate of carbon source substrates. The AWCD values of the rhizosphere soils were higher than those of the bulk soils. The values of amino acids in CRS and ZRS were slightly higher than those in CBS and ZBS. It meant that native plants have a certain positive effect on the metabolism of the microbial communities. *P. elliotii* have a weak influence on the functional structure of microbial communities.

Soil microbial communities have differences in carbon source usage, which make differences in the metabolic functions of the microbial communities (Figure 2). CRS, CMRS, PRS, and ARS had significantly higher usage efficiency of carboxylic acids than those of phenolic acids and amines. ZRS had a significantly higher usage of carboxylic acids and amines than those of phenolic acids and polymers. The usage of amino acids and carboxylic acids by ZBS, TRS and CMRS were significantly higher than that of carbohydrates, phenolic acids, and amines. CBS had no significant differences in the usage of carbon sources. The differences in the rhizosphere soils were more significant than those in the bulk soil. The native plants improved metabolic diversity of microbial communities of tailing soils.

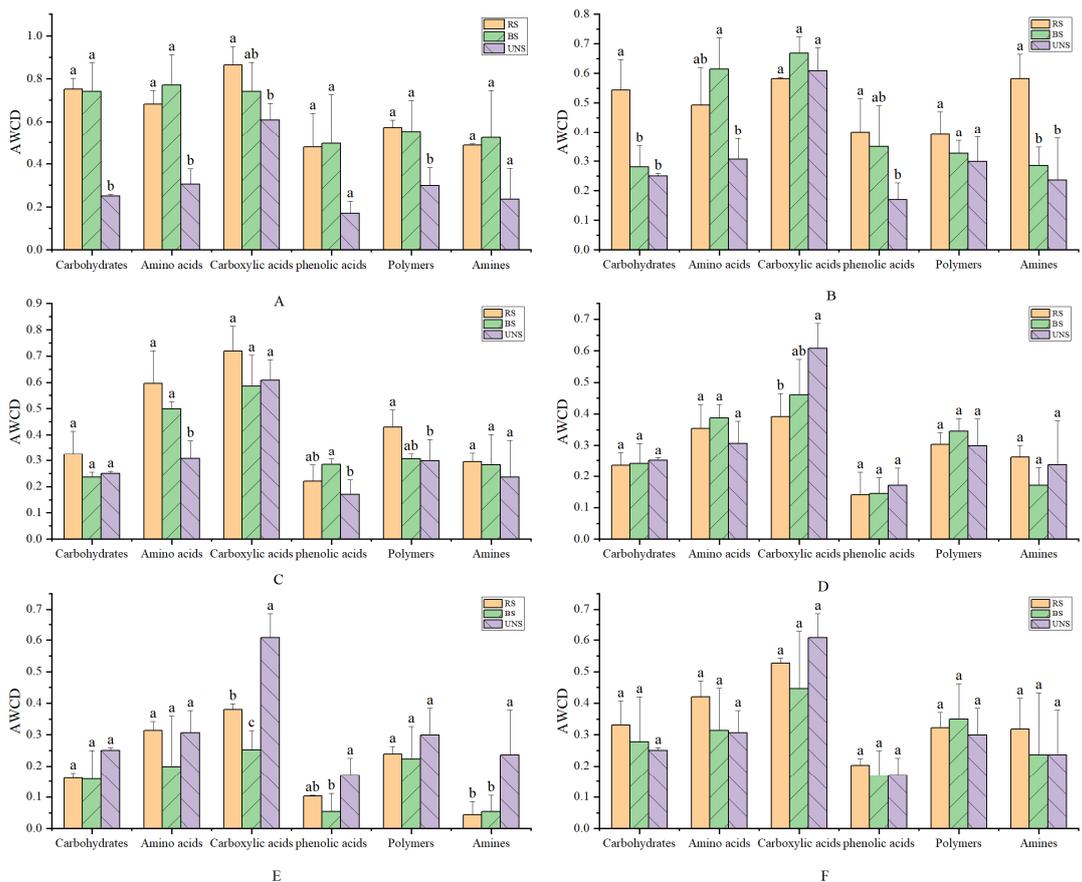
**Table 1.** Chemical characteristics of soil samples (rhizosphere and bulk soil) from iron mine tailing in Jiangxi.

Scheme	pH <sup>†</sup> Mean ± SD	Ec µs/cm Mean ± SD	SOC g/kg Mean ± SD	TN g/kg Mean ± SD	TP g/kg Mean ± SD	TK g/kg Mean ± SD	AN mg/kg Mean ± SD	AK mg/kg Mean ± SD	Cu mg/kg Mean ± SD	Pb mg/kg Mean ± SD	Zn mg/kg Mean ± SD	
CRS ‡	5.58 ± 0.03c §	134.97 ± 0.15a	7.63 ± 0.25b	0.82 ± 0.01b	0.41 ± 0.01c	20.41 ± 0.95bc	327.2 ± 47.90b	19.55 ± 4.52b	82.47 ± 2.45bc	50.36 ± 3.69b	238.34 ± 4.38b	
CBS	4.93 ± 0.02hi	87.43 ± 0.23c	3.47 ± 0.02d	0.42 ± 0.01h	0.37 ± 0.01c	22.98 ± 0.57ab	156.51 ± 3.71d	10.72 ± 0.66c	79.62 ± 1.74c	45.94 ± 1.71b	246.06 ± 2.38ab	
ZRS	4.95 ± 0.03hi	66.93 ± 0.15g	3.38 ± 0.18d	0.48 ± 0.00fg	0.66 ± 0.01a	20.97 ± 0.45bc	72.04 ± 1.33f	14.46 ± 0.72c	94.87 ± 1.43a	73.30 ± 1.71a	269.54 ± 18.32ab	
ZBS	5.20 ± 0.02f	70.57 ± 0.40f	4.93 ± 0.04cd	0.51 ± 0.02f	0.44 ± 0.02bc	20.04 ± 0.97bc	112.88 ± 6.13e	12.91 ± 0.82c	85.48 ± 2.51b	68.66 ± 15.74a	60.52 ± 3.43d	
TRS	5.49 ± 0.01d	85.37 ± 0.35d	6.24 ± 0.26bc	0.81 ± 0.02b	0.48 ± 0.02bc	20.69 ± 0.39bc	382.08 ± 1.01a	14.54 ± 1.26c	73.04 ± 0.63d	61.20 ± 1.58ab	100.82 ± 5.25d	
TBS	4.88 ± 0.03i	46.7 ± 0.35k	5.01 ± 0.18cd	0.63 ± 0.01d	0.54 ± 0.01b	23.61 ± 0.68a	67.96 ± 1.82f	13.71 ± 0.21c	60.44 ± 0.58e	73.42 ± 3.34a	290.16 ± 4.36ab	
CMRS	5.36 ± 0.03e	90.97 ± 0.15b	4.68 ± 0.20cd	0.62 ± 0.01d	0.32 ± 0.01c	18.43 ± 0.19c	372.26 ± 6.57a	23.45 ± 2.46ab	23.25 ± 0.32h	61.09 ± 0.96ab	268.76 ± 12.25ab	
CMBS	5.31 ± 0.02e	75.3 ± 0.00e	3.55 ± 0.15d	0.58 ± 0.01e	0.33 ± 0.01c	19.05 ± 0.51c	232.85 ± 0.00c	22.11 ± 0.70ab	28.45 ± 1.18g	64.10 ± 1.63a	297.14 ± 32.62a	
PRS	5.00 ± 0.03h	39.1 ± 0.44i	2.75 ± 0.39d	0.47 ± 0.02g	0.43 ± 0.00bc	24.33 ± 0.59a	56.00 ± 0.00f	13.04 ± 0.79c	25.82 ± 0.13gh	63.15 ± 2.88ab	282.68 ± 6.01ab	
PBS	5.08 ± 0.02g	63.37 ± 0.71h	4.61 ± 0.04cd	0.63 ± 0.03d	0.51 ± 0.16bc	21.3 ± 1.55b	162.75 ± 7.63d	13.42 ± 0.22c	26.12 ± 0.98gh	60.00 ± 1.60ab	270.24 ± 8.68ab	
ARS	6.26 ± 0.06a	62.73 ± 0.35hi	5.99 ± 0.29c	0.70 ± 0.03c	0.60 ± 0.01ab	22.92 ± 1.22ab	78.46 ± 3.54f	17.66 ± 0.57bc	60.00 ± 2.04e	65.53 ± 3.35a	170.83 ± 41.78c	
ABS	5.95 ± 0.07b	52.13 ± 0.31j	4.25 ± 0.08cd	0.69 ± 0.01c	0.60 ± 0.01ab	23.49 ± 0.20a	119.00 ± 4.63e	16.43 ± 0.21bc	63.44 ± 0.96e	71.84 ± 2.74a	164.96 ± 36.37c	
UNS	4.63 ± 0.02j	62.3 ± 0.40i	25.49 ± 2.22a	1.65 ± 0.01a	0.44 ± 0.01bc	14.81 ± 0.71d	168.47 ± 6.78d	23.95 ± 1.18a	34.92 ± 0.49f	65.50 ± 2.65a	148.24 ± 12.42c	
Risk screening value of heavy metal (GB15618-2018)						pH ≤ 5.5			50		70	
						5.5 < pH ≤ 6.5					90	
											200	

<sup>†</sup> Ec = electric conductivity; SOC = soil organic carbon; TN = total nitrogen; TP = total phosphorus; TK = total potassium; AN = available nitrogen; AK = available potassium; <sup>‡</sup> CRS/CBS = rhizosphere soil/bulk soil of *C. lanceolata*; ZRS/ZBS = rhizosphere soil/bulk soil of *Z. schneideriana*; TRS/TBS = rhizosphere soil/bulk soil of *T. ciliate*; CMRS/CMBS = rhizosphere soil/bulk soil of *C. myrsinifolia*; PRS/PBS = rhizosphere soil/bulk soil of *P. elliotii*; ARS/ABS = rhizosphere soil/bulk soil of *A. cremastogyne*; UNS = uncontaminated soil. <sup>§</sup> Values with the same lower case letters in a row are not significantly different at  $p < 0.05$ .



**Figure 1.** The AWCD values in the microbial community of soil samples increased quickly and then slowly during the cultivation in both rhizosphere soil (A); and bulk soil (B). The bars represent the means and SD of 3 replicates. CRS/CBS = rhizosphere soil/bulk soil of *C. lanceolata*, ZRS/ZBS = rhizosphere soil/bulk soil of *Z. schneideriana*, TRS/TBS = rhizosphere soil/bulk soil of *T. ciliata*, CMRS/CMBS = rhizosphere soil/bulk soil of *C. myrsinifolia*, PRS/PBS = rhizosphere soil/bulk soil of *P. Elliottii*, ARS/ABS = rhizosphere soil/bulk soil of *A. cremastogyne*, UNS = uncontaminated soil.



**Figure 2.** The AWCD values of soil microbial communities were calculated according to the type of carbon sources for comparison of differences in tailing soils under the influence of six native plants and uncontaminated soil. The bars represent the means and SD of 3 replicates. Means not sharing any letter are significantly different at the 5 % level of significance. (A–F): *C. lanceolata*; *Z. schneideriana*; *T. ciliata*; *C. myrsinifolia*; *P. Elliottii*; *A. cremastogyne*; RS = rhizosphere soil; BS = bulk soil; UNS = uncontaminated soil.

### 3.3. Soil Microbial Functional Diversity

The diversity index is used to reflect the richness and uniformity of a soil microbial community (Table 2). The Simpson dominance index had no significant difference between soil samples. The Pielou evenness index and the McIntosh evenness index of *C. lanceolata* soil were significantly higher than those of UNS, and the diversity indexes between the other samples and UNS had no significant difference. The overall trend was *C. lanceolata* > *Z. schneideriana* > *T. ciliata* > *A. cremastogyne* > *C. myrsinifolia* > *P. elliotii*. In all samples, the Pielou evenness index was between 0.85 and 0.95, the Simpson dominance index was between 0.89 and 0.95, and the McIntosh evenness index was between 0.88 and 0.96, which indicated the scope of changes was small in the uniformity and dominance of the microbial community among different soil samples, and the differences were mainly derived from the diversity of microbial communities.

**Table 2.** Soil microbial functional diversity index.

Samples	Shannon–Wiener Diversity Index	Pielou Evenness Index	Simpson Dominance Index	McIntosh Diversity Index	McIntosh Evenness Index
CRS †	3.19 ± 0.02a ‡	0.93 ± 0.01a	0.95 ± 0ab	3.64 ± 0.17b	0.96 ± 0a
CBS	3.20 ± 0.02a	0.94 ± 0a	0.95 ± 0a	4.17 ± 0.24a	0.96 ± 0a
ZRS	3.06 ± 0.09ab	0.91 ± 0.01b	0.95 ± 0ab	2.58 ± 0.31cd	0.95 ± 0ab
ZBS	2.88 ± 0.04bc	0.85 ± 0.01d	0.93 ± 0b	2.61 ± 0.1c	0.91 ± 0bc
TRS	2.96 ± 0.08b	0.88 ± 0.02cd	0.94 ± 0.01ab	2.41 ± 0.1cd	0.93 ± 0.02bc
TBS	2.84 ± 0.05bc	0.88 ± 0.01cd	0.93 ± 0b	2.08 ± 0.24d	0.93 ± 0.01b
CMRS	2.88 ± 0.07bc	0.86 ± 0.01cd	0.93 ± 0b	1.57 ± 0.08e	0.92 ± 0.01bc
CMBS	2.97 ± 0.05b	0.88 ± 0.01cd	0.94 ± 0ab	1.78 ± 0.2de	0.92 ± 0.01bc
PRS	2.76 ± 0.05bc	0.84 ± 0.01d	0.93 ± 0b	1.42 ± 0.02e	0.90 ± 0.01c
PBS	2.73 ± 0.21c	0.90 ± 0.03bc	0.92 ± 0.03b	1.40 ± 0.27e	0.92 ± 0.04bc
ARS	3.00 ± 0.04ab	0.88 ± 0.01c	0.94 ± 0ab	2.12 ± 0.3d	0.93 ± 0.01bc
ABS	2.77 ± 0.39bc	0.88 ± 0.01c	0.92 ± 0.03b	1.72 ± 0.79de	0.92 ± 0.02bc
UNS	2.89 ± 0.03bc	0.86 ± 0.01cd	0.93 ± 0b	2.00 ± 0.08de	0.91 ± 0.01bc

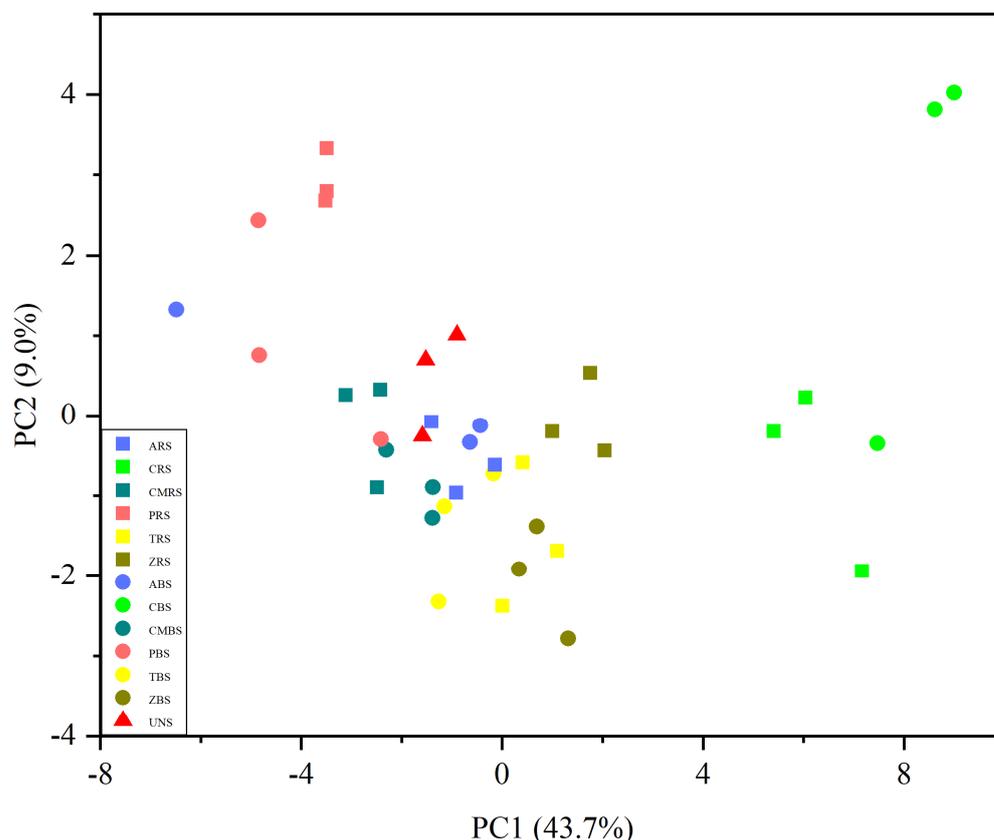
† CRS/CBS = rhizosphere soil/bulk soil of *C. lanceolata*; ZRS/ZBS = rhizosphere soil/bulk soil of *Z. schneideriana*; TRS/TBS = rhizosphere soil/bulk soil of *T. ciliate*; CMRS/CMBS = rhizosphere soil/bulk soil of *C. myrsinifolia*; PRS/PBS = rhizosphere soil/bulk soil of *P. elliotii*; ARS/ABS = rhizosphere soil/bulk soil of *A. cremastogyne*; UNS = uncontaminated soil. ‡ The data are shown as the means ± SD (n = 3). Values with the same lower-case letters in a row are not significantly different at  $p < 0.05$ .

PCA is a technique for analyzing and simplifying data sets. By decomposing the variance, the differences of multiple data sets are reflected in the two-dimensional coordinate graph, which can reflect the difference and distance of the samples; the coordinate axis can reflect the maximum variance eigenvalues (Figure 3). The closer the distance between the two samples is, the more similar the composition of the two samples will be [39]. The main difference is between different samples and the UNS. The closest to the UNS were *A. cremastogyne* and *C. myrsinifolia*, and the farthest was *C. lanceolata*. This indicates that the metabolic function structure of the rhizosphere soil and bulk soil of the same plant has a high similarity, and the most different from the UNS microbial metabolic diversity was *C. lanceolata*, followed by *P. elliotii*. *A. cremastogyne* and *C. myrsinifolia* had a high similarity with the functional structure of microbial metabolism in uncontaminated soil. Generally, the differences in the metabolic function diversity of different soil microbial communities are similar to the six types of carbon sources.

### 3.4. High-Throughput Sequencing Analysis

The high-throughput sequencing results indicated that the microbial communities of soil samples have high similarity in bacteria and obvious differences in fungi (Figure 4). It suggested that native plants affect the bacterial composition of soils, which is an important driver of the improvement of soil nutrients and microbial community metabolic diversity. The relative abundance of *Ascomycota* in the soil samples of *C. lanceolata* and *A. cremastogyne* was significantly lower than that of the UNS, and *Basidiomycota* was significantly higher

than that of the UNS. *Ascomycota* and *Basidiomycota* in the rhizosphere soil was significantly higher than that of bulk soil for *C. lanceolata* and *A. cremastogyne* (ARS > ABS, CRS > CBS), while *P. elliotii* had the opposite trend (PBS > PRS). The similarity of the microbial community diversity structure of CRS, ARS, PRS, and UNS decreased in turn. In the structural composition of fungi, CRS and UNS have smaller differences, which may also explain that *C. lanceolata* has higher carbon source usage and functional diversity in soil microbial communities than other plants.



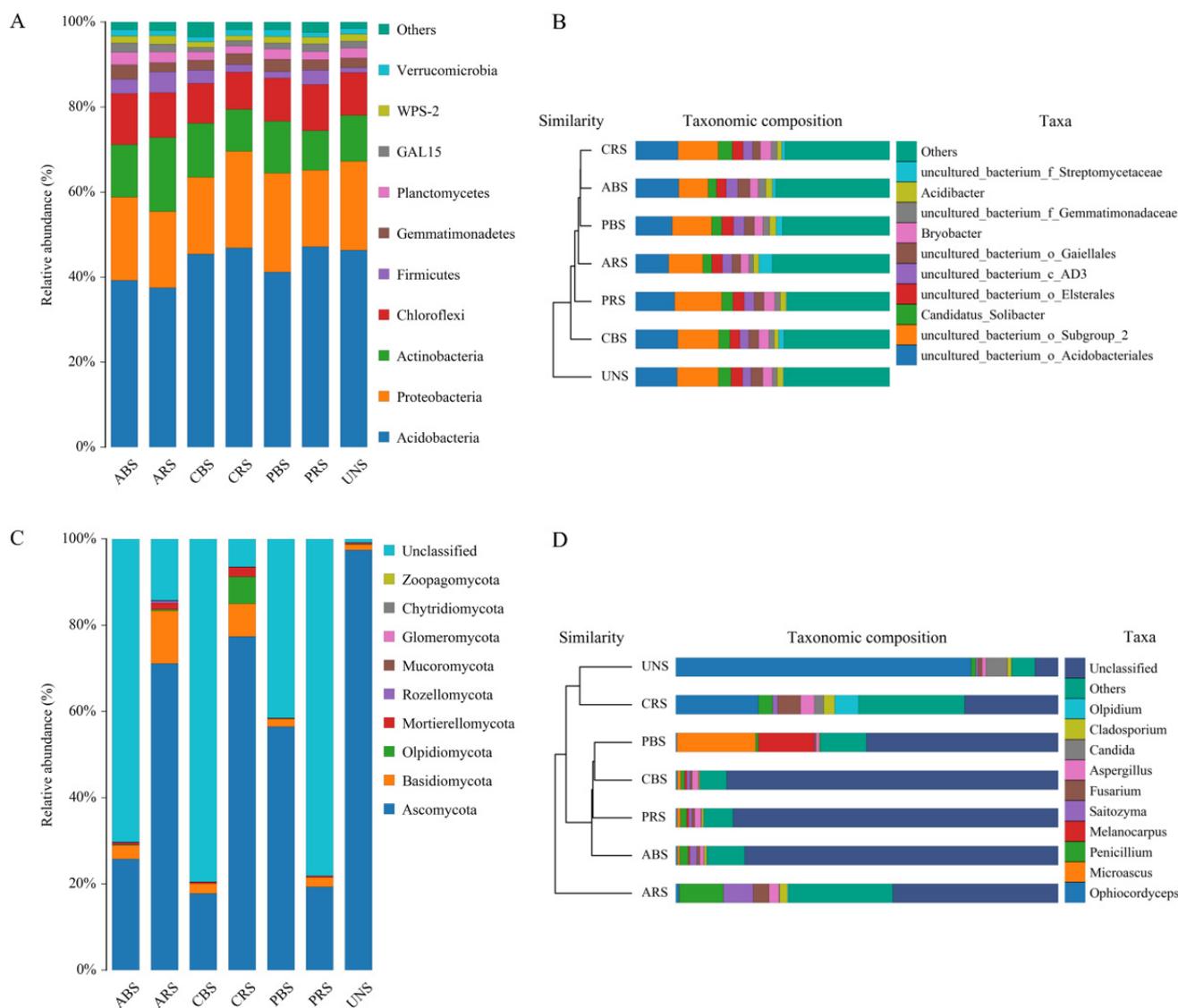
**Figure 3.** Principal component analysis of microbial carbon utilization patterns using Biolog™ EcoPlates from rhizosphere and bulk soils from six native plants and uncontaminated soil. Squares represent rhizosphere soil; circles represent bulk soil; triangles represent uncontaminated soil. For abbreviations see Table 1.

Redundancy analysis (RDA) and canonical correspondence analysis (CCA) [40] is the sorting method based on the development of correspondence analysis. The most influential environmental factors in RDA are AK, TN, and Ec (Figure 5). Ec has a strong influence on the changes in UNS, PBS, and CRS bacterial communities. AK, TN, AN, and SOC have a negative correlation with the changes of ABS and ARS bacterial communities. Similarly, the most influential environmental factor in CCA is pH. Ec has a strong influence on the diversity of the ABS and ARS fungal communities. Ec, TN, and SOC have a strong influence on the PRS and CBS fungal communities.

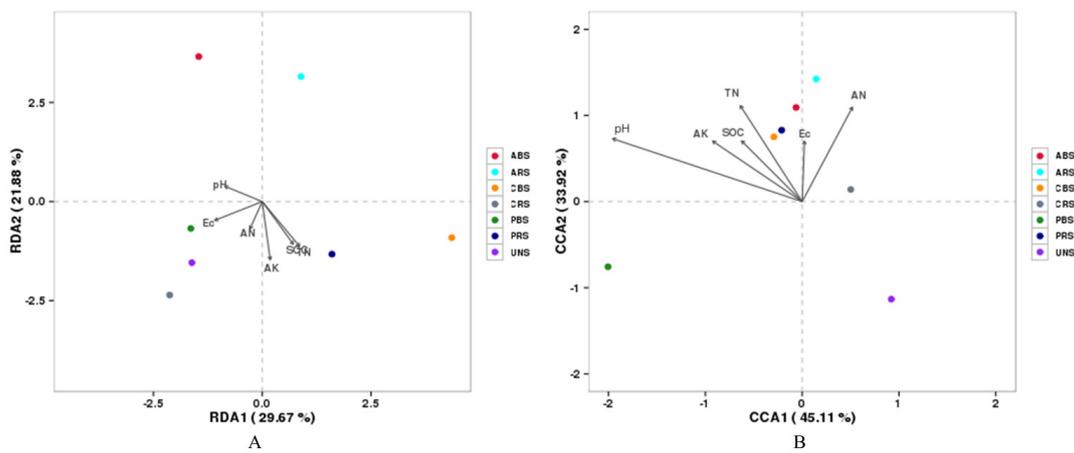
### 3.5. Correlation Analysis

The life activities of soil microorganisms are inseparable from the soil environment, and environmental factors have an important impact on the metabolism and community composition of soil microorganisms. Spearman correlation analysis was used to determine key factors, carbon sources, and microbial populations that play an important role in phytoremediation and soil stabilization. It is worth noting that most microorganisms showed no significant weak or no correlation with the six types of carbon sources available

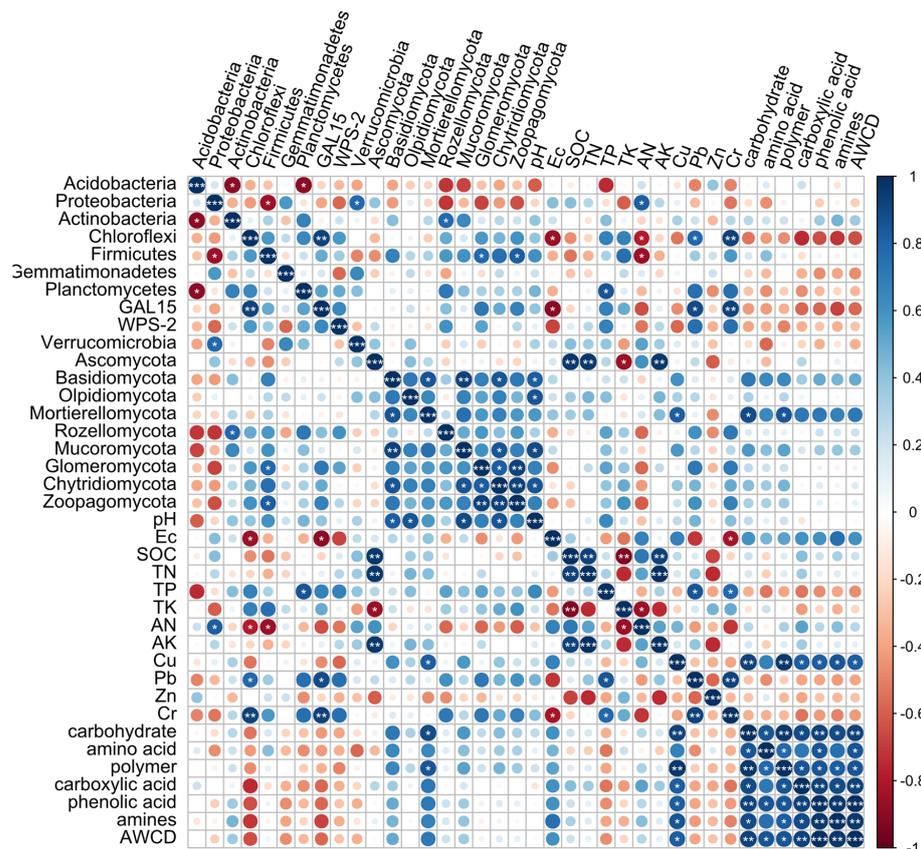
(Figure 6), and there was no significant correlation between carbon sources and pH, SOC, TN, etc. Similarly, Pb was significantly positively correlated with *Chloroflexi*, *GAL15*. We noted a very significant positive correlation between *Chloroflexi* and *GAL15* and a strong correlation with carboxylic acid, phenolic acid, and amines. Moreover, pH was significantly positively correlated with *Basidiomycota* and *Olpidiomycota*; *Mucoromycota*, *Chytridiomycota*, and *Ascomycota* were extremely significantly positively correlated with SOC, TN, and AK, and significantly negatively correlated with TK. This indicates that the utilization of carbon sources by soil microorganisms is mainly affected by specific microbial populations, rather than directly controlled by environmental factors such as pH, and these specific microbial populations may also be the key populations affecting soil heavy metals.



**Figure 4.** High-throughput sequencing to compare the relative abundance of bacteria (A) and fungi (C) at the phylum level in soil samples. Cluster analysis based on distance algorithm was used to further discriminate differences in bacterial (B) and fungal (D) communities at the genus level. For abbreviations see Table 1.



**Figure 5.** Regression analysis of correlation between bacterial (A) and fungal (B) composition of soil samples and environmental factors. The scale on the horizontal and vertical coordinates is the value generated by each sample during regression analysis and calculation with environmental factors; dots represent soil samples; arrows represent different environmental factors. The relationship between element points is represented by distance. CRS/CBS = rhizosphere soil/bulk soil of *C. lanceolata*; PRS/PBS = rhizosphere soil/bulk soil of *P. Elliottii*; ARS/ABS = rhizosphere soil/bulk soil of *A. cremastogyne*; UNS = uncontaminated soil.



**Figure 6.** Correlation of soil properties with microbial abundance and carbon source utilization. The color and size of the discs are used to represent the correlation between two variables. The darker the color and the larger the size is, the greater the absolute value of the correlation coefficient between variables. \*, \*\*, \*\*\* in white indicate significance at  $p < 0.05, 0.01, 0.001$ , respectively; Ec = electric conductivity; SOC = soil organic carbon; TN = total nitrogen; TP = total phosphorus; TK = total potassium; AN = available nitrogen; AK = available potassium; AWCD = the average well color development.

## 4. Discussion

### 4.1. Effects of Native Plants on Soil Chemical Characteristics

The most important point of soil remediation was the soil toxicity of heavy metals, which depends largely on the concentration of heavy metals. In this study, the values of Cu, Pb, and Zn in the rhizosphere soils and bulk soils were low. It suggested that native plants have soil remediation potential [41–44]. Moreover, pH and SOC are highly correlated with soil heavy metals and related microorganisms in the community. They may change the concentration of heavy metals and the structure of the microbial community in the soil [45–48]. Our results indicated that pH and SOC were highly correlated with fungi and not correlated with heavy metal content. Low SOC in the rhizosphere soil led to its limited effect on heavy metals. Improvements in soil nutrients and microbial diversity caused by native plants were considered as part of phytoremediation, and from this point of view, *C. lanceolata* was the more valuable native species. SOC, TP, and TN were affected by both heavy metal metals and plants. While plants improved soil nutrients, they were hindered by heavy metals and the consumption of organic matter and nitrogen [49]. Nevertheless, soil properties are indicators of the soil remediation capabilities of these plants, and the effect of plants on soil properties cannot be ignored [50,51].

Regarding changes in the content of heavy metals, it was highly accepted that plants, the environment, microbial communities, and so on may affect the ability of phytoremediation of soil heavy metal pollution [52–54]. For polluted soil, the change of pollutant content is the most intuitive indicator to measure the remediation effect. From this point of view, *C. myrsinifolia* and *P. elliotii* may have a better effect on decreasing the Cu content, but, it may be hard to solve the serious nutrient deficiencies faced by soil in tailing. Generally, our results confirm phytoremediation of native plants to heavy metal pollution but also indicate that native plants may affect soil microorganisms and soil nutrients. It is necessary to select the appropriate species according to the focus of soil remediation.

### 4.2. The Relationship between Microbial Metabolic Diversity and Soil Remediation

The impact of plants on soil is inseparable from the role of microorganisms. Understanding the metabolic changes of microorganisms is necessary for understanding phytoremediation. In this study, plants with high AWCD values in rhizosphere soil also have high AWCD values in bulk soil (Figure 1), which is clearly different from the performance in chemical properties (Table 1). This was also supported by the utilization of six basic carbon sources and the results of correlation analysis. It can be seen that the improvement of microbial metabolic diversity cannot be simply explained by the effects of environmental factors. It may be due to the influence of native plants.

The presence of plants can enhance the stability of the soil structure, accumulate and absorb heavy metal ions in soil, and synthesize phytochelatins [55,56]. These may alleviate the toxicity or reduce the pollution of heavy metals [57,58]. The reduction of Cu, Pb, and Zn can be used to explain the changes in the diversity of microbial communities, but the function of soil remediation does not depend on the role of the plant itself. Soil microorganisms directly or indirectly participate in this process, strengthening the plant's tolerance to heavy metal toxicity [59] and making it easier for plants to adapt to the contaminated soil and reduce heavy metal content more efficiently, which has an important assisting role in phytoremediation [60,61]. As our results show, the rhizosphere soil microbes of different plants have significant differences in their ability to metabolize carbon sources, mainly due to changes in microbial diversity. There was little difference in the preference for the certain type of carbon source metabolism. With the assistance of soil microorganisms, plants play a remediation function on heavy metal-contaminated soil. The functional diversity of soil microbial communities is an important reason for the differences in phytoremediation functions. The strength of soil microorganisms' assisting function in phytoremediation is related to certain specific microbial populations in the community [62,63], and related populations in these microbial communities are sensitive to plants [64]. Studies employed Biolog<sup>TM</sup> EcoPlate were not enough to detect small changes in the functional structure

of soil microbial communities [65,66]. In contrast, our results showed that the functional diversity of soil microbial communities and *Mortierellomycota* was positively correlated with the values of Cu, which indicated that *Mortierellomycota* was the key phylum for plants to regulate Cu and microbial community functions. There was not any correlation between Zn and impact factors. It suggested that differences in Zn content were more caused by the different absorption capacity of native plants. As the SOC and TN are important factors of microbial communities, they have no correlation with microbial populations. The rate of organic carbon accumulation in the rhizosphere varied due to differences in species and microbial community composition. It was indicated that the influence of native plants on the microbial community was the more decisive factor.

#### 4.3. The Relationship between Microbial Diversity and Soil Remediation

Studies have shown that the proportion of certain bacteria and fungi is significantly different between contaminated soil and healthy soil [67,68], and changes in their relative abundance can be used as a reference to measure soil restoration. *Proteobacteria* often dominate in soil microbial communities that have been contaminated with heavy metals for a long time, the Ascomycota:Basidiomycota and Saprotrophic:Ectomycorrhizal ratios for fungi, Cyanobacteria:Chloroflexi ratios for bacteria also largely reflect the differences between contaminated and healthy soils [69,70]. In this study, similar changes can be observed. In addition, *Ascomycota* has the most obvious change among different soil samples and was highly correlated with TN, TK, SOC, and AK. This was related to its dominant position in the microbial community and partly explains the improvement effect of *C. lanceolata* on soil nutrients. *Chloroflexi* and *GAL15* also play important roles in phytoremediation due to their association with Pb and Cr. It means that the results obtained by high-throughput sequencing and CLPP in the analysis of microbial community diversity are consistent to some extent. The positive correlation between pH and the microbial population suggested that native plants may have improved microbial community diversity and community structure by reducing soil acidity.

## 5. Conclusions

Selected native plants play a role in the mitigation of heavy metal and the improvement of soil nutrients and microbial diversity, and it is reasonable to be considered as a solution to the heavy metal problem in tailings soil in Jiangxi. *C. lanceolata* is a good choice for phytoremediation. The disadvantage of native plants was that the improvement of SOC would take longer.

Native plants have differences in improving soil nutrient status, affecting the rhizosphere microbial community, and reducing heavy metal content. The differences in the effect of native plants on Cu were mainly related to rhizosphere microorganisms, especially *Mortierellomycota*. The differences in the effect of native plants on Pb were mainly related to the difference in the effect of microorganisms and phosphorus. The differences in the effect of native plants on Zn, may be related to the uptake capacity of plant roots. They may achieve better results in the soil remediation of tailings under the action of a longer period of time. High-throughput sequencing and CLPP have good consistency in the analysis results of the microbial community diversity. The former is superior to the latter in studying subtle differences in soil microbial populations.

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