



Various Phyllosphere and Soil Bacterial Communities of Natural Grasses and the Impact Factors in a Copper Tailings Dam

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Abstract

Copper mining caused severe damage to the ecological environment of mining areas. The combination of microbe and plant remediation has an application potential in improving the absorption and transformation efficiency of heavy metals. The phyllosphere is the largest biointerface on the planet, and bacteria are the dominant microbial inhabitants of the phyllosphere, believed to be critical to plant growth and health. This study investigated the phyllospheric and soil bacteria communities using high-throughput sequencing, and endophyte infection statuses of four natural grasses by toluidine blue heparin assay. Results showed variation in phyllospheric bacterial community structure. Gammaproteobacteria were the most abundant bacterial population. Bacilli were found in the phyllosphere of *Bothriochloa ischaemum* and *Imperata cylindrica*, while *Clostridia* were only found in *Calamagrostis epigejos*. Alphaproteobacteria were the dominant bacteria in soil. In addition, bacterial communities were influenced by endophytic infection statuses. Oxalobacteraceae was associated with soil carbon and sulfur. Enterobacteriaceae had negative correlation with the ratio of soil carbon and nitrogen, and had positive correlation with Cd content. These results offer useful insights into phyllospheric bacterial community variance in four different natural grasses in a copper tailings dam.

Introduction

The underground copper mines in the Zhongtiao Mountains are the largest in China. They have consistently been increasing metal ore mining as well as gradually accelerating the speed of lift of their tailings dams. Such activities have led to large heavy metal inputs into the soil. This not only causes soil ecosystem degradation but also affects plant growth and development. Research on ecological phytoremediation and its practices in mining areas has resulted in increased interest in the restoration of heavy metal contaminated soils using plants and microbes [19, 40]. Both plants and soil microbes are capable of remediating heavy metals; therefore, the combination of microbe and plant remediation has a tremendous application potential in improving the absorption and transformation efficiency of heavy metals in the soil profile [6, 9]. However, most research studies have focused on microbial-mediated processes in the soil environment [17, 29] rather than the phyllosphere of natural grass species in

spite of the potential importance of phyllospheric bacterial communities. In recent years, the structure and function of phyllospheric microbial communities have attracted worldwide attention [28, 37].

Aerial components of living plants, including leaves, stems, buds, flowers, and fruits, provide a habitat for microorganisms, and this habitat is called the phyllosphere [37]. Bacteria are considered the dominant microbial inhabitants of the phyllosphere, but archaea, filamentous fungi, and yeasts could also be important inhabitants [37]. It has been shown that bacterial communities in the phyllosphere are able to promote plant growth [4, 13] and increase plant resistance to pathogen infection [3, 7]. Furthermore, studies have reported on a variety of effects associated with phyllospheric bacteria communities on plants, such as plant species [16, 20], environmental factors [27], microbial interactions [10], and the properties of the microbial colonists themselves. Previous studies have shown that phyllospheric bacteria communities are affected by climate changes whether directly or indirectly [5, 24]. In addition, the specific location of leaves on a plant will also influence bacterial colonists [23]. However, few studies have addressed the effects of endophytes on bacterial community structure of the phyllosphere, and this is in spite of its potential importance.

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Endophytic fungi are ubiquitous, residing in healthy plant tissue without causing obvious disease [1]. During the life-cycle of a plant host, the hypha of endophytic fungi will asymptotically colonize the intercellular spaces of plant tissues and organs, such as leaf sheaths and blades, and hypha density in stem meristems will be higher than in leaf sheaths and blades [33]. Numerous studies have showed that *Epichloë* endophytes affect host plant growth and fitness as well as the microbial communities in the soil [15, 42]. Accordingly, this study hypothesized that the status of endophytes could also influence phyllospheric bacterial communities in natural grass species. Soil microorganisms are the principal decomposers of plant, litter, and animal residue, and they play a major role in the native cycle of matter and the transformation of soil organic matter and inorganic matter, for example, soil microbial are the main participants in nutrient transformation, being the interface between water membrane biota and bacteria, protozoa and nematodes in soil [41].

High-throughput technology was used in this study to investigate phyllospheric bacterial communities and their influencing factors on different natural grass species as well as soil bacterial communities in a copper tailings dam. The objectives of this study were to (1) survey the composition and diversity of phyllospheric bacterial communities of four natural grass species, (2) examine the effects of endophytes on phyllospheric bacterial community structure, and (3) compare the dominant bacteria within phyllospheric bacterial communities and soil bacterial communities, as well as the impact factors in a copper tailings dam. This study could offer useful insights into phyllospheric bacterial community variance in four different natural grasses, and it was essential in understanding microbially mediated biogeochemical processes as well as the exploration on their potential usage in copper tailings dam remediation.

Materials and Methods

Site Description and Soil and Grasses Sampling

This study was conducted on the Eighteenth River tailings of the Northern Copper Mine in Yuanqu County, Shanxi Province, China. The copper tailings dam was built in 1969. Its present height is 23.0 m and its crest elevation is 509 m [36]. Each sub-dam has formed different vegetation domains following phytoremediation. In July 2016, the soil and four natural grasses including *Imperata cylindrical*, *Bothriochloa ischaemum*, *Elymus dahuricus*, and *Calamagrostis epigejos* from the same sub-dam had undergone 47 years of phytoremediation were sampling. We randomly amassed 30 grass samples collected during transect walks (100 × 10 m). To determine endophyte infection rates in

the four natural grass species, we analyzed *I. cylindrical* and *B. ischaemum*, which were determined to be endophyte free, and *E. dahuricus* (with an endophyte infection rate of 88.1%), and *C. epigejos* (with an endophyte infection rate of 47.8%). Sampled plants were spaced at a distance from 5 to 10 m. At the same time, we selected 16 individuals from each natural grass species and quickly sealed their leaves into sterile plastic bags using ethanol-sterilized tweezers. Samples were placed in an icebox and taken to the lab to be stored at −20 °C prior to high-throughput sequencing. Within this sub-dam, we also collected samples from the soil organic layer (from 0 to 5 cm depths directly below the litter layer) at three random points using a sterile blade. They were then composited together into a single sample. Visible roots and residues were removed prior to homogenizing the soil fraction of each sample. Fresh soil samples were filtered through a 2 mm sieve and divided into two subsamples. One subsample was stored at 4 °C to determine physiochemical properties, while the other was stored at −20 °C prior to DNA extraction.

Detection of Endophytes and Bacterial Community Structure of Phyllosphere and Soil

We randomly collected five tillers from each plant, and we used the outermost non-senescent leaf sheath of each tiller in this assay. A strip of epidermis was peeled from the inner surface of the leaf sheath close to the stem base. The strip was placed on a slide, mounted in aniline blue stain, and the slide was heated over a flame until the stain reached the boiling point. It was then examined for hyphae under ×400 magnification. We extracted microbial DNA from the four natural grass species samples using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA) according to the manufacturer's protocols. The V4–V5 region of the bacterial 16S ribosomal RNA (rRNA) genes was amplified using primers 515F 5'-barcode-GTGCCAGCMGCCGCGG-3' and 907R 5'-CCGTCAATTCMTTTRAGTTT-3', where the barcode was an eight-base sequence unique to each sample. For this study, we sent plant samples to the Lingen Biotechnology Co., Ltd (Shanghai, China) for high-throughput sequencing. Soil total microbial DNA was extracted using the DNA isolation kit for soil (Felix biotech, USA). The amplification of bacterial 16S rRNA was V3 fragments, and primers were 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 533R (5'-TTACCGCGGCTGCTGGCAC-3'). For this study, we sent soil samples to the Shanghai Personal Biotechnology Co., Ltd for high-throughput sequencing.

Determination of Soil Chemical Properties

We measured soil pH after shaking soil water (1:2.5 mass/volume) suspensions for 30 min. Soil moisture was measured

gravimetrically. We measured soil particle size using a Mastersizer 3000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Malvern, UK). Before obtaining the particle size measurements, each sample was weighed at 3 g, and the sediments were immersed in 10% H₂O₂, and then in 12.7% HCl to remove any plant debris and to disperse the aggregates within the sediments. The sample residue was finally treated with 10 mL of 0.05 M (NaPO₃)₆ in an ultrasonic vibrator for 10 min to facilitate the dispersion prior to the particle size analysis. Only slight differences (0.5%) were found in the repeated particles size measurements on each sample. We measured total soil carbon (C), total nitrogen (N), and total sulfur (S) content using the elemental analyzer (vario EL/MACRO cube, Elementar, Hanau, Germany). Soil ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), and Olsen P using the Automatic Discrete Analyzer (CleverChem 380, DeChem-Tech.GmbH, Germany). Heavy metal elements, which include As, Cd, Cr, Cu, Mn, Ni, Pb, and Zn, were measured after shaking in the reagent (3.0 mL HNO₃, 1.0 mL HF, and 2.0 mL H₂O₂) and left it for 30 min before microwave digestion. Samples were measured by inductively coupled plasma atomic emission spectrometry (iCAP 6000, Thermo Fisher, UK). For soil chemical properties, we conducted three repetitions in this sub-dam.

Statistical Analysis

We used Pearson product-moment correlation to determine whether there was significant correlation between tested abiotic characteristics, as well as the bacteria relative abundances and soil properties. These statistical analyses were conducted using SPSS 13.0 for Windows. For bacterial diversity in the phyllospheres of the different grass species, we conducted rarefaction analysis based on Mothur v.1.21.1 [32] to reveal diversity indices, including the Chao1, ACE, and Shannon indices.

Results and Discussion

In total, we obtained approximately 152,067 sequence reads of the 16S rRNA gene with an average length of approximately 376 bp after trimming and chimera removal in four natural grasses, and 43,437 sequence reads of the 16S rRNA gene with an average length of approximately 430 bp after trimming and chimera removal in soil. We used a 97% similarity cutoff value to delineate OTUs in our downstream analyses. Following subsampling, we acquired the total of 86 and 6118 OTUs for the bacteria in four natural grasses and the soil, respectively (Table 1). Previous studies had clearly shown that the structure of bacterial communities on leaves is similar for individuals of the same species, but this varies significantly between species [39]. This was in accordance with our results, which showed that the four natural grass species and soil characteristic in the copper tailings dam exhibited different phyllospheric and soil bacterial community structures. (Table 1).

We found that Gammaproteobacteria was the most abundantly represented bacterial population in the phyllospheres of the four natural grass species, with relative abundances from 45.5 to 52.4% (Fig. 1). Previous studies showed that Gammaproteobacteria has been found on other plant components, such as seeds [8, 38] and roots [18, 22]. Gammaproteobacteria dominance in these four natural grass species was also consistent with results from a previous study, which found Gammaproteobacteria dominance in the bacterial communities of leaf blades [12]. Gammaproteobacteria that inhabit phyllospheres can promote plant growth while both suppressing and stimulating the colonization and infection of tissues by plant pathogens [26].

We estimated ACE and Chao1 species richness indices at 17.36 and 17.00, respectively, in the phyllosphere of *I. cylindrica*. We found that *E. dahuricus* had the highest bacterial species richness of all phyllospheres investigated in this study (Table 1). However, diversity indices, such as the Shannon and Simpson indices, showed different trends

Table 1 Comparison between phylotype coverage and diversity estimators of phyllospheric bacterial communities of the four different natural grasses

Type	Sample	Reads ^a	OTUs ^b	Richness estimator		Diversity index	
				ACE	Chao1	Shannon	Simpson
Plant	<i>I. cylindrica</i>	42,650	17	17.36	17.00	0.56	0.71
	<i>B. ischaemum</i>	32,137	21	22.67	24.00	0.80	0.49
	<i>E. dahuricus</i>	34,457	26	26.51	26.00	0.61	0.68
	<i>C. epigejos</i>	42,823	22	23.24	22.25	0.53	0.73
Soil	Sub-dam	43,437	6118	18.05	17.66	9.99	0.99

^aTable 1 provides reads after trimming and chimera removal. The coverage percentage, richness estimators (ACE and Chao1), and diversity indices (Shannon and Simpson) were calculated using the Good's method and the Mothur program, respectively

^bOperational taxonomic units (OTUs) were defined at a 97% level of similarity

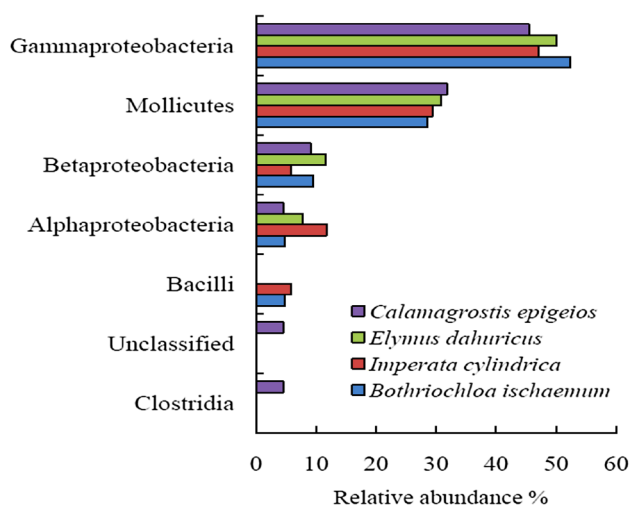


Fig. 1 Class rank of the relative abundance of phyllospheric bacterial taxa for the four different natural grass species

between the four natural grass species. Indicators of alpha diversity demonstrated that the bacterial community in the phyllosphere of *B. ischaemum* was higher compared to the other grass species, according to the Shannon index, and that the bacterial community in the phyllosphere of *C. epigeios* was higher compared to the other grass species, according to the Simpson index (Table 1). This could be explained by divergences in plant genotypes [37], environmental heterogeneity, plant species, and microbial interactions [10]. Furthermore, a previous study showed that plant genotypes can play a major role in determining the structure of microbial community phyllosphere [2]. On the other hand, the Simpson indices were significantly higher in soil than four nature grasses, which shows that the bacterial community was more diversity in soil than grasses. The main reason could be that the soil has more suitable habit for microbial growing and the environment of phyllosphere is not stable extremely, which can be changed by species, climate, and the different stages of leaves.

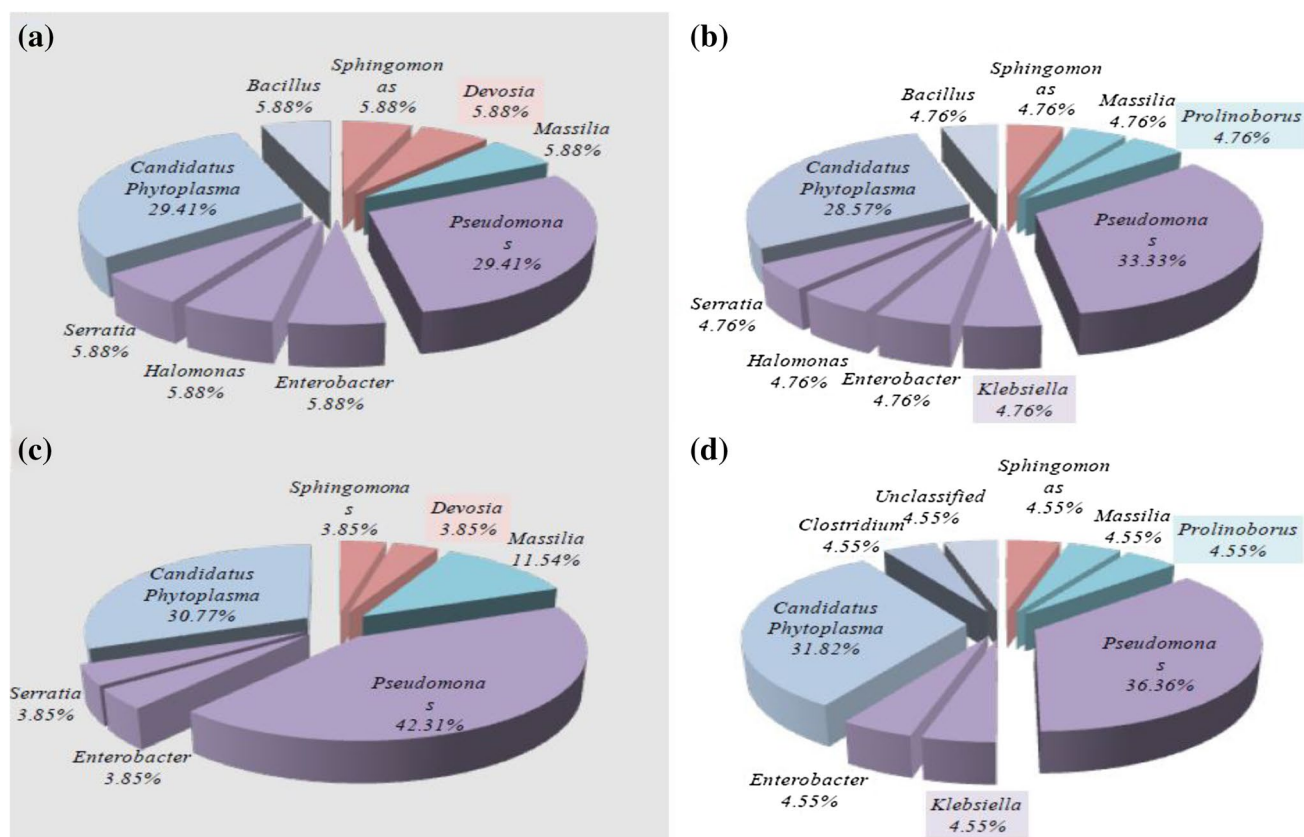


Fig. 2 Genus rank of the relative abundance of bacterial taxa in phyllospheres for the four different natural grass species. **a** *Imperata cylindrical*; **b** *Bothriochloa ischaemum*; **c** *Elymus dahuricus*; **d** *Calamagrostis epigeios*. The gray and white backgrounds represent

endophyte-infected and endophyte-free grass species, respectively. Red and green represent Alphaproteobacteria and Betaproteobacteria, respectively, and purple represents Gammaproteobacteria. (Color figure online)

In our study, Alphaproteobacteria, in the phylum of Proteobacteria, accounted for 11.8% of total detected OTUs in the phyllosphere of *I. cylindrical*, and Betaproteobacteria accounted for between 9.1% and 11.5% of total detected OTUs in the phyllosphere of the other three grass species investigated (Fig. 1). In general, Alphaproteobacteria and Gammaproteobacteria were the dominant bacterial inhabitants in the phyllospheres, and Bacteroidetes was also typically important. Under certain situations, Betaproteobacteria and Firmicutes can also form a large part of the bacterial community, with Acidobacteria, Actinobacteria, and Cyanobacteria occurring infrequently [11, 25].

It has been reported that endophytic fungi affect soil microbial community structure [35], and the impact of endophytic fungi on a community level is greater than on a population level [30]. Studies have shown that tall fescues infected by *Neotyphodium coenophialum* alter soil microbial communities [14]. Moreover, numerous studies have shown that host grass species infected with systemic endophytes have advantages over non-infected grass species [21, 31]. In our study, *Devosia*, in the phyllosphere of endophyte-infected grass species, is with a relative abundance of 5.88% in *I. cylindrical* and 3.85% in *E. dahuricus* (Fig. 2). However, we detected *Prolinoborus* and

Klebsiella in the phyllosphere of endophyte-free grass species, with little differences between relative abundances of *B. ischaemum* and *C. epigejos* (Fig. 2). These results showed that the status of endophyte infection could affect the structure of phyllospheric bacterial communities in the different natural grass species. For endophyte-infected grass species, we only detected *Halomonas* in the phyllospheric bacterial community of *I. cylindrical*, which had lower endophyte infection rates (47.8%) compared to *E. dahuricus* (88.1%) (Fig. 2). Thus, we speculated that the structure of phyllospheric bacterial communities was also influenced by endophyte infection rates.

Previous studies have shown that pH plays an important role in soil microbial communities [42]. In our experiments, the soil of the copper tailings dam was alkaline, with a soil pH of 7.9 (Table 2). This indicated a significant negative correlation between soil pH and NO₂⁻-N (Table 3). Microbes reach the phyllosphere by atmospheric deposition from plant and soil sources. The soil bacterial community itself could also play a role in shaping the structure of bacterial communities in the phyllosphere. Proteobacteria groups play important roles in supplying nutrients, such as carbon, nitrogen, sulfur, and phosphate [34]. In our study, Alphaproteobacteria was the dominant

Table 2 Soil physicochemical properties in the sub-dams with 47 years of restoration

Soil properties	Mean ±SD	Soil properties	Mean ±SD	Soil properties	Mean ±SD	Soil properties	Mean ±SD
NH ₄ ⁺ -N (mg/kg)	8.48 ± 1.05	N(g/kg)	0.69 ± 0.13	SWC (%)	0.018 ± 0.006	Cd (mg/kg)	5.27 ± 0.49
NO ₃ ⁻ -N (mg/kg)	5.12 ± 0.67	C(g/kg)	17.12 ± 0.54	pH	7.90 ± 0.07	Cu (mg/kg)	553.53 ± 106.78
NO ₂ ⁻ -N (mg/kg)	0.39 ± 0.06	C/N	27.41 ± 3.84	PS (µm)	37.34 ± 2.54	Pb (mg/kg)	267.62 ± 10.21
Olsen P (mg/kg)	7.21 ± 1.63	S (g/kg)	0.98 ± 0.29	As (mg kg ⁻¹)	9.62 ± 2.96	Zn (mg/kg)	109.79 ± 9.17

Physical and chemical properties of soil included ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), total nitrogen (N), total carbon (C), total sulfur (S), the ratio of carbon and nitrogen (C/N), soil water content (SWC), soil pH, particle size (PS)

Table 3 Pearson product-moment correlation between soil chemical properties

	NH ₄ ⁺ -N	NO ₃ ⁻ -N	NO ₂ ⁻ -N	Olsen P	N	C	C/N	S	SWC	pH
NO ₃ ⁻ -N	-0.215									
NO ₂ ⁻ -N	-0.274	0.888*								
Olsen P	0.940*	-0.232	-0.410							
N	0.006	0.918*	0.737	-0.044						
C	-0.899*	0.240	0.428	-0.877	-0.097					
C/N	-0.272	-0.85	-0.719	-0.178	-0.953*	0.272				
S	-0.889*	0.093	0.301	-0.862	-0.241	0.989**	0.411			
SWC	0.649	-0.271	-0.096	0.608	-0.37	-0.270	0.097	-0.233		
pH	-0.069	-0.666	-0.895*	0.159	-0.564	-0.191	0.675	-0.091	-0.254	
PS	-0.490	0.498	0.756	-0.736	0.435	0.514	-0.359	0.447	-0.384	-0.694

Physical and chemical properties of soil included ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), total nitrogen (N), total carbon (C), total sulfur (S), the ratio of carbon and nitrogen (C/N), soil water content (SWC), soil pH, particle size (PS), asterisk indicated significant correlation (*P < 0.05; **P < 0.01)

bacteria on a class rank, with a relative abundance of 23.1%. The relative abundances of Actinobacteria, Gammaproteobacteria, and Betaproteobacteria were 10.2%, 8.6%, and 7.8%, respectively (Fig. 3).

Soil pH was significantly associated with Sphingomonadaceae and Comamonadaceae, respectively, at family level

(Table 4). Without considering host genotype, Oxalobacteraceae was significantly associated with soil C and S. Enterobacteriaceae had negative correlation with the ratio of soil carbon and nitrogen, and had a significant positive correlation with Cd content (Table 5). These results reflect indirect effects associated with differences in phyllospheric

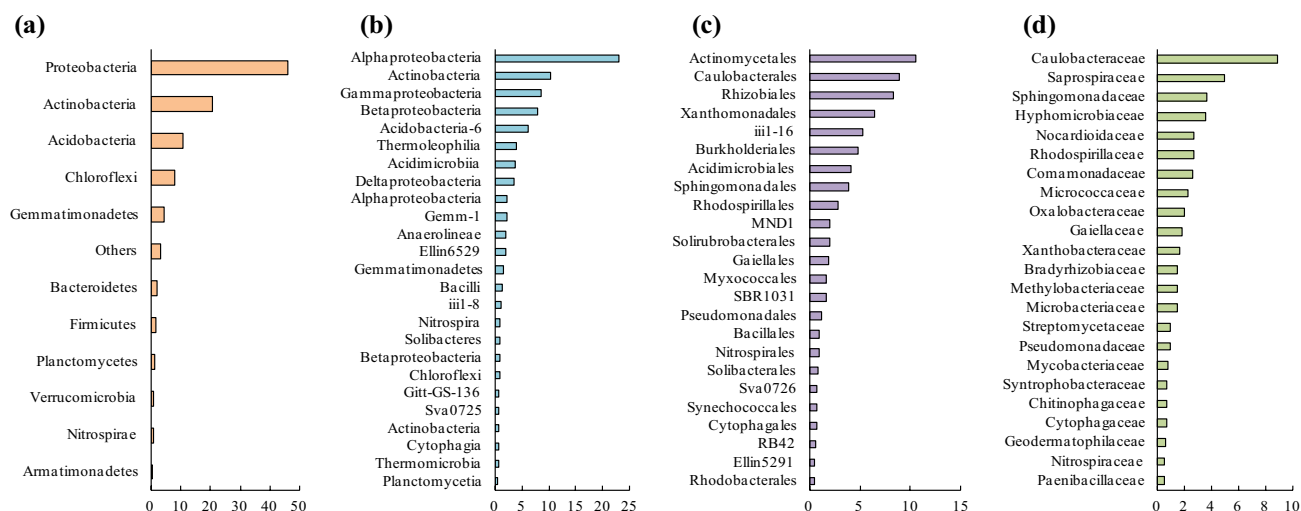


Fig. 3 Different levels of relative abundance of identified soil bacteria $\geq 0.5\%$ in the copper tailings dam. **a** Phylum; **b** class; **c** order; **d** family

Table 4 Pearson product-moment correlation between dominant soil bacterial communities on family level and soil properties

Relative	Micrococcaceae	Microbacteriaceae	Nocardioidaceae	Caulobacteraceae	Methylobacteriaceae	Sphingomonadaceae	Comamonadaceae
Abundance	(2.0–2.6%)	(1.4–1.7%)	(2.6–3.4%)	(8.4–9.3%)	(1.2–1.7%)	(3.3–3.9%)	(2.3–2.9%)
NH ₄ ⁺ -N	-0.984	0.879	0.963	0.958	0.983	0.999*	0.992
NO ₃ ⁻ -N	0.092	-0.400	-0.188	0.205	0.100	0.043	-0.039
NO ₂ ⁻ -N	-0.255	-0.062	0.161	-0.143	-0.247	0.383	0.306
Olsen P	-0.905	0.724	0.859	-0.850	-0.901	0.954	0.926
N	-0.211	-0.107	0.115	-0.098	-0.202	0.341	0.263
C	0.888	-0.699	-0.840	0.830	0.884	-0.942	-0.912
C/N	0.487	-0.187	-0.400	0.383	0.479	-0.600	-0.532
S	0.831	-0.614	-0.774	0.762	0.826	-0.898	-0.860
SWC	-0.716	0.899	0.780	-0.791	-0.722	0.616	0.678
pH	0.992	-0.901	-0.975	0.971	0.991	-0.999**	-0.997*
PS	-0.853	0.974	0.899	-0.907	-0.857	0.775	0.824
As	0.719	-0.901	-0.783	0.794	0.725	-0.619	-0.651
Cd	-0.851	0.643	0.796	-0.786	-0.847	0.914	0.878
Cu	-0.714	-0.458	-0.643	0.629	0.708	-0.802	-0.750
Pb	-0.822	0.960	0.874	-0.882	-0.827	0.738	0.791
Zn	-0.962	0.827	0.931	-0.924	-0.959	0.990	0.975

Physical and chemical properties of soil included ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), Olsen P, total nitrogen (N), total carbon (C), total sulfur (S), the ratio of carbon and nitrogen (C/N), soil water content (SWC), soil pH, particle size (PS), and heavy metal of soil As, Cd, Cu, Pb, and Zn

*Correlations were significant at a level of 0.05 (2-tailed). The relative abundance of dominant family was $\geq 1.2\%$

**Correlations were significant at a level of 0.01 (2-tailed)

Table 5 Pearson product-moment correlation between dominant phyllospheric bacterial communities on family level of the four different natural grass species and soil physical and chemical properties

Relative	Acholeplasmataceae	Enterobacteriaceae	Oxalobacteraceae	Pseudomonadaceae	Sphingomonadaceae
Abundance	(28.6–31.8%)	(7.7–14.3%)	(4.5–11.5%)	(29.4–42.3%)	(3.8–5.9%)
NH ₄ ⁺ -N	-0.103	0.494	-0.925	-0.837	0.771
NO ₃ ⁻ -N	-0.863	0.702	0.015	-0.133	-0.021
NO ₂ ⁻ -N	-0.908	0.597	0.211	-0.236	0.199
Olsen P	-0.105	0.374	-0.945	-0.612	0.494
N	-0.868	0.884	-0.347	-0.369	0.159
C	-0.015	-0.456	0.970*	0.665	-0.536
C/N	0.918	-0.971*	0.492	0.592	-0.403
S	0.120	-0.579	0.995**	0.706	-0.55
SWC	0.042	-0.049	-0.176	-0.593	0.784
pH	0.908	-0.648	-0.022	0.561	-0.584
PS	-0.784	0.392	0.361	-0.258	0.328
As	-0.665	0.330	0.456	0.224	-0.291
Cd	-0.795	0.958*	-0.744	-0.913	0.784
Cu	0.653	-0.939	0.875	0.811	-0.617
Pb	-0.019	0.085	-0.345	-0.708	0.860
Zn	0.425	0.055	-0.790	-0.366	0.289

Physical and chemical properties of soil included ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), Olsen P, total nitrogen (N), total carbon (C), total sulfur (S), the ratio of carbon and nitrogen (C/N), soil water content (SWC), soil pH, particle size (PS), and heavy metal of soil As, Cd, Cu, Pb, and Zn

*Correlations were significant at a level of 0.05 (2-tailed). The relative abundance of dominant family was $\geq 3.8\%$

**Correlations were significant at a level of 0.01 (2-tailed)

bacteria community responses to soil chemical properties. Further research is necessary to determine the causal factors that drive growth stages, temporal shifts, and root-zone microbial communities, to ascertain whether the structure and composition of phyllospheric bacterial communities follow a predictable pattern over time, and to assess their applicability in copper tailings dams.

In conclusion, determining the taxonomic identity of phyllospheric bacterial communities in different natural grass species and what is the impact factors in phyllospheric bacterial taxa respond to environmental factors are essential in understanding microbially mediated biogeochemical processes as well as the exploration on their potential usage in copper tailings dam remediation.

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