

## Research Article

# The Pioneering Role of Bryophytes in Ecological Restoration of Manganese Waste Residue Areas, Southwestern China

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The mining of manganese brings excellent wealth to humankind. However, it destroys the ecological environment, mainly manifested as heavy metal pollution and vegetation destruction. The restoration of ecological vegetation in manganese mining areas has become an important work after mineral exploitation. The effect of bryophytes on ecological restoration in mining areas is irreplaceable. The bryophytes diversity and its pioneering role in two types of manganese waste residue areas were investigated in Guizhou province, China. The results showed that there were 24 species of mosses in mine waste slag areas, and all of them belonged to 6 families and 15 genera; the species *Gymnostomum subrigidulum*, *Pohlia gedeana*, and *Bryum atrovirens* were the dominant mosses. There were 6 species of mosses in electrolytic manganese slag areas, and all of them belonged to 5 families and 5 genera. The dominant moss was *B. atrovirens*. The bryophytes diversity in the electrolytic manganese slag areas with lower pH was poorer than that in mine slag areas. The accumulation of heavy metals in mosses showed that *B. atrovirens* collected from two types of areas had a strong ability to accumulate Mn with the cumulants 5588.00  $\mu\text{g/g}$  and 4283.41  $\mu\text{g/g}$ , respectively. All mosses had a strong enrichment ability to Cd. It indicated that mosses had strong tolerance to heavy metals. Bryophytes increased the available nutrients and bacterial community diversity of mosses growth substrates in two types of areas. Besides, we studied the relationships between bacterial community structure and soil factors. The main soil factor affecting the bacterial community structure was available nitrogen (AN) in mine waste slag areas, while it was pH in the electrolytic manganese residue areas. The systematic study suggested that bryophytes increased the available nutrients and the microbial community diversity of the growth substrates in manganese waste residue areas, which provided the basic conditions for the growth of vascular plants.

## 1. Introduction

Guizhou province, located in southwest China, has high levels of metallic minerals in its soil. The manganese reserves in Guizhou province account for about 60% of the national manganese reserves and mainly concentrate in Tongren city [1]. Manganese mining activities can produce a lot of solid waste, including mine waste slag, tailing slag, and electrolytic manganese slag [2]. Solid waste can bring damage to the ecological environment, such as soil erosion, heavy metal pollution, and groundwater pollution [3, 4]. In 2014, the production of electrolytic manganese slag was about millions of tons in Tongren city [5], and the heavy metal pollution in mining areas has attracted the attention of many scholars. Most of the heavy metals of manganese solid waste exceed

the standard seriously, among which Mn pollution is the most serious, followed by Pb, Cd, Zn, and Cu [6, 7]. The soils are thin and lack nutrient elements in manganese mining areas, which restrict the growth of plants and causes desertification [8].

Bryophytes, the most primitive higher plants with strong water holding capacity, are effective accumulators of elements and play an important role in restoring soil fertility [9–11]. Bryophytes play producer, prospector, monitor, controller, and so on in the metal mining areas [12–18]; they have been widely used to monitor heavy metal pollution in the atmosphere [19–22]. Bryophytes have a wide range of adaptability and strong reproductive capacity and play an irreplaceable role in promoting biological crusts, water conservation, improvement of ecological environment, and

so forth [23]. As an advanced stage of biological crust development, bryophytes can improve the availability of nutrients in soil [24]. The diversity of bryophytes is influenced by the structure of vascular plants and microbial communities. Bryophytes can indicate the ecological restoration of karst caves [25].

The interface between bryophytes and its growth substrate is an important part, and the highly enriched symbiotic microorganisms in them have caught the attention of many scholars in recent years. The research about bryophytes and symbiotic bacteria mainly concentrated on deserts, forests, grasslands, and degraded karst areas [26–29]. Microorganisms such as bacteria and fungi that are symbiotic with bryophytes are related to its arid habitats [30]. These studies are numerous, but studies on the accumulation of heavy metals in bryophytes in manganese solid wastes areas and their effects on growth substrate are lacking. We found that bryophytes crust exists in all the places where vascular plants grow in the manganese solid wastes areas. This phenomenon prompts us to realize the following questions: (1) What was the biodiversity of bryophytes and dominant species in manganese solid wastes areas? (2) What characteristics do bryophytes have for heavy metal accumulation? (3) What were the effects of bryophytes on the growth substrate and the symbiotic bacterial community structure? (4) How was microbial community structure related to soil factors influenced by bryophytes? The findings from this study should provide a theoretical basis for bryophytes to be the typical stress-resistant plants and the pioneers of ecosystem reconstruction in mining areas.

## 2. Materials and Methods

**2.1. Research Areas.** The study sites are located in Yinjiang county and Songtao county (27°35' to 28°10' N, 108°17' to 109°12' E), Tongren city, Guizhou province, southwestern China (Figure 1). The altitudes range from 406 m to 1600 m, and the average annual temperature is 16.5°C. Tongren city is an integral part of China's "Manganese triangle" (namely, Hunan Huayuan, Guizhou Songtao, and Chongqing Xiushan), which has rich manganese reserves.

**2.2. Field Quadrat Setup and Sample Collection.** Samples were collected in July 2020. The study involved two different types of manganese solid wastes areas: mine waste slag areas (A, site in Yinjiang county), which is composed of waste rocks and a small amount of tailings produced during manganese mining; electrolytic manganese slag areas (B, site in Songtao county), which is a kind of solid wastes with high moisture contents. Manganese dioxide was electrolyzed from manganese carbonate ore by the sulfuric acid method. The above process can produce a lot of electrolytic manganese slag. The sample quadrat was set according to the actual investigation situation, and each quadrat was 1 m × 1 m. All kinds of bryophytes samples with about 2 cm thick growth substrate were collected and put in valve bags. The samples were placed in a portable

freezer and transported back to the laboratory for storage at 4°C [30].

We collected three composite bare soil samples as a control in each area to measure the available nutrients and microbial community structure of bryophytes growth substrate. Each sample was a mixture of three samples from the same quadrat. Approximately, 30 g of each mixture was collected. In total, 6 composite bare soil samples (ANS1, ANS2, ANS3, BNS1, BNS2, and BNS3) were collected in the two areas and stored at 4°C until further processing. The basic properties of mine waste slag and electrolytic manganese slag are shown in Table 1.

**2.3. Statistics of Dominant Bryophytes.** In order to investigate the species of bryophytes, bryophyte specimens were collected from two types of manganese solid wastes areas in Tongren city, Guizhou province. Specimens were identified by the classical morphological classification method. All bryophytes species were identified in each quadrat. The bryophytes and their coverage areas were recorded in each quadrat [25, 31]. Dominant bryophytes species were determined according to the following criteria: (1) frequency, it means the number of quadrats a species is in/the total number of quadrats × 100 [30]; and (2)  $S_a/S_t$ , the ratio was the meaning of coverage of each species in all quadrats. The total quadrats in this study are 20 m<sup>2</sup>. Combining the two indexes, the dominant species were screened out.

**2.4. Accumulation of Heavy Metals in Bryophytes.** In order to study the heavy metal accumulation of bryophytes in manganese solid wastes areas, representative bryophytes were selected for heavy metal analysis. In the laboratory, bryophytes were isolated from the growth substrate using forceps, rinsed with tap water until free of impurities, and then rinsed 2 times with ultrapure. Place the kraft paper bag with clean bryophytes in the oven at 75°C for 24 hours and then grind and homogenize it by 0.18 mm nylon sieve. The corresponding growth substrate samples were naturally air-dried and homogenized using 0.15 mm nylon sieve [14].

Digestion of Cu, Zn, Cd, Pb, Mn, Ni, and Cr in bryophytes and growth substrate samples: take 0.1 g of each sample and put it into the teflon digestion tank, add 3 mL of nitric acid and 1 mL of hydrofluoric acid; add 1 mL of perchloric acid after digestion at 180°C for 20 h, dissolve it on the electric heating plate at 200°C for 2 h, remove the acid until it was nearly dry, and then let its volume be 50 mL by using deionized. Three parallels were prepared for each sample, and two sample blanks were prepared for analysis. The quality of the whole experiment was controlled by the national environmental standard sample ESS-3 (GSBZ 50013-83) and the biological component analysis-spinach-GBW10015 (GSB-6) [4, 32, 33]. Flame atomic absorption spectrometry (AAS ZEE nit-700P) was used to analyze the concentrations of Mn and Fe. An inductively coupled plasma mass spectrometer (ICAP TQ ICP-MS Thermo Scientific) was used to analyze the concentrations of Ni, Cr, Cu, Zn, Cd, and Pb [34].

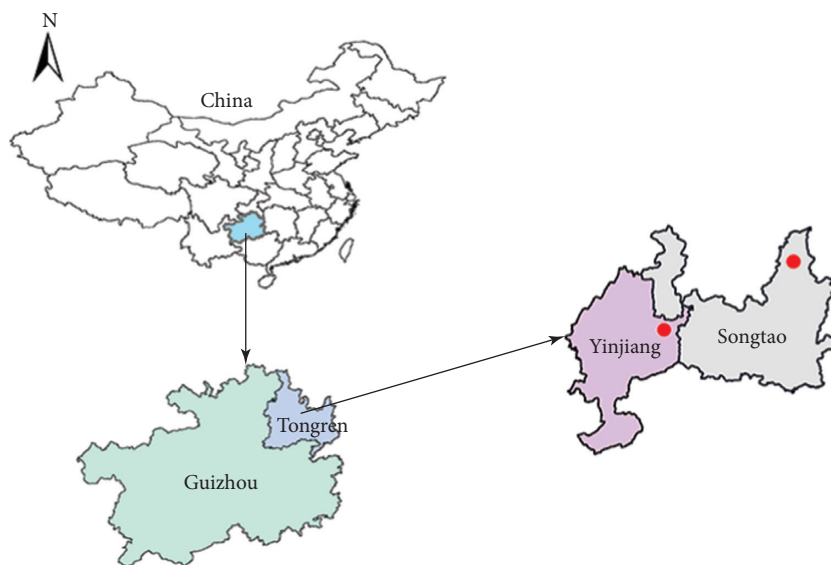


FIGURE 1: Sampling collected sites: Yinjiang means the sites of mine waste slag areas and Songtao means the sites of electrolytic manganese slag areas.

Bioaccumulation factors (BCF) are the ratio of the heavy metal content in the aboveground parts of plants to the corresponding heavy metal content in the soil. This ratio represents the plant's ability to absorb heavy metals from the soil [35]. Its calculation formula is as follows:  $BCF = C_p/C_s$ .

$C_p$  represents the content of heavy metal in the aboveground part of the bryophytes ( $\mu\text{g/g}$ ) and  $C_s$  represents the content of heavy metal in growth substrate ( $\mu\text{g/g}$ ).

#### 2.5. Growth Substrate Available Nutrients of Bryophytes.

To investigate the influence of bryophytes on the growth substrate, representative bryophytes growth substrate collected from two types of manganese waste residue areas was selected to study the available nutrients. The growth substrate samples were naturally dried and passed through 2 mm sieve. Samples treated above were used for the analysis of the available nutrients in the soil, mainly including pH, available nitrogen (AN), available phosphorus (AP), available potassium (AK), and total organic matter (TOC). At the same time, we analyzed the availability of Fe, Mn, Cu, and Zn extracted by DTPA. All the analysis followed the protocols of Lu, 1999 [36].

#### 2.6. Microbial Diversity Analysis. Sample Selected.

To clarify the effects of bryophytes on bacterial communities in two types of manganese waste residue areas, growth substrate samples of 5 bryophytes and 2 bare soil samples collected from two types of manganese waste residue areas were measured. The samples' information is shown in Table 2.

**DNA Extraction and High-Throughput Sequencing.** Microbial DNA extraction from the growth substrate was carried out according to the E.Z.N.A.<sup>TM</sup>, Mag-Bind Soil DNA Kit (OMEGA, M5635-02). DNA quality and

concentration were checked by 1% agarose gel electrophoresis and Qubit 3.0. Using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') to amplify the V3-V4 hypervariable regions of bacteria 16S rRNA. PCR system is as follows: 15  $\mu\text{L}$  of 2  $\times$  Hieff<sup>®</sup> Robust PCR Master Mix, 1  $\mu\text{L}$  of Index-PCR primer F, 1  $\mu\text{L}$  of primer R, and 20–30 ng of template DNA; add H<sub>2</sub>O until the total volume reached 30  $\mu\text{L}$ . The PCR operation conditions are as follows: 3 min of denaturation at 95°C, 25 cycles of 20 s at 94°C, 20 s for annealing at 55°C, and 30 s for elongation at 72°C, and a final extension at 72°C for 10 min. Using 2% agarose gel electrophoresis to detect the final PCR products, the concentration was determined by Qubit 3.0 fluorescence quantifier. The above process was commissioned by Shanghai Sangon Biological Engineering.

**2.7. Data Analysis.** A radar map of BCF was performed in SigmaPlot 12.5 to demonstrate the enrichment of heavy metals in bryophytes. Significant difference analysis was finished by SPSS 23.0 to reveal the effects of bryophytes on available nutrients of growth substrate in manganese solid wastes areas. Venn diagram of OTUs level demonstrated the microbial community characteristics of bryophytes growth substrate and bare soil. Relative abundance of major microflora at phylum level and class level in different species of bryophytes growth substrate has been generated. Linear discriminant analysis effect size (LEfSe) was performed to find the functional characteristics that can well explain the differences between groups in two or more groups of samples in different biological conditions or environments. The relationship between microbial community structure and soil factors has been analyzed by canonical correspondence analysis (CCA) or redundancy analysis (RDA).



TABLE 2: Samples of microbial diversity analysis.

Samples	Source
ANS	Bare soil samples collected from mine waste slag areas
BNS	Bare soil samples collected from electrolytic manganese slag areas
APS	Growth substrate of <i>P. gedeana</i> collected from mine waste slag areas
AGS	Growth substrate of <i>G. subrigidulum</i> collected from mine waste slag areas
ARS	Growth substrate of <i>B. atrovirens</i> collected from mine waste slag areas
BRS	Growth substrate of <i>B. atrovirens</i> collected from electrolytic manganese slag areas
BFS	Growth substrate of <i>Funaria microstoma</i> collected from electrolytic manganese slag areas

### 3. Results

#### 3.1. The Pioneering Role of Dominant Bryophytes in Mine Waste Slag Areas

**3.1.1. The Dominant Species of Bryophytes in Mine Waste Slag Areas.** There were 24 species of mosses in mine waste slag areas, and all of them belonged to 6 families and 15 genera (Table 3). The families of Pottiaceae and Bryaceae were the dominant families, which all contained 8 bryophytes species. There were 16 species in the two families, which was 66.67% of the total species. There were 3 life types, including dwarf type (less than 1.5 cm), high cluster type (more than 1.5 cm), and wefts type. The dwarf type was 66.67%, high cluster type 12.5%, and wefts type 20.83%. About 93.75% of the species in dominant families were of the dwarf type. The dwarf type of bryophytes was conducive to the absorption of water and the improvement of their adaptability. In the dry and severely polluted environment, the dwarf type was usually the absolute dominant [14].

In Table 4, the top 9 dominant bryophytes of mine waste areas were listed combining frequency and coverage ( $S_a/S_t$ ), namely, *G. subrigidulum*, *P. gedeana*, *B. atrovirens*, *C. schmidii*, *H. rosea*, *T. brachydontium*, *C. gratum*, *M. hornschiuchiana*, and *B. jilinense*. *G. subrigidulum* was the species with the highest frequency and the extensive coverage in manganese waste areas; *P. gedeana* ranked second, followed by *B. atrovirens*. We identified the above three species (*G. subrigidulum*, *P. gedeana*, and *B. atrovirens*) as the dominant species in mine waste slag areas.

**3.1.2. The Heavy Metal Accumulation of Bryophytes in Mine Waste Slag Areas.** To clarify the accumulation of heavy metals in mosses collected from mine waste slag areas, the total amount of heavy metals in mosses and their growth substrate ranking in the top 9 mosses was measured (Table 5), and the BCF was calculated. Radar maps were made according to the BCF of the top 9 mosses (Figure 2(a)). All mosses had a stronger enrichment effect on Cd, and the minimum enrichment coefficient of Cd exceeded 1.0. Among them, *H. rosea* had the most potent ability, with an enrichment coefficient of 5.36 (Table 6). However, its enrichment ability on other elements was poor. The species *P. gedeana* and *C. schmidii* had strong enrichment ability for Pb, whose enrichment coefficients were 1.387 and 0.921, respectively. The dominant mosses *B. atrovirens* had a strong ability to accumulate Mn with the cumulants 5588.00  $\mu\text{g/g}$ ,

which indicated that *B. atrovirens* had strong tolerance to Mn.

**3.1.3. Effects of Bryophytes on the Available Nutrients of Growth Substrate in Mine Waste Slag Areas.** To explore the effects of mosses on growth substrate, the growth substrate available nutrients of 6 dominant mosses (*C. schmidii*, *P. gedeana*, *H. rosea*, *B. jilinense*, *G. subrigidulum*, and *B. atrovirens*) collected from mine waste slag areas were analyzed, including pH, AN, AP, AK, and TOC. The availabilities of Fe, Mn, Cu, and Zn extracted by DTPA were analyzed too. The control samples were collected from mine waste slag areas, namely, ANS. All samples were set to three replicates, and the results were displayed as mean  $\pm$  standard deviation (SD) (Table 7).

**pH.** The mean pH of bare soil (ANS) was 7.41, and mosses increased the pH of the growth substrate, among which the pH of *G. subrigidulum* was the highest with the mean value of 7.77. However, there was no significant difference in the effects of mosses on pH.

**AN.** The mean content of available nitrogen (AN) in bare soil was 23.50  $\mu\text{g/g}$ , and the content of AN in mosses growth substrate was higher than bare soil with the content range of 36.62–179.85  $\mu\text{g/g}$ . All the mosses increased the content of AN significantly except for *P. gedeana* and *B. jilinense* ( $P < 0.05$ ), and the content of AN in *G. subrigidulum* growth substrate was the highest with the mean 150.46  $\mu\text{g/g}$ .

**AP.** The content of available P (AP) in the growth substrate was 5.4  $\mu\text{g/g}$  in the bare soil. Different moss species had different effects on the content of AP in the growth substrate. The content of AP in the growth substrate of *P. gedeana* and *H. rosea* was lower than that in the bare soil, while the content of AP in the other 4 mosses growth substrate was higher than that in the bare soil. *B. atrovirens* significantly increased the content of AP growth substrate with the mean 12.57  $\mu\text{g/g}$  ( $P < 0.05$ ).

**AK.** The content of K in the control bare soil was 72.61  $\mu\text{g/g}$ . With the participation of mosses, the content of available potassium (AK) in the growth substrate was higher than that in the bare soil. It ranged from 72.69  $\mu\text{g/g}$  to 270.01  $\mu\text{g/g}$ . The AK contents of *C. schmidii*, *H. rosea*, and *B. atrovirens* were significantly greater than those in the bare soil ( $P < 0.05$ ), which were 197.53  $\mu\text{g/g}$ , 232.05  $\mu\text{g/g}$ , and 223.53  $\mu\text{g/g}$ , respectively.

TABLE 3: Statistics of bryophytes, genera, and species in mine waste slag areas.

Family	Genus	Species	Life style
Pottiaceae	<i>Molendoa</i>	<i>Molendoa hornschurchiana</i>	Dwarf cluster
	<i>Gymnostomum</i>	<i>Gymnostomum subrigidulum</i>	Dwarf cluster
	<i>Trichostomum</i>	<i>Trichostomum brachydontium</i>	High cluster
	<i>Hydrogonium</i>	<i>Hydrogonium pseudoehrenbergii</i>	Dwarf cluster
	<i>Hyophila</i>	<i>Hyophila rosea</i>	Dwarf cluster
	<i>Weissia</i>	<i>Weissia semipallida</i>	Dwarf cluster
			<i>Weissia controversa</i>
		<i>Weissia brachycarpa</i>	Dwarf cluster
Thuidiaceae	<i>Thuidium</i>	<i>Thuidium assimile</i>	Wefts
	<i>Haplocladium</i>	<i>Haplocladium strictulum</i>	Wefts
	<i>Cyrtohypnum</i>	<i>Cyrtohypnum contortulum</i>	Wefts
		<i>Cyrtohypnum gratum</i>	Wefts
Bryaceae	<i>Pohlia</i>	<i>Pohlia gedeanana</i>	Dwarf cluster
	<i>Bryum</i>	<i>Bryum pallescens</i>	Dwarf cluster
		<i>Bryum algovicum</i>	Dwarf cluster
		<i>Bryum dichotomum</i>	Dwarf cluster
		<i>Bryum atrovirens</i>	Dwarf cluster
		<i>Bryum tuberosum</i>	Dwarf cluster
		<i>Brachymenium</i>	<i>Brachymenium pendulum</i>
Leucobryaceae		<i>Brachymenium jilinense</i>	Dwarf cluster
	<i>Campylopus</i>	<i>Campylopus irriggatus</i>	High cluster
		<i>Campylopus schmidii</i>	High cluster
Hypnaceae	<i>Hypnum</i>	<i>Hypnum cupressiforme</i>	Wefts
Funariaceae Schwagr	<i>Physcomitrium</i>	<i>Physcomitrium sphaericum</i>	Dwarf cluster
6 families	15 genera	24 species	

TABLE 4: Top 9 bryophytes species collected from mine waste slag areas.

Number	Species	Frequency (%)	$S_a/S_t$
1	<i>Gymnostomum subrigidulum</i>	20	0.160
2	<i>Pohlia gedeanana</i>	15	0.135
3	<i>Bryum atrovirens</i>	15	0.130
4	<i>Campylopus schmidii</i>	10	0.095
5	<i>Hyophila rosea</i>	10	0.060
6	<i>Trichostomum brachydontium</i>	10	0.030
7	<i>Cyrtohypnum gratum</i>	10	0.030
8	<i>Molendoa hornschurchiana</i>	10	0.030
9	<i>Brachymenium jilinense</i>	5	0.025
Total	—	—	0.695

TABLE 5: Content of heavy metals in bryophytes and growth substrate from mine waste areas.

Samples		Mn ( $\mu\text{g/g}$ )	Cr ( $\mu\text{g/g}$ )	Ni ( $\mu\text{g/g}$ )	Cu ( $\mu\text{g/g}$ )	Zn ( $\mu\text{g/g}$ )	Cd ( $\mu\text{g/g}$ )	Pb ( $\mu\text{g/g}$ )
<i>M. hornschurchiana</i>	In-plant	293.51	283.31	14.02	13.14	123.86	1.19	33.45
	Substrate	1384.96	3385.94	51.95	17.12	258.05	1.58	96.66
<i>C. gratum</i>	In-plant	392.88	4.26	7.55	10.31	40.00	1.16	15.09
	Substrate	45050.00	168.75	39.46	36.32	141.84	1.49	87.46
<i>T. brachydontium</i>	In-plant	4346.88	36.19	16.43	15.03	39.11	1.74	11.61
	Substrate	58487.39	175.10	99.44	231.25	241.56	0.53	28.90
<i>C. schmidii</i>	In-plant	3083.82	17.53	9.87	16.10	83.04	2.85	20.80
	Substrate	36242.62	32.21	27.39	55.72	218.73	1.43	22.58
<i>P. gedeanana</i>	In-plant	2941.91	16.15	9.09	14.21	79.35	3.03	19.51
	Substrate	25385.17	23.59	9.79	39.88	183.03	1.23	14.06
<i>H. rosea</i>	In-plant	3282.74	9.50	9.47	11.21	78.85	5.78	6.60
	Substrate	114591.71	58.35	43.94	57.23	413.16	1.08	38.39
<i>B. jilinense</i>	In-plant	5442.64	15.12	8.25	12.52	86.52	1.98	10.37
	Substrate	124085.87	37.55	25.28	38.38	273.49	0.80	21.06
<i>G. subrigidulum</i>	In-plant	2737.28	7.64	6.01	8.82	70.69	5.10	9.89
	Substrate	19587.96	40.41	27.84	55.03	296.98	2.34	23.77
<i>B. atrovirens</i>	In-plant	5588.00	7.26	5.73	6.58	65.96	2.10	13.57
	Substrate	51028.68	103.75	56.33	171.59	231.41	1.03	23.20
Background of Guizhou province	Soil	591.00	86.60	33.70	25.70	82.40	0.13	29.30

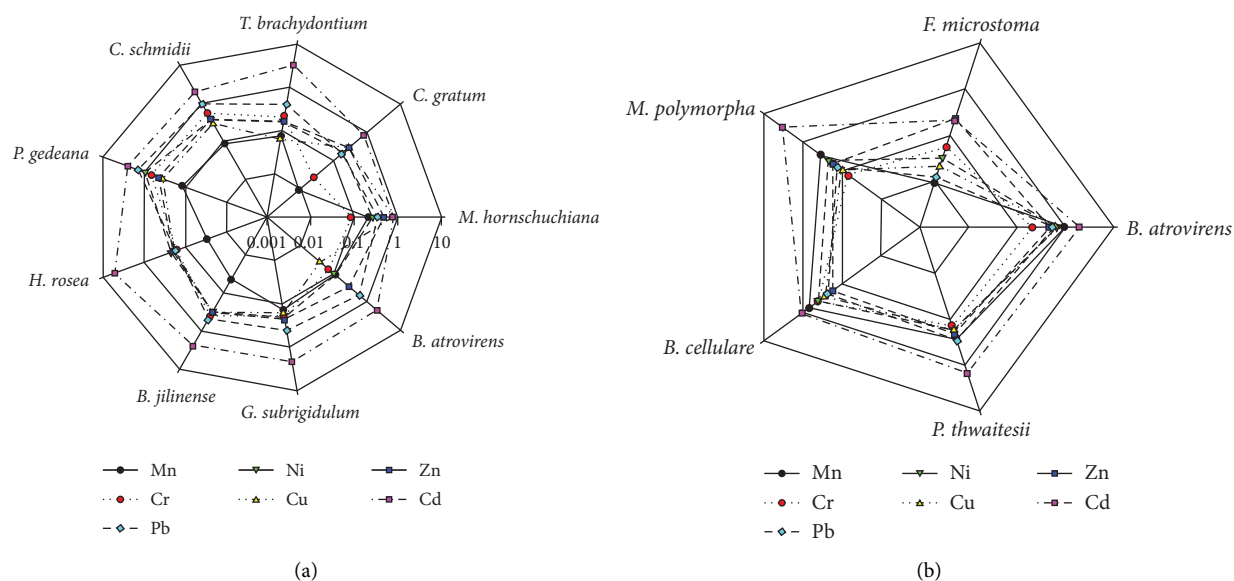


FIGURE 2: Bioaccumulation factors (BCF) of mosses for heavy metals. (a) The BCF of moss samples collected from mine waste slag areas. (b) The BCF of moss samples collected from electrolytic manganese slag areas.

TABLE 6: The enrichment coefficient of heavy metals in Bryophytes from mine waste areas.

Samples	Mn	Cr	Ni	Cu	Zn	Cd	Pb
<i>M. hornsuschiana</i>	0.212	0.084	0.270	0.768	0.480	0.754	0.346
<i>C. gratum</i>	0.009	0.025	0.191	0.284	0.282	0.781	0.173
<i>T. brachydontium</i>	0.074	0.207	0.165	0.065	0.162	3.281	0.402
<i>C. schmidii</i>	0.085	0.544	0.360	0.289	0.380	1.992	0.921
<i>P. gedeanana</i>	0.116	0.685	0.929	0.356	0.434	2.459	1.387
<i>H. rosea</i>	0.029	0.163	0.216	0.196	0.191	5.360	0.172
<i>B. jilinense</i>	0.044	0.403	0.327	0.326	0.316	2.462	0.493
<i>G. subrigidulum</i>	0.140	0.189	0.216	0.160	0.238	2.179	0.416
<i>B. atrovirens</i>	0.110	0.070	0.102	0.038	0.285	2.032	0.585

**TOC.** The organic matter content was 28.79 g/kg in bare soil. The TOC content was higher than bare soil except for *P. gedeanana* and *B. jilinense*; the TOC contents of *C. schmidii*, *H. rosea*, and *B. atrovirens* were significantly greater than those in bare soil ( $P < 0.05$ ). The content of TOC in *G. subrigidulum* growth substrate was the highest with the mean 54.65 g/kg, and the change of TOC was consistent with AN.

**Fe.** The content of DTPA-Fe in bare soil was 10.38  $\mu\text{g/g}$ . With the participation of mosses, the content of DTPA-Fe in the growth substrate was increased in all the other 5 bryophytes except for *B. jilinense*, and the DTPA-Fe content of *B. atrovirens* was significantly greater than that in bare soil ( $P < 0.05$ ), which was 22.84  $\mu\text{g/g}$ .

**Mn.** The content of DTPA-Mn in bare soil was 100.52  $\mu\text{g/g}$ , and the effect of mosses on the DTPA-Mn in the growth substrate did not show regularity.

**Cu.** The content of DTPA-Cu in bare soil was 0.64  $\mu\text{g/g}$ , and the content of DTPA-Cu was increased with the participation of mosses. The content in *G. subrigidulum* was significantly greater than that in bare soil ( $P < 0.05$ ), which was 1.69  $\mu\text{g/g}$ .

**Zn.** The content of DTPA-Zn in bare soil was 1.51  $\mu\text{g/g}$ , and mosses increased the content of available Zn in the growth substrate, with a range of 1.68–12.99  $\mu\text{g/g}$ . In addition, except for *P. gedeanana* and *B. atrovirens*, the content of the other four mosses growth substrate was significantly greater than that in bare soil ( $P < 0.05$ ), and the highest was *G. subrigidulum* with an average content of 7.98  $\mu\text{g/g}$ .

**3.1.4. Effects of Bryophytes on Bacterial Community Structure in Mine Waste Slag Areas.** In this study, growth substrate samples of three dominant mosses (*G. subrigidulum*, *B. atrovirens*, and *P. gedeanana*) and bare soil samples collected from mine waste slag areas were measured. After screening the sequencing data, 900,118 sequences from 12 samples were clustered into 5415 bacterial OTUs. The alpha diversity index of bacterial showed that Shannon, Chao, and Ace index all increased with the effects of mosses (Table 8). Compared with bare soil (ANS), the Shannon index of *G. subrigidulum* (AGS), *P. gedeanana* (APS), and *B. atrovirens* (ARS) increased by 6.42%, 9.27%, and 1.07%, respectively; the Chao index increased by 49.99%, 53.22%, and 29.28%, respectively. The results indicated that mosses increased the microbial community diversity of growth substrate.

TABLE 7: Effects of bryophytes on available nutrients of growth substrate in mine waste slag areas.

Samples	pH	AN ( $\mu\text{g/g}$ )	AP ( $\mu\text{g/g}$ )	AK ( $\mu\text{g/g}$ )	TOC (g/kg)	DTPA-Fe ( $\mu\text{g/g}$ )	DTPA-Mn ( $\mu\text{g/g}$ )	DTPA-Cu ( $\mu\text{g/g}$ )	DTPA-Zn ( $\mu\text{g/g}$ )
ANS	7.41 $\pm$ 0.60a	23.50 $\pm$ 1.73a	5.40 $\pm$ 3.36ab	72.61 $\pm$ 48.78a	28.79 $\pm$ 6.95a	10.38 $\pm$ 1.32ab	100.52 $\pm$ 50.99ab	0.64 $\pm$ 0.13a	1.51 $\pm$ 0.44a
<i>C. schmidii</i>	7.36 $\pm$ 0.17a	120.17 $\pm$ 45.72c	5.60 $\pm$ 2.60ab	197.53 $\pm$ 30.22b	50.93 $\pm$ 16.19b	11.70 $\pm$ 7.61ab	103.39 $\pm$ 57.98ab	0.74 $\pm$ 0.22a	7.37 $\pm$ 2.09c
<i>P. gedecana</i>	7.73 $\pm$ 0.06a	44.14 $\pm$ 10.76a	1.27 $\pm$ 0.38a	108.89 $\pm$ 52.60ab	20.46 $\pm$ 6.18a	12.10 $\pm$ 4.45ab	73.68 $\pm$ 13.73a	0.80 $\pm$ 0.21a	2.15 $\pm$ 0.5ab
<i>H. rosea</i>	7.61 $\pm$ 0.07a	115.31 $\pm$ 45.38bc	4.39 $\pm$ 0.63ab	232.05 $\pm$ 79.60b	37.51 $\pm$ 6.87ab	11.00 $\pm$ 1.01ab	110.46 $\pm$ 42.02ab	0.73 $\pm$ 0.25a	7.11 $\pm$ 0.48c
<i>B. jilimense</i>	7.60 $\pm$ 0.10a	57.51 $\pm$ 19.23ab	10.07 $\pm$ 9.55ab	120.53 $\pm$ 78.71ab	24.84 $\pm$ 4.45a	6.66 $\pm$ 0.90a	82.50 $\pm$ 22.92ab	0.42 $\pm$ 0.13a	5.89 $\pm$ 1.06b
<i>G. subrigidulum</i>	7.77 $\pm$ 0.18a	150.46 $\pm$ 25.63c	9.81 $\pm$ 2.68ab	139.06 $\pm$ 55.70ab	54.65 $\pm$ 11.26b	16.75 $\pm$ 2.02bc	52.00 $\pm$ 11.66a	1.69 $\pm$ 0.40b	7.98 $\pm$ 4.81c
<i>B. atrovirens</i>	7.52 $\pm$ 0.21a	114.15 $\pm$ 25.34bc	12.57 $\pm$ 5.80b	223.53 $\pm$ 65.45b	38.33 $\pm$ 6.82ab	22.84 $\pm$ 8.94c	156.83 $\pm$ 27.01b	0.73 $\pm$ 0.19a	1.97 $\pm$ 0.27ab

Different lowercase letters in the same column indicate significant differences ( $P < 0.05$ ).



TABLE 8: Effects of bryophytes on bacterial community diversity index in mine waste slag areas.

Samples	Shannon	Chao	Ace	Simpson	Coverage (%)
ANS	5.61 ± 0.40a	2159.44 ± 827.14a	2135.22 ± 832.90a	0.019 ± 0.00a	99.34 ± 0.32a
AGS	5.97 ± 0.51a	3238.43 ± 611.74a	3194.18 ± 615.37a	0.016 ± 0.01a	98.45 ± 0.50ab
APS	6.13 ± 0.44a	3308.68 ± 415.43a	3287.30 ± 471.35a	0.009 ± 0.01a	98.56 ± 0.48ab
ARS	5.67 ± 0.29a	2791.66 ± 259.02a	2754.15 ± 264.97a	0.014 ± 0.01a	98.52 ± 0.24b

Different lowercase letters in the same column indicate significant differences ( $P < 0.05$ ).

However, the overall difference did not reach a significant level, and the difference between different mosses species was not significant ( $P > 0.05$ ).

The Venn diagram demonstrated that OTUs varied among different mosses species (Figure 3(a)). The OTUs of bare soil (ANS) were 3284, and the OTUs of *G. subrigidulum*, *P. gedeanana*, and *B. atrovirens* were 4137, 3942, and 3158, respectively. All the mosses increased the OTUs except for *B. atrovirens*. The number of unique OTUs ranged from 219 (ARS) to 387 (AGS). A total of 1673 OTUs appeared simultaneously in each sample, which accounted for 30.89% of the total OTUs. The taxonomic results showed that Proteobacteria and Acidobacteria were the richest in all samples at the phylum level (Figure 4(a)), with the ratios of 34.79–49.30% and 10.02–25.45%, respectively. In addition, Bacteroidetes, Actinobacteria, Gemmatimonadetes, Chloroflexi, and Planctomycetes were the richest phyla in all samples (>1.5% was identified as a dominant phylum). Compared with bare soil, mosses increased the relative abundance of Acidobacteria; AGS and ARS reached a significant level ( $P < 0.05$ ). Meanwhile, all the mosses reduced the relative abundance of Proteobacteria, and the difference between moss species was not significant ( $P > 0.05$ ). At the class level (Figure 4(b)), the main dominant classes were Alphaproteobacteria, Gammaproteobacteria, Acidobacteria\_Gp4, Betaproteobacteria, Actinobacteria, and Sphingobacteriia, with the proportions of 18.44%–24.27%, 7.17%–14.68%, 4.10%–17.19%, 5.26%–9.76%, 4.76%–7.38%, and 4.36%–6.83%, respectively. Compared with bare soil, mosses reduced the relative abundance of Gammaproteobacteria, and AGS reached a significant level ( $P < 0.05$ ). At the same time, mosses increased the abundance of Acidobacteria\_Gp4, and ARS reached a significant level ( $P < 0.05$ ). The above taxonomic analysis showed that mosses significantly influenced the microbial community structure, but there was no significant difference among different moss species.

In addition, LEfSe analysis revealed that AGS had no sensitive biomarkers comparing to other samples. The phylum Acidobacteria was significantly enriched in ARS and APS. Two orders, Acidimicrobiales and Rhodobacterales, were sensitive to ANS (Figure 5(a)). The sensitive biomarkers of bacteria were significantly different between bare soil and mosses, but there was no difference in different species of mosses.

Different groups are represented by different colors, the different color nodes in the branches represent the microorganism groups that play an important role in the corresponding color grouping. The yellow nodes denote that microorganism groups do not play an important role. The

species names represented by English letters are shown in the legend on the right.

**3.1.5. Effects of Soil Factors on Bacterial Community Composition in Mine Waste Areas.** The relationship between bacterial community structure and soil factors was analyzed by CCA or RDA. According to the detrended correspondence analysis (DCA), if the length of the first axis was more than 3.5, we should select CCA, but if it was less than 3.5, RDA should be selected [37].

In the analysis of samples from mine waste slag areas, with the DCA results (axis lengths = 2.107) less than 3.5, RDA was carried out on the bacterial community (Figure 6(a)). The RDA analysis showed that axis 1 and axis 2 explained the variance for 30.81% and 16.08%, respectively. The bacterial community structure in different mosses (AGS, APS, and ARS) and bare soil (ANS) had obvious differences in response to soil factors. Bacteria community structure of bare soil was greatly affected by DTPA-Zn and clustered in the negative direction of axis 1 alone; however, all the mosses clustered in the positive direction of axis 1 and positively correlated with most soil factors. AN ( $P = 0.04$ ,  $R^2 = 0.517$ ) was the main soil factor affecting the composition of the bacterial community, and other soil factors did not reach the significant levels ( $P > 0.05$ ). RDA analysis showed that, with the participation of mosses, the bacteria community structures varied with the changing of available nutrients of the growth substrate.

### 3.2. The Pioneering Role of Bryophytes in Electrolytic Manganese Slag Areas

**3.2.1. The Dominant Species of Bryophytes in Electrolytic Manganese Slag Areas.** There were 6 species of mosses in electrolytic manganese slag areas, and all of them belonged to 5 families and 5 genera (Table 9). The family of Pottiaceae was the dominant family, which contained 2 bryophytes species. There were 3 life types, including the dwarf type (less than 1.5 cm), high cluster type (more than 1.5 cm), and tiled type. The dwarf type was 66.67%, the high cluster type was 16.67%, the tiled type was 16.67%. All of the species in dominant families were of dwarf type. The results were the same as those in mine waste areas. According to the frequency and coverage, we identified *B. atrovirens* as the dominant species in electrolytic manganese slag areas.

**3.2.2. The Heavy Metal Accumulation of Bryophytes in Electrolytic Manganese Slag Areas.** The total amount of heavy metals in 5 bryophytes (except for

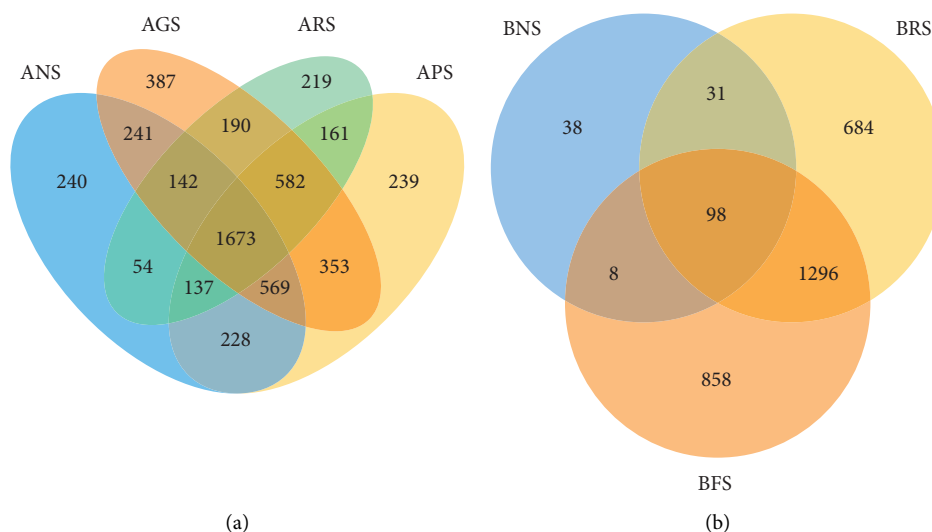


FIGURE 3: Venn diagram of exclusive and shared bacterial in growth substrate of moss samples (at the OTUs level). (a) Venn diagram analyzed composition similarities and differences of bacteria in growth substrate of mosses (AGS, ARS, and APS) and bare soil (ANS) samples collected from mine waste slag areas. (b) Venn diagram analyzed composition similarities and differences of bacteria in growth substrate of mosses (BRS, BFS) and bare soil (BNS) samples collected from electrolytic manganese slag areas.

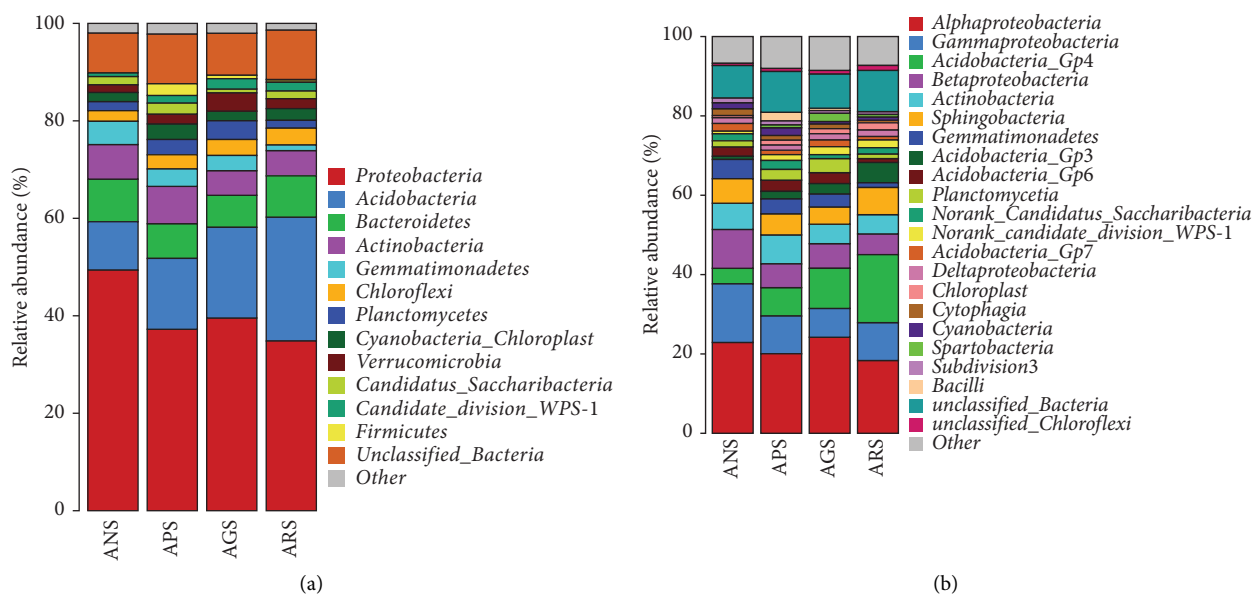
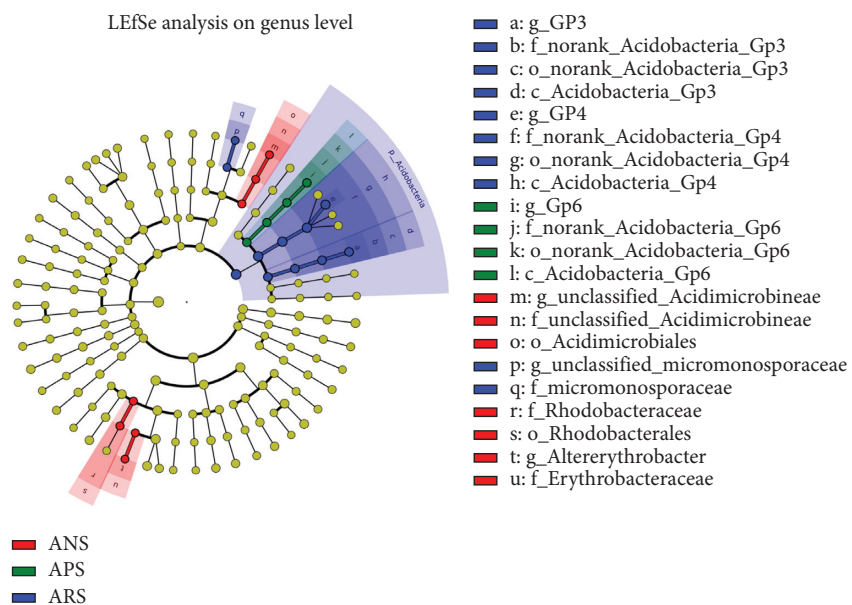


FIGURE 4: Relative abundance of the taxonomic composition of the bacteria communities at different levels of classification in mine waste slag areas. (a) At the phylum level. (b) At the class level.

*C. lonchophyllum*) and their growth substrate collected from electrolytic manganese slag areas were measured (Table 10), and the radar map was made according to the BCF of the 5 bryophytes (Figure 2(b)). All mosses had a strong enrichment effect on Cd except for *F. microstoma*. *M. polymorpha* had the strong ability with an enrichment coefficient of 3.23 (Table 11). The species *B. atrovirens* had a strong enrichment ability for all the 7 heavy metals, with the highest enrichment ability for Mn, and the coefficient was 0.953.

3.2.3. *Effects of Bryophytes on the Available Nutrients of Growth Substrate in Electrolytic Manganese Slag Areas.* To explore the effects of mosses on growth substrate, the growth substrate available nutrients of 2 mosses (*B. atrovirens* and *F. microstoma*) collected from electrolytic manganese slag areas were analyzed, including pH, AN, AP, AK, and TOC. The availability of Fe, Mn, Cu, and Zn extracted by DTPA was analyzed. The control sample was BNS. All the parameters were measured in triplicate; the results were reported as mean  $\pm$  standard deviation (SD) (Table 12).



(a)

FIGURE 5: Continued.

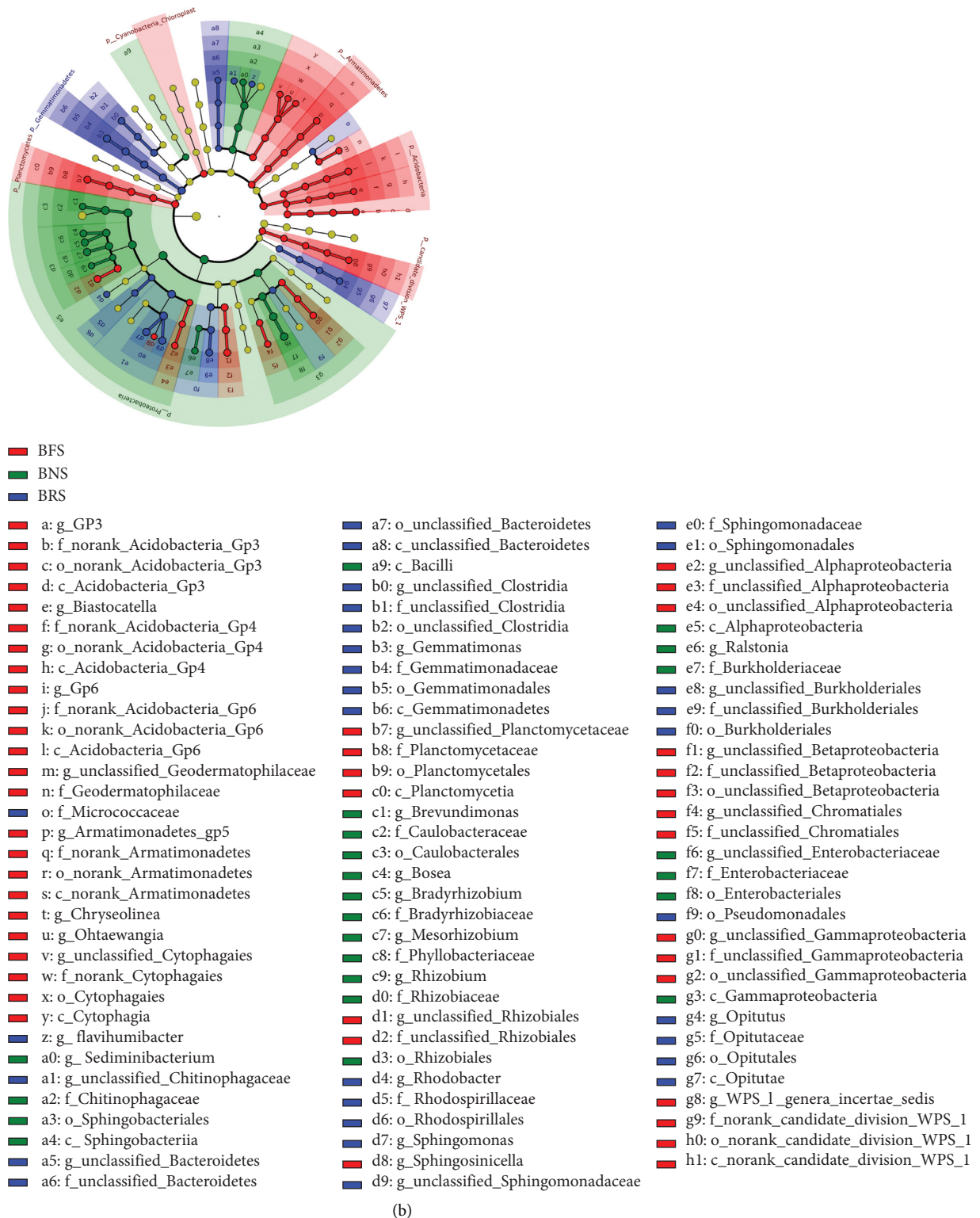


FIGURE 5: LEfSe results revealed bacteria biomarkers that were sensitive to growth substrate of mosses or bare soil at the genus level in two types of manganese waste residue areas. (a) The LEfSe of bacteria in mine waste slag areas. (b) The LEfSe of bacteria in electrolytic manganese slag areas.

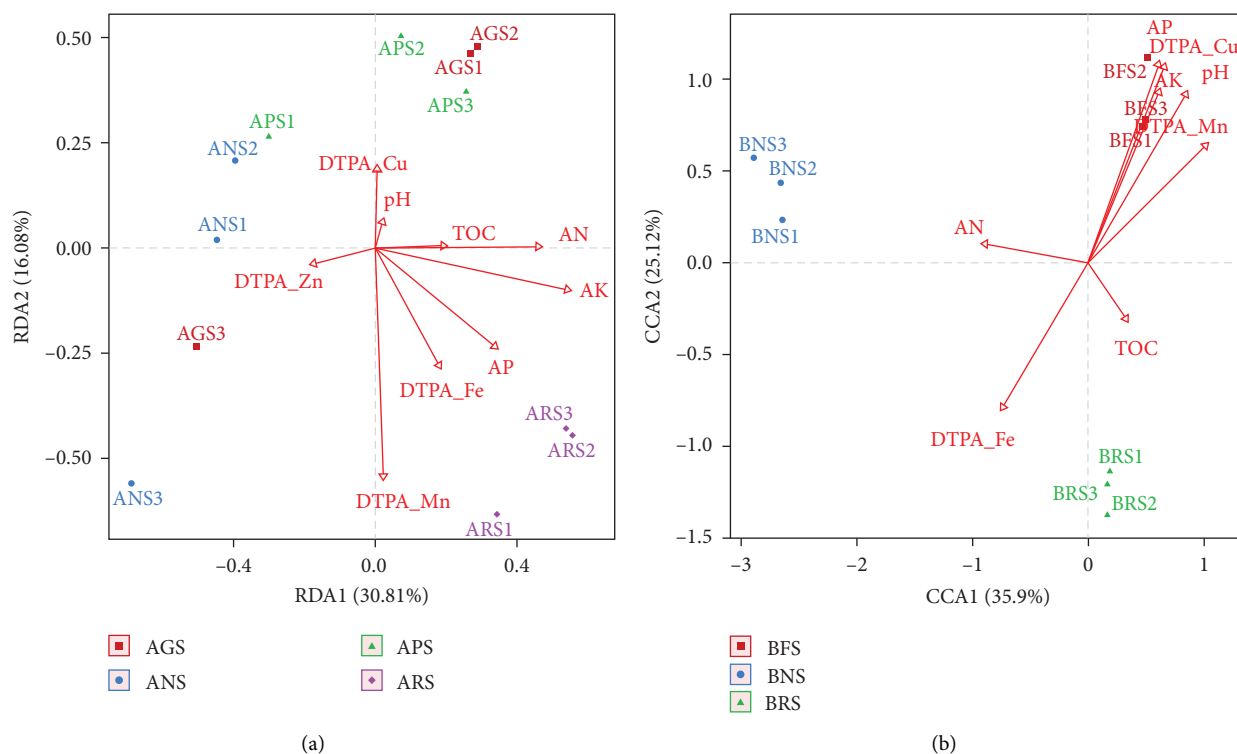


FIGURE 6: Redundancy analysis to show relationships among soil factors and moss species and bacterial community structures. (a) The DCA analysis of samples collected from mine waste slag areas. (b) The CCA analysis of samples collected from electrolytic manganese slag areas.

TABLE 9: Statistics of bryophytes, genera, and species in electrolytic manganese slag areas.

Family	Genus	Species	Life style
Bryaceae	<i>Bryum</i>	<i>Bryum atrovirens</i> <i>Bryum cellulare</i>	Dwarf cluster Dwarf cluster
Funariaceae	<i>Funaria</i>	<i>Funaria microstoma</i>	Dwarf cluster
Marchantiaceae	<i>Marchantia</i>	<i>Marchantia polymorpha</i>	Tiled
Calymperaceae	<i>Calymperes</i>	<i>Calymperes lonchophyllum</i>	Dwarf cluster
Bartramiaceae	<i>Philonotis</i>	<i>Philonotis thwaitesii</i>	High cluster
5 families	5 genera	6 species	

TABLE 10: Content of heavy metals in bryophytes and growth substrate from electrolytic manganese slag areas.

Samples	Mn ( $\mu\text{g/g}$ )	Cr ( $\mu\text{g/g}$ )	Ni ( $\mu\text{g/g}$ )	Cu ( $\mu\text{g/g}$ )	Zn ( $\mu\text{g/g}$ )	Cd ( $\mu\text{g/g}$ )	Pb ( $\mu\text{g/g}$ )	
<i>B. atrovirens</i>	In-plant	4283.41	21.26	24.93	40.84	124.60	3.10	31.77
	Substrate	5910.74	103.54	38.42	65.69	260.76	1.56	57.24
<i>F. microstoma</i>	In-plant	1124.36	32.58	7.96	17.96	42.67	0.46	9.00
	Substrate	120290.76	596.79	245.32	846.06	193.54	2.22	737.02
<i>M. polymorpha</i>	In-plant	1892.03	6.99	7.60	6.15	44.24	4.78	7.06
	Substrate	5456.27	98.33	35.67	63.02	268.32	1.48	54.32
<i>B. cellulare</i>	In-plant	4083.22	42.23	15.42	18.33	45.35	1.68	13.25
	Substrate	5910.74	103.54	38.42	65.69	260.76	1.56	57.24
<i>P. thwaitesii</i>	In-plant	1643.61	14.21	6.65	11.43	55.01	2.34	16.47
	Substrate	5910.74	103.54	38.42	65.69	260.76	1.56	57.24

*pH*. The mean pH of bare soil (BNS) was 5.07, and mosses increased the pH of the growth substrate. The pH of *B. atrovirens* and *F. microstoma* was 6.09 and 7.66, respectively. Mosses can increase the pH significantly ( $P < 0.05$ ).

*AN*. The mean content of AN in bare soil was  $519.98 \mu\text{g/g}$ , and the content of AN in mosses growth substrate was lower than bare soil with the content  $284.31 \mu\text{g/g}$  and  $278.96 \mu\text{g/g}$ . All the mosses significantly reduced the content of AN in electrolytic manganese slag areas ( $P < 0.05$ ).

TABLE 11: The enrichment coefficient of heavy metals in bryophytes from electrolytic manganese slag areas.

Samples	Mn	Cr	Ni	Cu	Zn	Cd	Pb
<i>B. atrovirens</i>	0.953	0.205	0.649	0.622	0.478	1.986	0.555
<i>F. microstoma</i>	0.009	0.055	0.032	0.021	0.221	0.207	0.012
<i>M. polymorpha</i>	0.347	0.071	0.213	0.098	0.165	3.230	0.130
<i>B. cellulare</i>	0.691	0.408	0.401	0.279	0.174	1.077	0.231
<i>P. thwaitesii</i>	0.278	0.137	0.173	0.174	0.211	1.500	0.288

AP. The content of AP in the growth substrate was 43.09  $\mu\text{g/g}$  in the bare soil. Different moss species had different effects on the content of AP in the growth substrate. The content of AP in the growth substrate of *B. atrovirens* was lower than that in the bare soil, while the content of AP in the *F. microstoma* growth substrate was higher than that in the bare soil. *F. microstoma* significantly increased the content of AP ( $P < 0.05$ ).

AK. The content of AK in the control bare soil was 37.82  $\mu\text{g/g}$ . The content of AK in mosses growth substrate was greater than that in the bare soil with 64.65  $\mu\text{g/g}$  and 426.36  $\mu\text{g/g}$ . *F. microstoma* increased the content of AK significantly ( $P < 0.05$ ).

TOC. The organic matter content was 36.27 g/kg in bare soil. With the participation of mosses, the TOC content of growth substrate was higher than bare soil. However, it was not a significant increase ( $P > 0.05$ ).

Fe. The content of DTPA-Fe in bare soil was 144.18  $\mu\text{g/g}$ . With the participation of mosses, the iron content in the growth substrate was reduced.

Mn. The content of DTPA-Mn in bare soil was 38.44  $\mu\text{g/g}$ , and mosses significantly increased the content of Mn in growth substrate with 176.63 and 299.68  $\mu\text{g/g}$  ( $P < 0.05$ ).

Cu. The content of DTPA-Cu in bare soil was 1.32  $\mu\text{g/g}$ , and the content of DTPA-Cu was increased significantly with the participation of mosses.

Zn. The content of DTPA-Zn in bare soil was 0.98  $\mu\text{g/g}$ , and mosses all increased the content of available zinc with 5.42  $\mu\text{g/g}$  and 2.44  $\mu\text{g/g}$ ; the content of *B. atrovirens* growth substrate was significantly greater than that in bare soil ( $P < 0.05$ ).

**3.2.4. Effects of Bryophytes on Bacterial Community Structure in Electrolytic Manganese Slag Areas.** To investigate the effects of mosses on bacterial community structure in electrolytic manganese slag areas, two mosses (*B. atrovirens* and *F. microstoma*) and bare soil samples collected from electrolytic manganese slag areas were measured. After screening the sequencing data, 731,286 sequences from 9 samples were clustered into 3013 bacterial OTUs. The alpha diversity index of bacterial showed that Shannon, Chao, and Ace index all increased with the effect of mosses (Table 13). Compared with bare soil (BNS), the Shannon index of *B. atrovirens* (BRS) and *F. microstoma* (BFS) increased by 73.28% and 112.21%, respectively, and the Chao index all increased 22 times. The results indicated that mosses significantly increased the microbial community diversity of growth substrate ( $P < 0.05$ ).

The Venn diagram demonstrated that mosses increased OTUs (Figure 3(b)). The OTUs of bare soil (BNS) were 175, and the OTUs of *B. atrovirens* (BRS) and *F. microstoma* (BFS) were 2109 and 2260, respectively. The number of unique OTUs ranged from 38 (BNS) to 858 (BFS). A total of 98 OTUs appeared simultaneously in each sample, which accounted for 3.25% of the total OTUs. The taxonomic results showed that Proteobacteria was the richest in all samples at the phylum level (Figure 7(a)), with the ratio of 41.49–83.24%. Mosses significantly increased the relative abundance of Actinobacteria ( $P < 0.05$ ). Meanwhile, the mosses significantly reduced the relative abundance of Proteobacteria and Bacteroidetes. At the class level (Figure 7(b)), the main dominant classes were Alphaproteobacteria, Gammaproteobacteria, and Betaproteobacteria, with the proportions of 20.09%–59.00%, 6.98%–20.96%, and 2.44%–9.02%, respectively. Compared with bare soil, mosses significantly reduced the relative abundance of Gammaproteobacteria and Alphaproteobacteria ( $P < 0.05$ ). At the same time, mosses increased the abundance of Betaproteobacteria. The above taxonomic analysis showed that mosses significantly influenced the microbial community structure.

In addition, LEfSe analysis revealed that the phylum Proteobacteria was significantly enriched in BNS. Gemmatimonadetes were significantly enriched in BRS, and the 5 phyla, namely, Acidobacteria, Armatimonadetes, candidate.division.WPS.1, Cyanobacteria, Chloroplast, and Planctomycetes, were significantly enriched in BFS (Figure 5(b)). The sensitive biomarkers of bacteria were significantly different between bare soil and mosses in electrolytic manganese slag areas.

**3.2.5. Effects of Soil Factors on Bacterial Community Composition in Electrolytic Manganese Slag Areas.** The DCA analysis of samples from electrolytic manganese slag areas was also performed. As the DCA results (axis lengths = 4.580) were more than 3.5, CCA was performed to analyze the bacterial community (Figure 6(b)). The CCA analysis showed that axis 1 and axis 2 explained the variance for 35.9% and 25.12%, respectively. Bacteria community structures of bare soil (BNS) were greatly affected by AN and clustered in the negative direction of axis 1 alone. However, the mosses clustered in the positive direction of axis 1 and positively correlated with most soil factors. pH ( $P = 0.001$ ,  $R^2 = 0.984$ ) was the main soil factors affecting the composition of the bacterial community. The bacterial community in different types of waste residues areas had different responses to soil factors. In the mine waste slag areas, the main

TABLE 12: Effects of mosses on available nutrients of growth substrate in electrolytic manganese slag areas.

Samples	pH	AN ( $\mu\text{g/g}$ )	AP ( $\mu\text{g/g}$ )	AK ( $\mu\text{g/g}$ )	TOC (g/kg)	DTPA-Fe ( $\mu\text{g/g}$ )	DTPA-Mn ( $\mu\text{g/g}$ )	DTPA-Cu ( $\mu\text{g/g}$ )	DTPA-Zn ( $\mu\text{g/g}$ )
BNS	5.07 $\pm$ 0.28a	519.98 $\pm$ 95.66a	43.09 $\pm$ 3.42a	37.82 $\pm$ 25.72a	36.27 $\pm$ 4.87a	144.18 $\pm$ 21.25a	38.44 $\pm$ 10.99a	1.32 $\pm$ 0.39a	0.98 $\pm$ 0.42a
<i>B. atrovirens</i>	6.09 $\pm$ 0.33b	284.31 $\pm$ 110.43b	30.64 $\pm$ 7.92a	64.65 $\pm$ 33.12a	49.72 $\pm$ 2.56a	105.38 $\pm$ 51.42a	176.63 $\pm$ 48.14b	3.34 $\pm$ 1.01b	5.42 $\pm$ 1.94b
<i>F. microstoma</i>	7.66 $\pm$ 0.08c	278.96 $\pm$ 60.38b	2024.05 $\pm$ 244.01b	426.36 $\pm$ 135.74b	43.99 $\pm$ 15.19a	17.61 $\pm$ 3.23b	299.68 $\pm$ 14.43c	36.2 $\pm$ 1.3c	2.44 $\pm$ 0.5a

Different lowercase letters in the same column indicate significant differences ( $P < 0.05$ ).

TABLE 13: Effects of bryophytes on bacterial community diversity index in electrolytic manganese slag areas.

Samples	Shannon	Chao	Ace	Simpson	Coverage (%)
BNS	2.62 ± 0.16a	91.00 ± 19.58a	61.80 ± 57.12a	0.15 ± 0.01a	1 ± 0a
BRS	4.54 ± 0.20b	2107.55 ± 123.85b	2382.51 ± 119.11b	0.04 ± 0.02b	0.99 ± 0b
BFS	5.56 ± 0.11c	2077.56 ± 668.55b	2091.62 ± 735.43b	0.01 ± 0c	0.99 ± 0b

Different lowercase letters in the same column indicate significant differences ( $P < 0.05$ ).

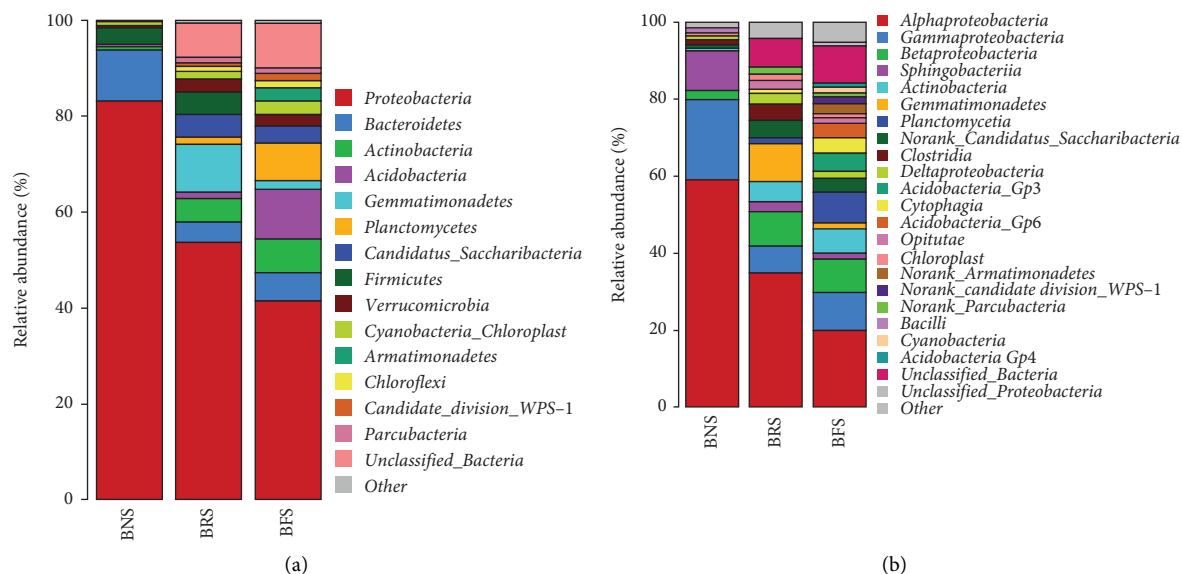


FIGURE 7: Relative abundance of the taxonomic composition of the bacteria communities at different levels of classification in electrolytic manganese slag areas. (a) At the Phylum level. (b) At the class level.

environmental factor affecting the structures of the microbial community was AN, while in the electrolytic manganese slag areas, the main soil factor was pH.

#### 4. Discussion

As the earliest plants on land, bryophytes are comprised of three large phyla: mosses, liverworts, and hornworts [38, 39]. For a long time, bryophytes have been regarded as the pioneers of vegetation restoration with the function of water retention and pedogenesis [30, 40]. As the important component of biological crusts, bryophytes have been widely studied in desert ecological restoration and karstification [40–42]. It plays an essential role in soil nutrient cycling, and it is effective in increasing soil nutrients and is beneficial for the retention of soil nutrients [43, 44]. On the one hand, after the decomposition of bryophytes, the dead parts can increase the thickness of the soil layer and release nutrients (N, P, K, C) into the soil at the same time. On the other hand, bryophytes' physiological and metabolic activities enhance the biological dissolution of rocks [10, 42, 45]. Our survey showed that mosses had different effects on the available nutrients of the growth substrate in the two areas. In mine waste areas, mosses increased pH, AN, AP, AK, and TOC. *G. subrigidulum* was the most effective for increasing AN and TOC. It was deficient in soil nutrients and nutrient elements in mine waste areas, and the larger biomass and

higher coverage of *G. subrigidulum* resulted in an effective increase in organic matter content. However, in electrolytic manganese residue areas, it contained about 1% ammonium nitrogen and had the potential to be used as plant growth substrate or fertilizer [46, 47], the growth of mosses made use of AN in the matrix and significantly reduced the content of AN. But mosses raised the pH and TOC that were the same as mine waste areas.

Mosses not only improved the available nutrients of the growth substrate but also changed the microbial community diversity in the matrix. The previous reports showed that the bacteria that inhabit in/on bryophytes were related to the species of bryophytes and ecosystem. Our results showed that soil factors can affect microbial community structure significantly. Overall, mosses effectively enriched the diversity of bacterial community structure in the growth substrate (Figures 4 and 7). The relative abundance of Acidobacteria was improved significantly at the phylum level. As the important dominant bacterium group of soil, Acidobacteria has the function of breaking down the remains of plants and animals in the soil. The relative abundance of Acidobacteria in wasteland soil was lower than that in the garden and cultivated land [48–51]. Mosses provide nutrient substance for the colonization of a large number of Acidobacteria in manganese waste residue areas. Proteobacteria includes four classes:  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ , and the ratio of the phylum was differentially varied across the



different hosts [27, 30, 52–54]. The proportion of each class varied according to the species of moss, and the relative abundance of  $\alpha$  and  $\gamma$  classes was greater. With the involvement of mosses, the proportions of Proteobacteria were limited. The RDA/CCA analysis showed that AN was the main soil factor that affected the structure of the bacterial community in mine waste areas. However, it was pH that affected electrolytic manganese residue areas. Due to the characteristics of the two types of waste slag, electrolytic manganese residue has lower pH and the mine waste areas have a lower content of AN. With the participation of bryophytes, these two indexes are greatly improved. Mosses effectively changed the available nutrients of the growth substrate, thus affecting the diversity of microbial community structure.

Bryophytes are an effective accumulator of elements. This particular physiological characteristic is due to its special morphological characteristics, such as relatively simple morphology, one-cell-thick (lack of cuticle), and no vascular tissue. Bryophytes have a strong accumulation effect on Cd and it can be used as an indicator of Cd pollution [14, 17, 21]. In our study, all the mosses in two types of manganese waste residue areas had a strong accumulation effect on Cd, which is similar to previous results [55]. The accumulation of heavy metals in bryophytes is related to stormwater runoff and growth matrix [56]. Our study showed that the content of Cd in manganese waste residue areas seriously exceeds the soil background value. Previous studies proved that the content of Cd in rice rhizosphere soil increased with the increase of water content, but Zn, Cu, and As showed no such trend [57]. Higher Cd content of growth substrate and strong water retention of mosses may make it have strong enrichment for Cd. Compared with other heavy metal elements, mosses had a strong ability to accumulate manganese. It indicated that mosses had strong tolerance to Mn. But the BCF of Mn was lower, which was mainly caused by the high content of Mn in the growth substrate in manganese waste residue areas.

Previous studies have been conducted on bryophytes biodiversity in mining areas, including gold, copper, and mercury in Guizhou province. Pottiaceae and Bryaceae were the dominant families [15, 58–60]. Water and lower soil pH can cause the decline of bryophytes diversity, and the acidified soil matrix is one of the main reasons for the loss of bryophytes diversity [9, 61]. In our survey, bryophytes' biodiversity varied greatly in two types of manganese waste residue areas. The bryophytes species in the electrolytic manganese residue areas were poorer than those in mine waste areas, and the moss species similarity coefficient was lower between the two areas. The electrolytic manganese process means that manganese ore is extracted by acid to obtain manganese salt and then send it to an electrolytic cell for electrolytic precipitation of single manganese metal. It can produce a large amount of waste residue, including acid leaching residue, sulfurized residue, and chromium containing waste residue [62]. So, the electrolytic manganese residue areas had a lower pH than mine slag areas, which significantly affected the bryophytes distribution. However, the living types of bryophytes in the two areas were similar,

and both were dominated by the dwarf type. This physiological feature was conducive to the adaptation of bryophytes to the harsh environment, such as drought and strong light in the mining areas [14].

## 5. Conclusion

The systematic study of the pioneering role of mosses in ecological restoration in two types of manganese waste residue areas was carried out in Guizhou province, China. The bryophytes species are different in two types of manganese waste residue areas. Bryophytes have a strong enrichment of Cd while they have a strong accumulation of Mn. Mosses can increase the available nutrients and the bacterial community diversity of the growth substrate in the two areas. AN was the main soil factor that affected the microbial community structure in the mine waste residue areas, while it was pH in the electrolytic manganese residue areas. Bryophytes provide conditions for the colonization of other vascular plants by improving the available nutrients and microbial community structure of the growth substrate in manganese waste residue areas. Different mosses have different effects on available nutrients and microbial diversity, which need further study. The study provides a theoretical basis for bryophytes to become a typical stress-resistant plant and a pioneer of ecosystem reconstruction in the mining area.

## Data Availability

The numerical data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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