Article

Primary Succession Changes the Composition and Functioning of the Protist Community on Mine Tailings, Especially Phototrophic Protists

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ABSTRACT: Primary succession in mine tailings is a prerequisite for tailing vegetation. Microorganisms, including bacteria, fungi, and protists, play an important role in this process in the driving force for improving the nutritional status. Compared to bacteria and fungi, protist populations have rarely been investigated regarding their role in mine tailings, especially for those inhabiting tailings associated with primary succession. Protists are the primary consumers of fungi and bacteria, and their predatory actions promote the release of nutrients immobilized in the microbial biomass, as well as the uptake and turnover of nutrients, affecting the functions of the wider ecosystems. In this study, three different types of mine tailings associated with three successional stages (original tailings, biological crusts, and *Miscanthus sinensis* grasslands) were selected to characterize the protistan community diversity, structure, and function during primary succession. Some members classified as consumers dominated the network of microbial communities in the tailings, especially in the original bare land tailings. The keystone phototrophs of Chlorophyceae and Trebouxiophyceae showed the highest relative abundance in the biological crusts and grassland rhizosphere, respectively. In addition, the co-occurrences between protist and bacterial taxa demonstrated that the proportion of protistan phototrophs gradually increased during primary succession. Further, the metagenomic analysis of protistan metabolic potential showed that abundances of many functional genes associated with photosynthesis increased during the primary succession of tailings. Overall, these results suggest that the primary succession of mine tailings drives the changes observed in the protistan community, and in turn, the protistan phototrophs facilitate the primary succession of tailings. This research offers an initial insight into the changes in biodiversity, structure, and function of the protistan community during ecological succession on tailings.

KEYWORDS: mine tailings, primary succession, protistan community, biological crusts, Miscanthus sinensis, metagenomic analysis, interaction networks, keystone taxa

INTRODUCTION

Tailings are slurry wastes from ore processing and contain abundant amounts of metal(loid)s, sulfates, as well as other minerals and salts. Tailings are also considered to be the major anthropogenic cause of soil erosion.[1,2] Around 5−7 billion tons of waste tailings are produced annually, causing damage to extensive areas of land resources and mined lands.[3,4] Therefore, large land areas have been set aside for storing tailings in Australia, China, Europe, Peru, South Africa, and the USA.[5,6] However, the tailings not only cover a large amount of mining land resource area and waste precious resources but also cause catastrophic environmental events due to the leaching of toxic metal(loid)s.[7] Therefore, it is crucial to improve the management of tailings using appropriate management and treatment strategies.[8]

Bioremediation provides a promising remediation strategy at contaminated tailing sites because some microorganisms may contribute substantially to a wide range of ecological services, including nitrogen fixation,[9] carbon fixation,[10] and metal(loid) cycling,[11] thereby affecting the ecosystem of tailing sites and promoting tailing vegetation. For instance, *Meiothermus* and *Thiobacillus* from alkaline tailings have shown potential as nitrogen- and carbon-fixing organisms as well as in metal

transformation and pollution tolerance; these bacteria were even beneficial for phytoremediation by providing nutrients for plants.[10] Some bacteria that evolved to fix nitrogen autotrophically in response to nutrient deficiencies (e.g., nitrogen and carbon) were often detected in tailings, which could play important roles in facilitating nutrient acquisition by plants and ecological succession in tailings.[6] In addition, some ectomycorrhizal fungi could exude ergosterol and low-molecular-mass organic acids that help to solubilize tailings; these fungi exhibited a potential to improve the survival rate, growth, and health of white spruce seedlings in tailings.[12] However, a growing number of researches have mainly focused on the communities of fungi and bacteria in tailing habitats, but little research has been done on the protistan community, which limits our overall understanding of the ecological roles of the microbiome in tailings.

As a major group of microbes, protists are diverse in taxonomy and rich in function.[13−15] Protists are the main consumers of fungi and bacteria,[13,16] and their predation activities facilitate the release of nutrients immobilized in the microbial biomass while promoting nutrient uptake as well as turnover, influencing the functions of a wide range of ecosystems.[17−21] In addition, protists can also facilitate nutrient recycling by regulating the fixation of carbon and degradation of organic matter.[22,23] Therefore, it is fair to propose that protists play an important role in the ecological succession of tailings. However, only limited studies have focused on the protists inhabiting mine tailings.[24,25] Therefore, it is crucial and necessary to elucidate the role of the protistan community in tailings, which can provide new insights into the bioremediation of tailings.

Mine tailings can be colonized by vegetation as a consequence of primary succession, which is desirable for stabilizing the tailing surfaces and has been the subject of intensive research recently.[26,27] However, the microbial community dynamics in the primary succession of tailings have received less attention and are more poorly understood than those involved in the establishment and succession of natural vegetation.[28,29] Notably, the composition, function, and shift of the protistan community associated with ecosystem development in mine tailings have been rarely documented. Therefore, in this study, we selected three different types of tailings (e.g., mine tailings contaminated with antimony (Sb), lead/zinc (Pb/Zn), and gold (Au)) and collected samples in three different stages of primary succession (e.g., original tailings from bare land, tailings of biological crusts, and tailings from the plant rhizosphere). The 18S rRNA and 16S rRNA analysis, shotgun metagenomics, and multivariable statistics were performed to (i) investigate the changes in the composition of taxonomic and functional communities of protists, (ii) elucidate interactions between protistan and bacterial communities, and (iii) detect the most important functional protists found during the primary succession of tailings.

■ MATERIALS AND METHODS

Tailing Sample Collection and Geochemical Analyses

Tailing samples were collected from mining sites across Southern China. The sampling information is provided (Figure S1 and Table S1). In brief, the samples were collected from three metal(loid)-contaminated mining areas containing Sb (Xikuangshan), Pb/Zn (Fankou), and Au (Huangjia). Three sample types (e.g., original tailings from bare land (BL), tailings of biological crusts (BC), and tailings from the *Miscanthus sinensis* rhizosphere (MS)) were collected. A total of 98 samples were collected; these were transported to the

laboratory within 24 h and stored at 4 °C. For analyzing pH, 2 g of freeze-dried tailings was fully mixed with 10 mL of distilled water, and then pH was determined using an HQ30d pH meter (Hach, CO, USA). To analyze the total contents of S and P, the mixture was centrifuged and measured by ion chromatography on an ICS-40 system (DIONEX, Sunnyvale, USA). The total concentrations of metal(loid)s in tailing samples were measured by digesting the tailings with a mixture of HNO₃ and HCl (1:3) using ICP-OES (Vista MPX, Varian, USA).[30]

DNA Extraction and Illumina MiSeq Sequencing of 18S rRNA

Genomic DNA was extracted from tailings with a DNeasy Powersoil kit (QIAGEN, Dresden, Germany) following the manufacturer's instructions.[31,32] The DNA quality and quantity were measured using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).[33] The 18S rRNA V9 was amplified on the ABI 2720 thermocycler (Applied Biosystems, Carlsbad, CA, USA) using the primer pair 1380F (5′-CCCTGCCHTTTGTACACAC-3′) and 1510R (5′-CCTTCYGCAGGTTCACCTAC-3′).[34] The 16S rRNA gene (V4−V5 region) was amplified with a 515F/806R primer pair.[35] Amplicons were quantitated using an FLX800T spectrophotometer (BioTek Instruments, Inc., Winooski, Vermont, USA) and pooled equimolarly for paired-end sequencing on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) at Personal Biotechnology (Shanghai, China). QIIME2 was applied to analyze the sequencing reads. Protistan and bacterial representative amplicon sequence variants (ASVs) were assigned against the Protist Ribosomal Reference (PR2) database[36] and SILVA database,[37] respectively. The trophic functional groups, including parasites, consumers, and phototrophs, were assigned at the genus level according to previous reports (Table S2).[38−40]

Shotgun Metagenomic Sequencing

Three samples (BL = 1, BC = 1, and MS = 1) were selected for shotgun metagenomic sequencing at Personal Biotechnology (Shanghai, China). The characterization of functional genes of the protistan community was conducted according to a previous description.[41] The sequences were quality-controlled by the Trimmomatic software.[42] The clean reads were evaluated for quality using a FastQC toolkit, and the community composition was classified using Kraken2.[43] The reference databases (e.g., bacteria, fungi, protozoa, and archaea) were searched using the parameters ″−paired −use-names −use-mpa-style −report-zero-counts″. Furthermore, the output files of Kraken2 were adjusted using the highly accurate statistical method Bracken to gain the final read count table by the ″kreort2mpa.py″ function.[44] The protist-classified reads were filtered using the Kraken2 database with the parameter ″−classified-out″[41] and were assembled to contigs using Megahit with parameters ″−k-min 21 −k-max 121 −k-step 10″.[45] Finally, the gained contigs were subjected to the annotation of genes using the KofamKOALA web server (https://www.genome.jp/tools/kofamkoala/) against the KEGG database with default parameters.[46] The count number of KEGG annotation was filtered for downstream comparison. The raw data were submitted to the NCBI database (Accession No. PRJNA793085).

Statistical Analysis

The alpha diversity was calculated using the QIIME2 microbiome bioinformatics platform. Principal coordinate analysis (PCoA) was done using ade4 v1.7.13, and permutational multivariate analysis of variance was taken out with the vegan package. Statistical analysis was performed by a Student's *t* test in the SPSS software v.20. The niche breadth and generalist/specialist species were calculated based on the previous descriptions.[47−49] The generalist species can thrive in a wide range of environmental conditions and utilize a variety of different resources. However, specialist species are able to only thrive in a limited range of environmental conditions or have a narrow diet.[50,51] The interactions of ″microbe−microbe″ and ″environment−microbe″ were analyzed using a co-occurrence network.[52,53] A connected link denotes a high and significant Spearman's correlation between variables. The size of each node is positively related to the count of connections—degree. A larger node represents a node with more connections. The thickness of each link is positively proportional to the absolute
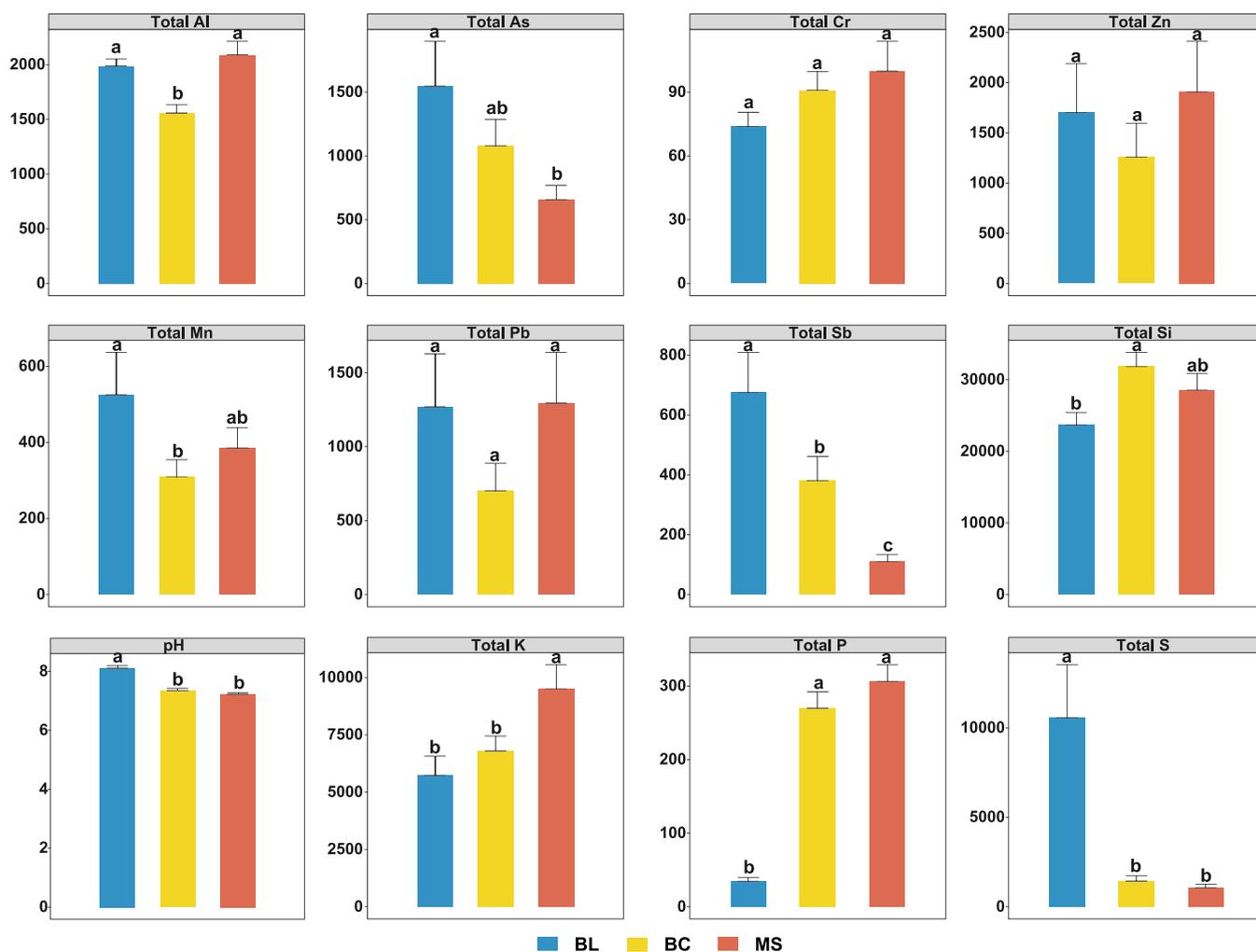
Figure 1. Geochemical parameters of samples. Different letters (a, b, c) indicate significant difference based on a least significant difference (LSD) test ($p < 0.05$). BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.
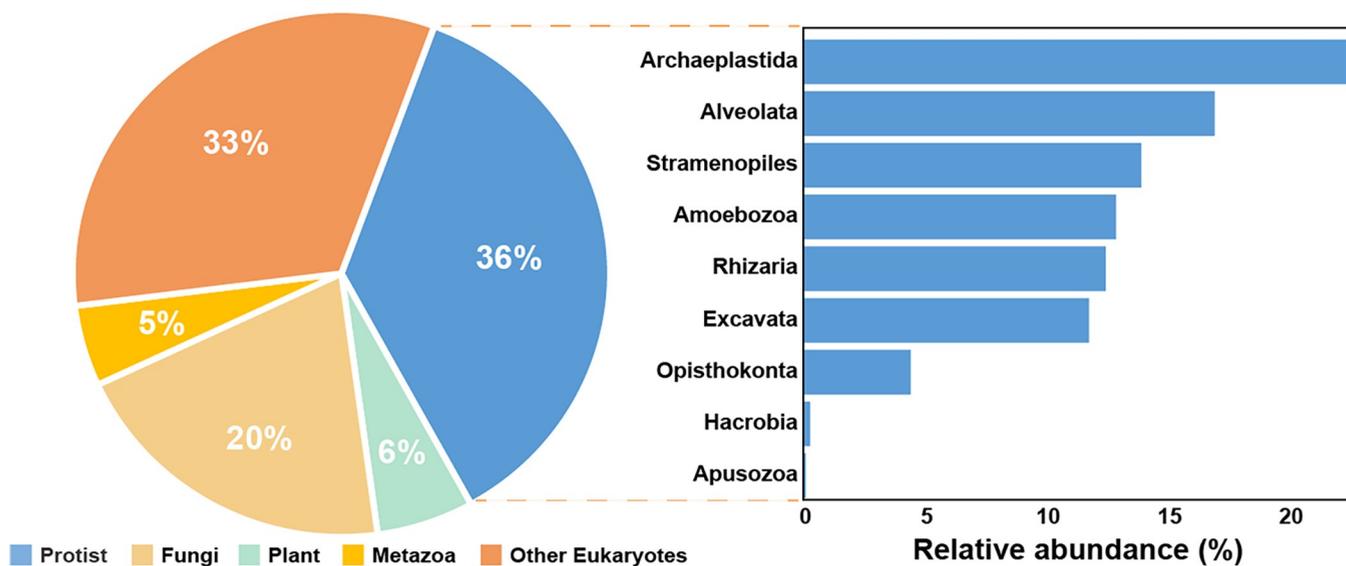

Figure 2. Compositions of eukaryotic communities in mine tailing samples.
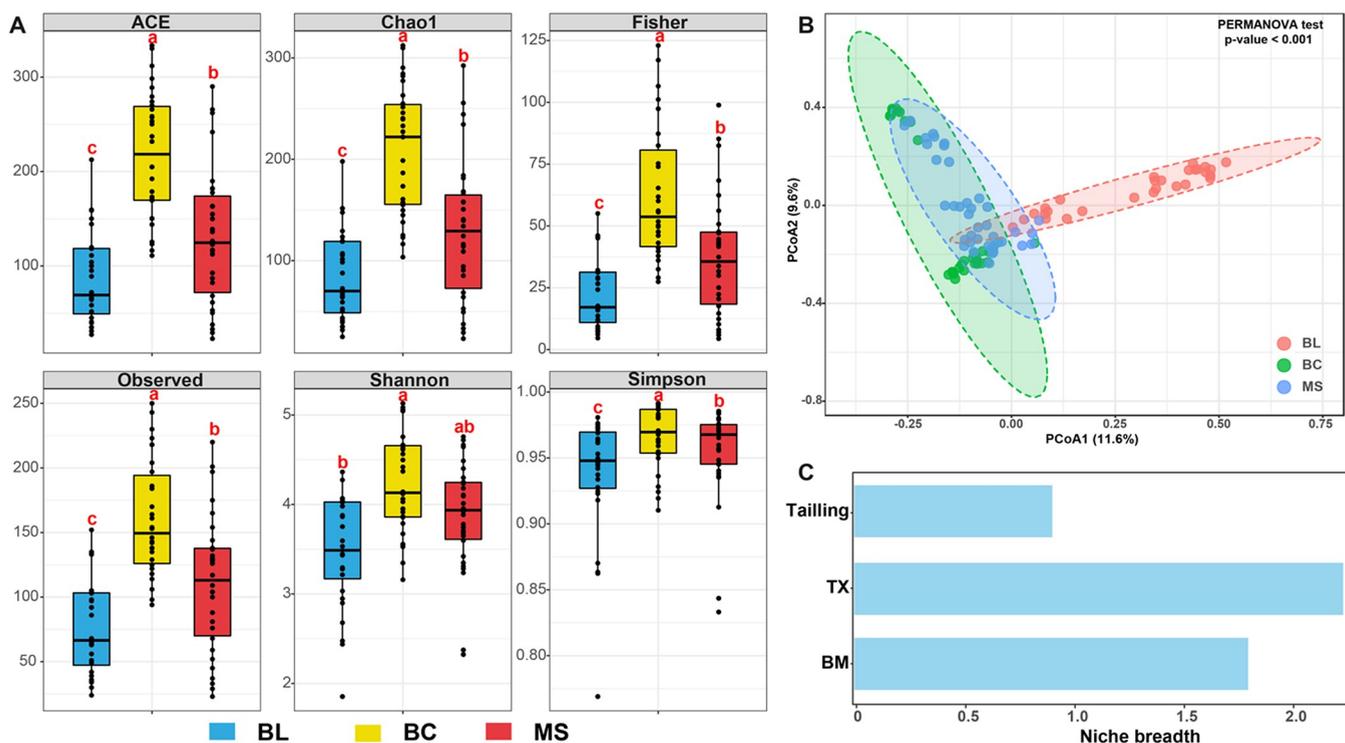
Figure 3. Comparisons of (A) the diversity indices, (B) the principal coordinate analysis based on the Bray–Curtis distance, and (C) the niche breadth of the whole protistan community. Different letters (a, b, c) indicate significant difference based on a least significant difference (LSD) test ($p < 0.05$). BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.

Figure 4. Relative abundances at the supergroup level: (A) bar graph and (B) box plot. Different letters (a, b, c) indicate significant difference based on a least significant difference (LSD) test ($p < 0.05$). BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.
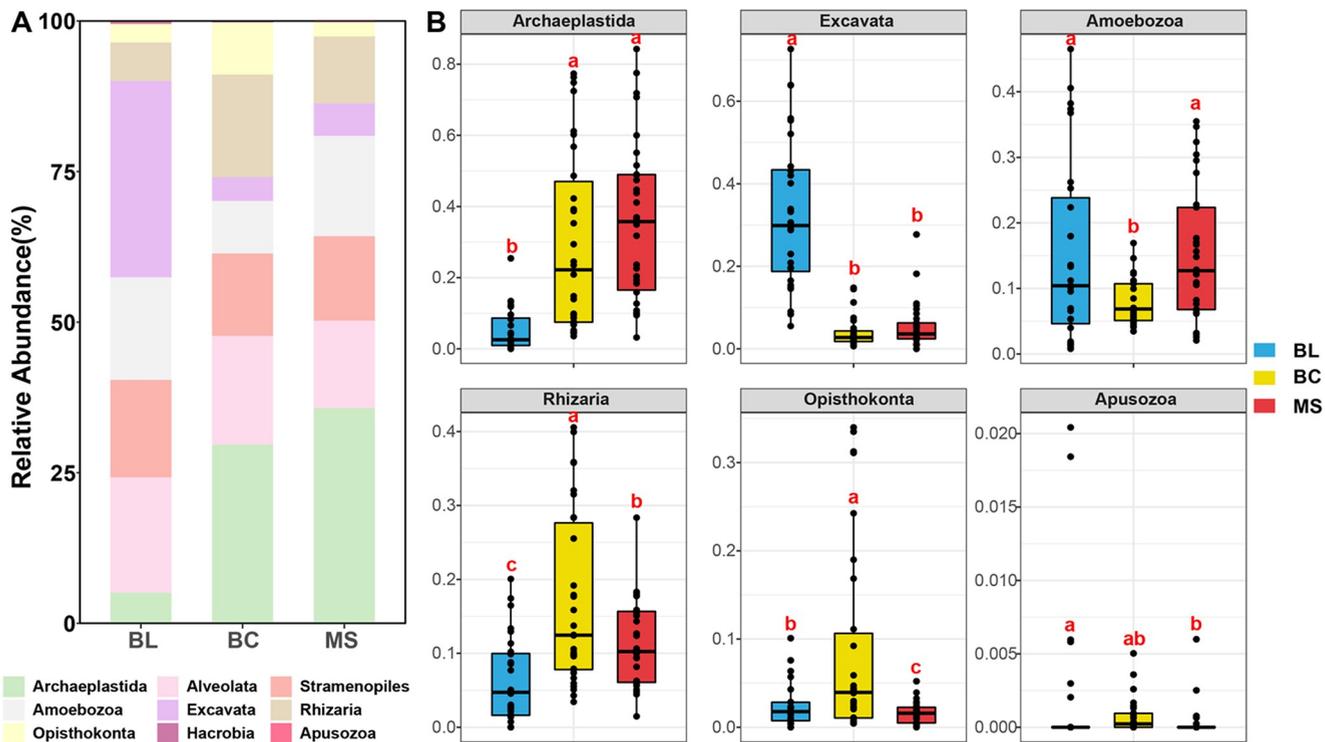
Figure 5. Comparisons of the diversity indices and the PCoA based on the Bray−Curtis distance of the functional protistan communities. Different letters (a, b, c) indicate significant difference based on a least significant difference (LSD) test ($p < 0.05$). BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.
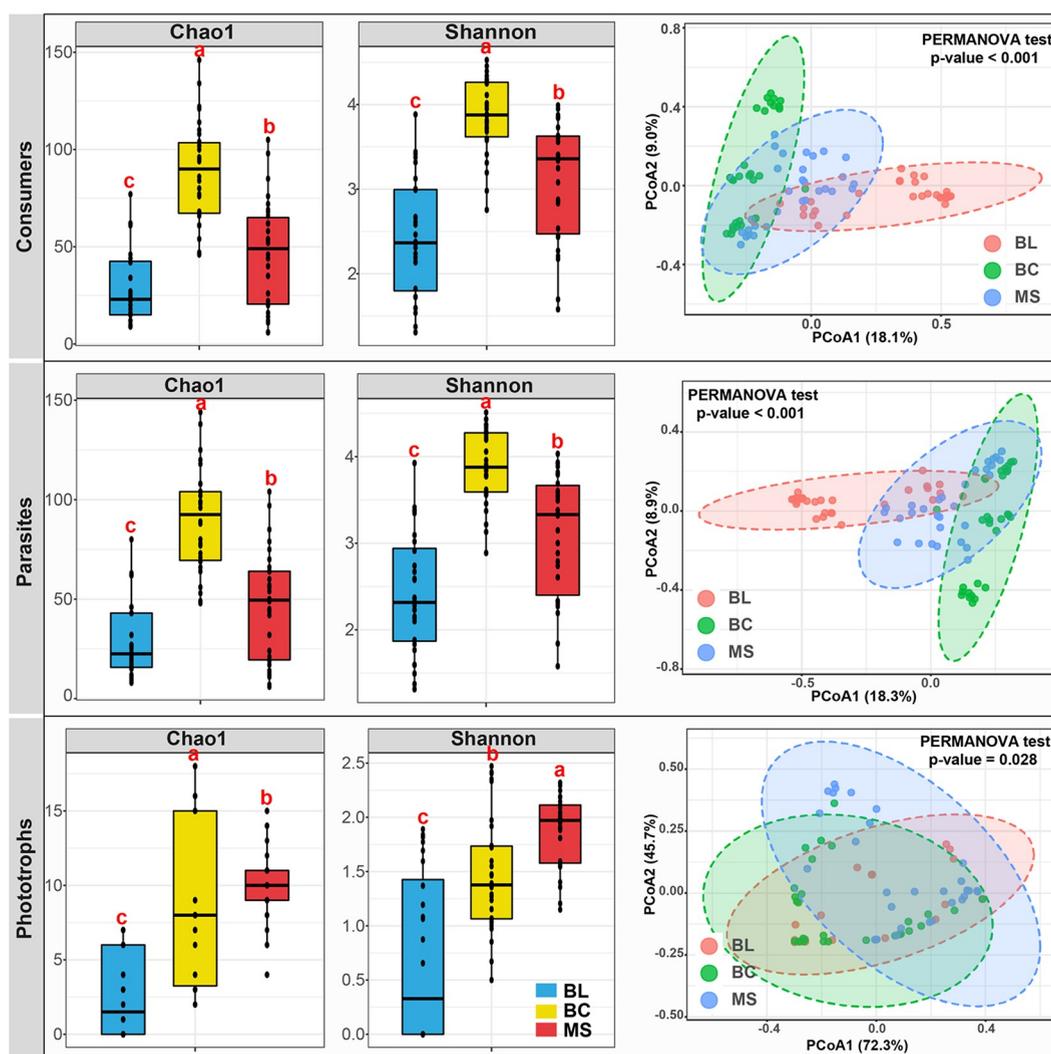
Spearman value. The network plots were modeled by the interactive Gephi platform.[54] Redundancy analysis (RDA) was conducted by OmicStudio (https://www.omicstudio.cn/tool).

■ RESULTS

Geochemical Analyses

This study analyzed the total concentration of Al, As, Cr, Mn, Pb, Sb, Si, Zn, pH, K, P, and S (Figure 1). As, Sb, pH, and S concentrations decreased as the primary succession progressed from BL to BC and finally to MS. However, contrary patterns of K and P were observed. Lower Al and Mn contents were observed in BC compared to those in BL or MS. The highest Si concentration was observed in BC followed by MS and BL. In addition, the total concentration of Cr, Pb, and Zn showed no significant difference between the series.

Characterization of the Overall Protistan Community

Based on the analysis of eukaryotic sequences, protists were found to be the most dominant eukaryotes (36%) (Figure 2). Sequences of Archaeplastida were the most abundant supergroup of protists followed by Alveolata, Stramenopiles,

Amoebozoa, Rhizaria, Excavata, Opisthokonta, Hacrobia, and Apusozoa (Figure 1).

For the whole protistan community, the alpha diversities (ACE, Chao1, Fisher, Observed, Shannon, and Simpson) were the highest in BC followed by BL and MS (Figure 3A). Protistan community compositions varied significantly among different stages of tailings during primary succession, as evidenced by the obvious separation between the cluster of BL samples and the clusters of BC and MS samples ($p < 0.001$) (Figure 3B). In addition, the different niche breadths of BL, BC, and MS furthermore demonstrated the differences of the protistan communities among the three stages of tailings during primary succession (Figure 3C). Community composition analysis indicated that Excavata sequences were the most abundant in the BL samples, while Archaeplastida sequences became the most abundant in BC and MS (Figure 4A). In the primary succession samples, significant differences were observed in the supergroup (Figure 4B). The relative abundance of Archaeplastida was 4.8- and 6.0-fold higher in BC and MS samples than in BL, while the relative abundance of Excavata was 0.9- and 0.8-fold lower, respectively. In addition, the highest and lowest
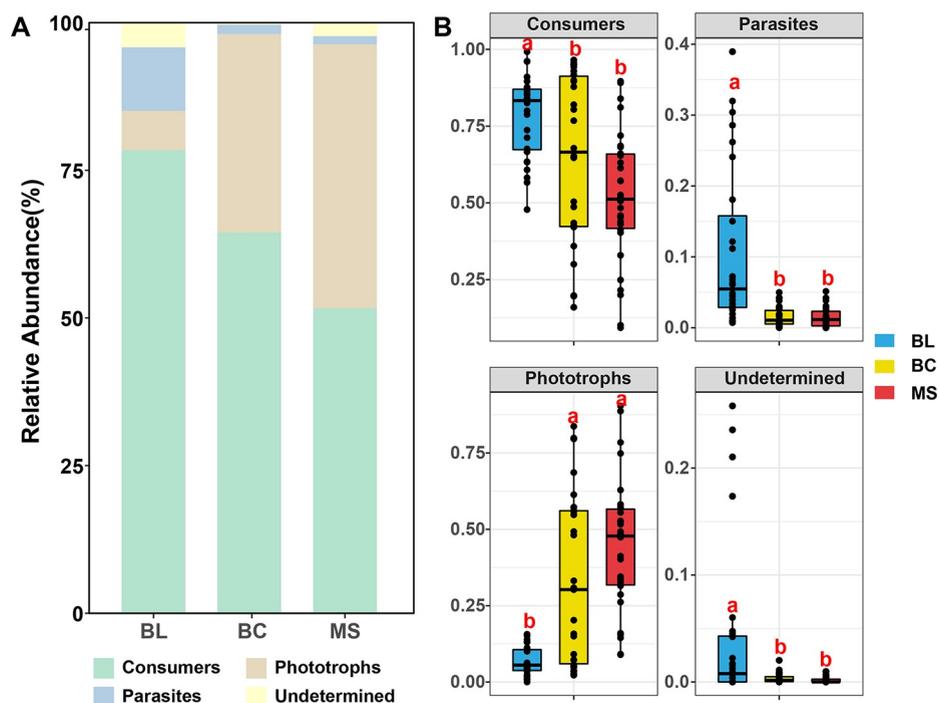
Figure 6. The relative abundances of functional protists. (A) Bar graph and (B) box plots. Different letters (a, b, c) indicate significant difference based on a least significant difference (LSD) test ($p < 0.05$). BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.
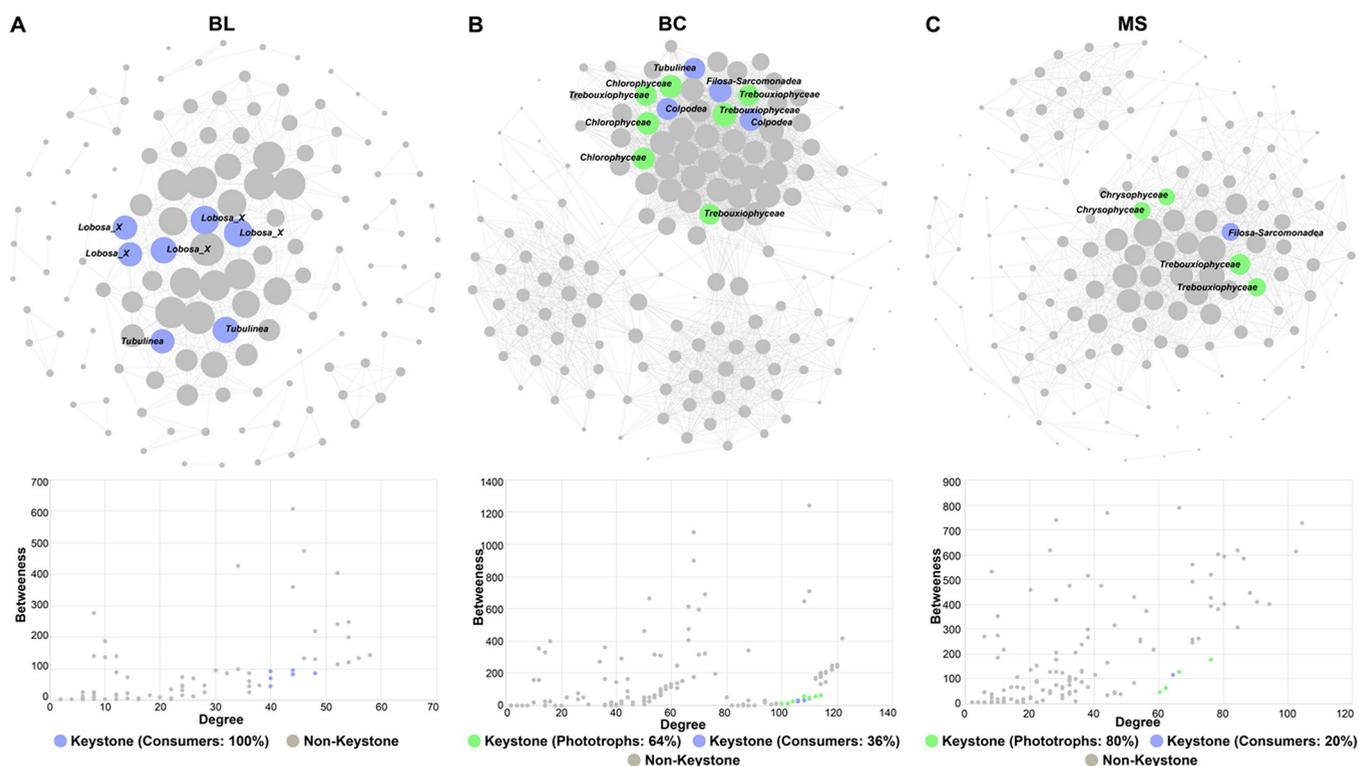

Figure 7. Co-occurrence network analysis of protist−protist showing the biological interactions in (A) BL, (B) BC, and (C) MS. Edges show significant connections. The size of each node is proportional to the number of connections to it. The scatter plot below the network plot shows criteria used to select the keystone taxa. BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.
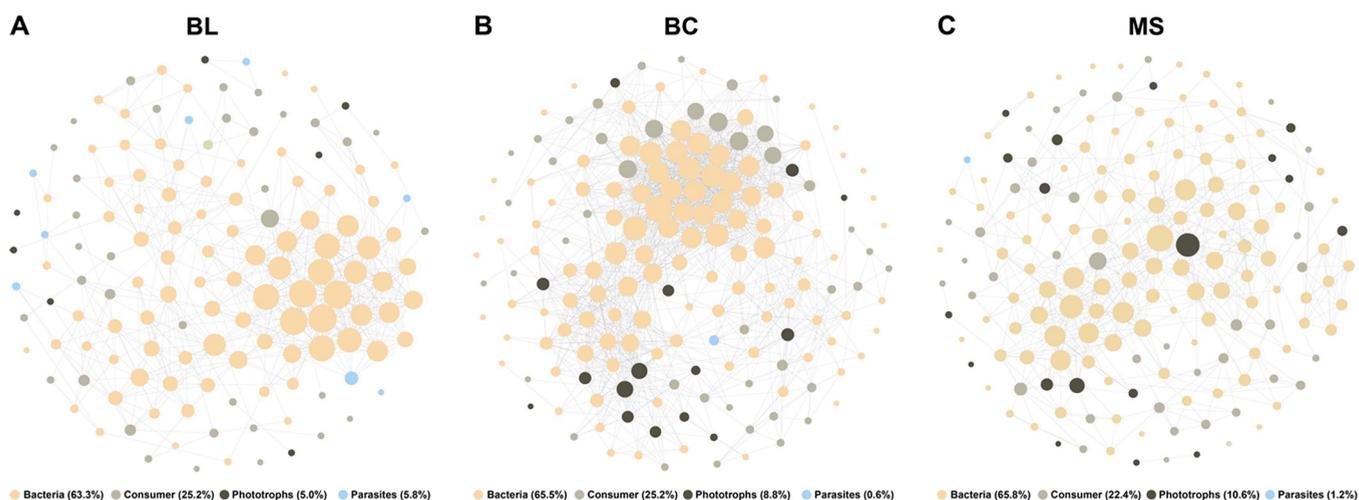
Figure 8. The co-occurrence network of protist–bacteria showing the biological interactions in (A) BL, (B) BC, and (C) MS. Edges only show significant connections. The size of each node is proportional to the number of connections to it. BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.

relative abundance of Rhizaria and Amoebozoa was observed in the BC samples, respectively.

Functional Protistan Community Compositions

The assignment of the taxonomic profiles to their individual functions illuminated the impacts of primary succession on the potential functions of protists. The protistan community was functionally divided into consumers, parasites, and phototrophs (Figure 5). The alpha diversities and PCoA revealed significant variations in the functional community composition of protists among the primary succession of the three types of tailings. Thereby, the protistan functional community composition was furthermore summarized (Figure 6A). The consumers were observed to be the most dominant functional group in all tailing samples. The relative abundances of consumers and parasites were significantly higher in BL samples than those in BC and MS samples, while the relative abundances of phototrophs were significantly lower in BL than those in BC and MS samples (Figure 6B).

Interaction Networks and Key Functional Protists

The interaction networks of the functional community of protists were generated. Consistent with the functional community composition, the consumers accounted for more than 60% in each network of BL, BC, and MS samples (Figure S2). Importantly, the ratio of phototrophs was increasing over time with the primary succession of tailings (from BL to MS). In addition, the proportion of positive links occupied more than 90% in all three networks. The keystone taxa were identified according to the criteria of the nodes with high degrees and low betweenness centralities.[55] The keystone taxa are highly connected microorganisms, which individually or within a community have a significant impact on the structure and function of the microbial communities regardless of their abundance across space and time.[52] These taxa play a unique and critical role in the microbiome, and their removal can lead to great changes in the structure and function of microbial communities.[55] In BL, all identified keystone taxa were consumers, including Lobosa_X and Tubulinea (Figure 7A and Table S3). In BC (Figure 7B and Table S4) and MS (Figure 7C and Table S5), the identified keystone taxa were classified as consumers and phototrophs, in which the keystone phototrophs become dominant (BC: 64%, MS: 80%). The keystone

phototrophs were classified as Chlorophyceae and Trebouxiophyceae at the class level. The highest relative abundance of Chlorophyceae was observed in BC; the relative abundance of
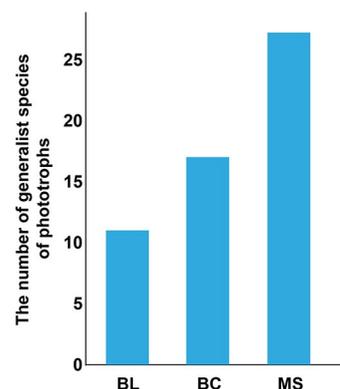

Figure 9. The number of generalist species of phototrophs in BL, BC, and MS. BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.

Trebouxiophyceae was increasing with the primary succession of tailings (Figure S3).

Furthermore, the interactions between protistan and bacterial communities were analyzed to assess the ecological roles of protists in soil microbial communities (Figure 8). The percentage of consumers was reasonably similar in all three networks, while the ratio of phototrophs increased (from 5.0 to 10.6%) during the primary succession of tailings. Generalist species have wider fundamental niches than specialist species, leading to the strong competitiveness of generalists.[56] The largest generalists of phototrophic protists were observed in MS followed by BC and BL (Figure 9). Taken together, these results suggest that phototrophic protists are becoming more and more competitive over time and may play important ecological functions during the primary succession of the tailings.

The interactions between the geochemical parameters and the functional community of protists are shown in Figure 10. The Sb, pH, and P contents were the main factors, which affected 55.7% of ASVs. Other geochemical parameters including Si, Zn, and Mn also had great influences on the functional community.
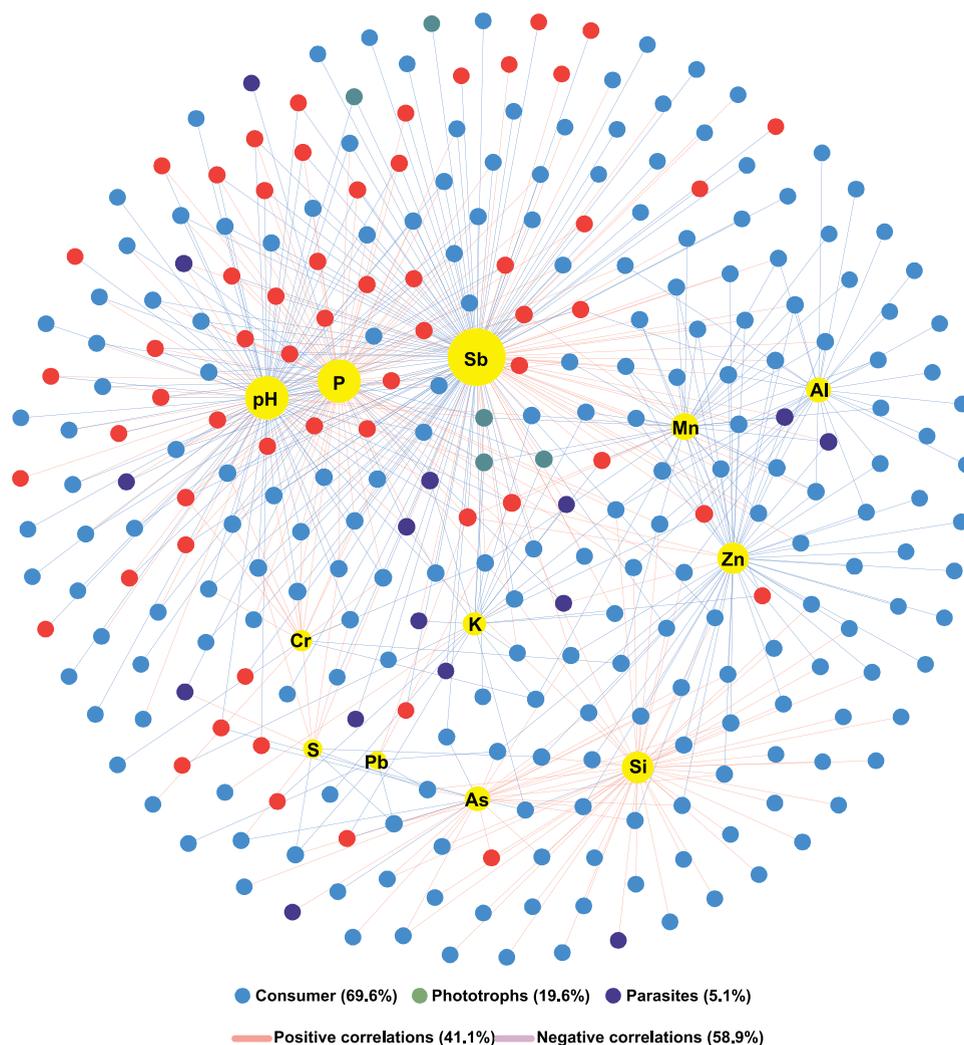
Consumer (69.6%) ● **Phototrophs (19.6%)** ● **Parasites (5.1%)**

━━ **Positive correlations (41.1%)** ━━ **Negative correlations (58.9%)**

Figure 10. Co-occurrence network of environment−microbe showing the correlations between the geochemical parameters and the relative abundances (ASV level).

The above results were also demonstrated by the RDA analysis (Figure S4).

Functional Genes of the Protistan Community

The reads classified as protists from the metagenomes were retained for the annotation of functional genes (Figure 11). In general, BL samples had the highest relative abundance of genes associated with metal tolerance (Figure 11A), including Zn, nickel, cobalt, copper, As, Sb, and cadmium, except for manganese (Figure 11B), which indicated that the presence of vegetation may provide a better niche for protists. Importantly, we focused on genes associated with photosynthesis (Figure 11C). The highest relative abundance was observed in MS followed by BC and BL, which further demonstrated that phototrophic protists may play important roles during the primary succession of mine tailings.

■ DISCUSSION

Compared to bacteria, archaea, and fungi, our understanding of protists in environmental samples is limited, especially for those inhabiting tailings. Protists may play a key role in the primary succession of mine tailings by adjusting the food web and carbon flow. In this study, three different types of tailings including those with Sb, Pb/Zn, and Au contamination from mines with three remarkably different stages (original tailings from bare land, biological crusts, and tailings from the rhizosphere of *Miscanthus sinensis* grasslands) were selected to investigate the protistan community structure and function associated with ecosystem development in mine tailings. The microbial interactions, keystone of functional protists, and functional genes were also evaluated.

Dominant Protists in the Tailings

Clear differences were observed among the protistan community in the three stages of tailings as described above (Figure 3), suggesting that the primary succession of tailings serves as a strong selection force in shaping the protistan community composition. This result is in line with prior reports that different stages of primary succession of tailings typically harbor unique microbiomes, though the protistan community was excluded in these studies,[5,29,57,58] except that a dynamic and diverse eukaryotic community containing protists was described in oil sand tailings at Northern Alberta.[59]

Our research indicated that the protistan community in tailing samples was predominated by supergroups Archaeplastida, Excavata, Amoebozoa, and Rhizaria (Figure 4). A significantly enriched Excavata group was observed in the BL samples (Figure 4). Excavata showed highly unusual mitochondrial,
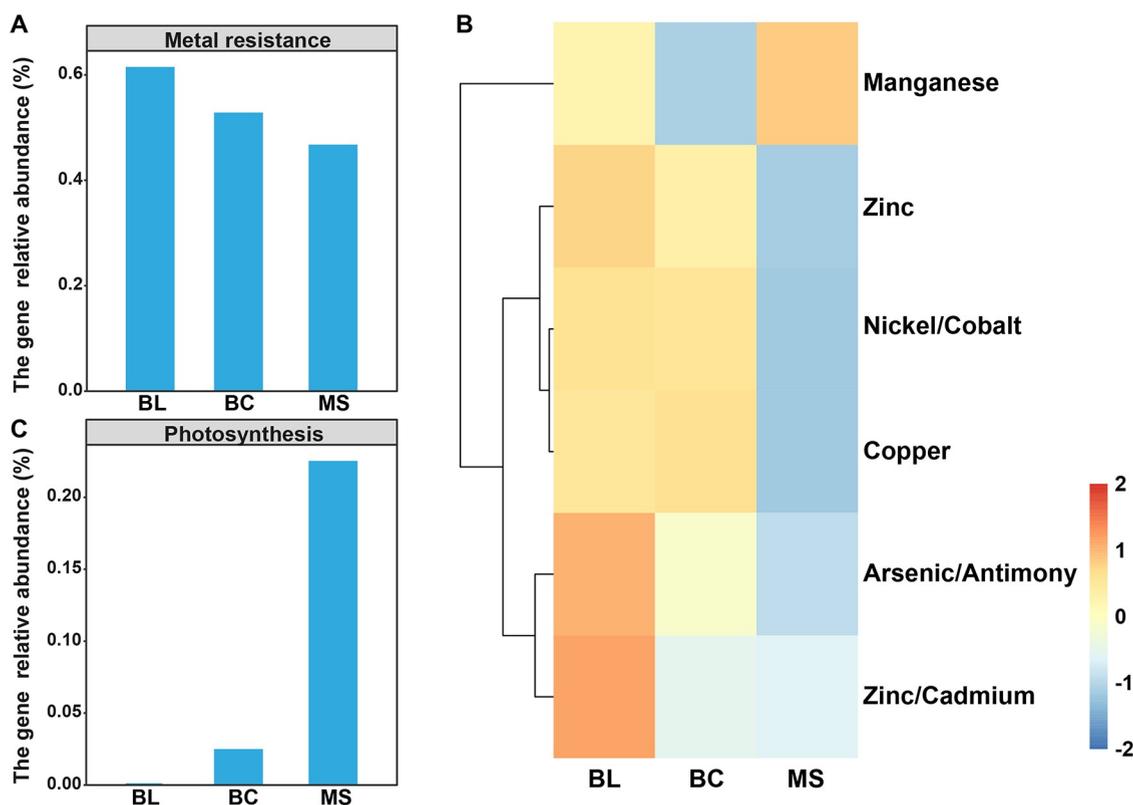
Figure 11. Metagenomic analysis indicates the metabolic potentials of the protistan community inhabiting mine tailings. (A) Total genes providing metal tolerance, (B) individual genes providing metal tolerance, and (C) photosynthesis genes. BL: original tailings from bare land, BC: tailings of biological crusts, and MS: tailings from the *Miscanthus sinensis* rhizosphere.

nuclear, and chloroplast genomes.[60] The phylogenetic relationship of Excavata members indicated that these members are more diverse than previously thought.[60] Some Excavata have been isolated from various environmental samples such as soils, sediments, and AMD,[41,60] but only a few have been found in the tailing samples.[59] Therefore, our results suggest that the protist Excavata might play important roles in the tailings, especially in bare land.

The relative abundances of Archaeplastida and Rhizaria were significantly higher in the BC and MS samples compared to BL (Figure 4). The Archaeplastida, containing a large proportion of the large seaweeds, algae, and plants that support life on earth, are part of the main evolutionary lineages of the photosynthetic species and are critical to the world's ecosystems and human welfare and health.[61] Some members of Archaeplastida may even invade the land and set the stage for the evolutionary movement onto the land of many life groups.[61] Therefore, it is reasonable that the Archaeplastida would be enriched during ecosystem development on mine tailings. Fine-tuning between microbe genomic traits and plant immune response is a well-known method used to mediate microbial colonization.[62] Interestingly, in the Rhizaria supergroup, the cell-surface G-protein-coupled receptor mediated signaling pathway was found to be significantly enriched.[63] The cell-surface G-protein-coupled receptor could sense various extracellular signals from the plant host and has been shown to facilitate colonization by microorganisms.[64] Thus, the enriched Rhizaria may have a better colonization capacity in plants, even in biological crusts. Further studies are necessary to elucidate molecular plant–protists (even biological crusts–protists) and the interactions

leading to the selective recruitment of some functional protists in the plant rhizosphere and biological crusts.

Functional Protists in the Tailings

Taxonomically related protists can serve different functions from microbial predators (consumers) to plant parasites and pathogens, and similarly, organisms that belong to different taxonomic groups may have developed to share similar patterns of nutritional feeding.[13] Therefore, studying not only the taxonomy but also the potential function of protists would provide invaluable information.[15,65,66] In addition, the co-occurrence network, in ecosystem studies, has become an ever more important tool for exploring the symbiosis modes of the microbial community and identifying keystone taxa.[9,11,67] It has been reported that the biotic interactions of microorganisms are the most essential factors in the microbial community composition and function.[68] The identification of keystone taxa is also critical because these taxa are essential for regulating the structure, composition, and functions of microbial communities[52] and for facilitating the revegetation because they may be beneficial for plant growth and development.[69]

Methods for grouping protists by function and analyzing interactions within and between functional groups offered a better visualization of the correlation between protistan community members. In all three stages of primary succession of tailings, most interactions and nodes were linked to consumers (predators) (Figure S2), and some consumers were identified as the keystone taxa (Figure 7). It has been reported that predator–prey interactions increase the biotic network complexity and stability,[70] and some protists belonging to consumer groups have been indicated to be the keystone taxa in the microbial networks.[66] This is not surprising because

consumers influence almost all microorganisms within their network.[15] Populations of microorganisms preyed upon by consumers may be drastically reduced in size below the detection limit.[15,71] Other microbes that have not preyed (i.e., nonpreferred or predation-resistant microbes) may benefit from the release of nutrients from the protist-preyed microbial biomass and/or obtain a competitive advantage due to protistan predation on their strong competitors.[15,72,73] Therefore, our results suggest that consumers may have an important role in driving the microbial community network in mine tailings, especially in the stage of bare land.

The phototrophic protists may play the most important roles in the primary succession of mine tailings based on the identification of keystone phototroph taxa (Figure 7) and genes associated with photosynthesis (Figure 11C). Most metal(loid)s can impair photosynthesis in microorganisms.[74] However, siderophores can protect microorganisms against various types and levels of metal(loid) toxicity.[75] Siderophores can not only chelate Fe(III) with an extremely high affinity but also chelate numerous other metal(loid)s with variable affinities.[76,77] With a high affinity for iron and other metal(loid)s and their substantial production and secretion into the extracellular medium, siderophores apparently provide a kind of extracellular protection for bacteria by blocking external metal(loid)s from entering bacteria and helping bacteria to avoid the diffusion of metal(loid)s through porins into the bacteria.[78] Some microorganisms isolated from biological soil have been reported to produce siderophores.[74] The rhizospheric microorganisms and the plant itself could produce siderophores. These secreted siderophores could also be used by other microorganisms.[74,79] Therefore, our results may imply that the niches of biological crusts and the rhizosphere provided better environments for protistan phototrophs than bare land in mine tailings. Further research will be needed to illuminate the molecular mechanisms leading to the selective recruitment of protistan phototrophs in biological crusts and plant rhizospheres as well as to understand the ecological roles of protistan phototrophs in tailings.

To date, the ecology of protists in mining areas has been less studied, and the shift of the protist community across various geochemical gradients in mining areas is scarce. Protistan communities were investigated on a terrace contaminated by acid mine drainage (AMD) (AMD study hereafter).[41] The intrusion of AMD created a sharp geochemical gradient along the terrace. In this study (Tailing study hereafter), the primary succession changes also created a sharp geochemical gradient. Therefore, these two studies provided an opportunity to investigate the response of protists inhabiting mining areas to a sharp geochemical shift, and their responses were compared here. In the AMD study, pH was identified as the major driving force, but Sb and P were identified as the major environmental factors in the Tailing study. Different geochemical conditions enriched different dominant protistan taxa. It was observed that *Leishmania*, *Plasmodium*, and *Besnoitia* were the most abundant protistan genera in AMD contaminated terrace, while Archaeplastida, Alveolata, Stramenopiles, Amoebozoa, and Excavata were the dominant taxa in the tailings. In addition, both the metabolic potentials of the innate protistan communities were investigated in the two studies, providing an in-depth analysis to understand the response of innate protists to various geochemical changes. In AMD studies, the enrichment of many functional genes suggested the unique metabolic potential of protistan communities to adapt to the extreme AMD environment. For example, genes for acid stress

(e.g., *kdpA*) and metal resistance (e.g., *ASNA1*, *ArsR*, and *mmtH*) were detected in the AMD study. Like the AMD study, genes for metal resistance were also prevailed in the Tailing study. But genes for photosynthesis increased from BC to MS, suggesting that phototrophic protists may play an important role in tailing vegetation. The comparison of two mining contaminated sites suggested that the geochemical conditions substantially shaped the innate protistan communities. Innate protistan communities may develop various survival strategies such as metal resistance, acid stress resistance, and photosynthesis to respond to extreme geochemical conditions.

■ CONCLUSIONS

In summary, this study provided novel insights and proof that primary succession within mine tailings induces changes in the alpha diversity, structure, composition, and biotic interaction of the protistan community based on 18S rRNA sequencing. Furthermore, taxonomic profiles were analyzed as they relate to functionality. Some members classified as consumers showed that they served vertical roles and dominated the network of microbial communities in mine tailings, especially in the bare land stage. Some phototrophic protists (e.g., Chlorophyceae and Trebouxiophyceae) could be selectively enriched in the biological crusts and plant rhizosphere, which may be beneficial for the successful colonization of biological crusts and pioneer plants on mine tailings. Overall, these results suggest that the primary succession of mine tailings is a strong driver of the protistan community, facilitating the functioning of the tailing ecosystem.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsenvironau.1c00066.

> Sampling locations; co-occurrence network analysis of protist−protist; relative abundance of keystone taxa of phototrophs; and redundancy analysis (Figures S1−S4) (PDF)

> Sample information of sites; trophic functional groups at the genus level; and node table for protist−protist interaction network in bare land/biological/*M. sinensis* rhizosphere (Tables S1−S5) (XLSX)

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