Soil Biology & Biochemistry 114 (2017) 52–58

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Changes in microbial community composition following phytostabilization of an extremely acidic Cu mine tailings

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A R T I C L E I N F O

Article history:
Received 31 October 2016
Received in revised form
2 July 2017
Accepted 5 July 2017
Available online 12 July 2017

Keywords:
Archaea-dominated community
Extremely acidic
Microbial community structure
Mine wastelands
Phytostabilization

A B S T R A C T

Phytostabilization of an extremely acidic (pH < 3) mine tailings via direct revegetation remains a challenge, which can perhaps be attributed largely to a limited understanding of the responses of microbial communities in extremely acidic mine tailings to practical phytostabilization. In order to shed light on the mechanism by which amending materials used in phytostabilization practice help to improve the growth conditions in reconstructed root zones and thereby facilitate the initial establishment of vegetation in extremely acidic mine tailings, 4000 m^2 of vegetation was established successfully within six months on an extremely acidic (pH 2.5) Cu mine tailings amended directly with ameliorants. Further, a comparative analysis of the microbial communities in amended layer of the reclaimed tailings (ALRT), unamended layer of the reclaimed tailings (ULRT) and the unreclaimed tailings (UT) was performed. The results revealed intriguing changes in microbial community composition following the phytostabilization. While UT communities were dominated by *Euryarchaeota* with relative abundances varying from 57% to 84%, *Proteobacteria* was the most predominant phylum in those of both ULRT and ALRT with relative abundances ranging from 29% to 53%. *Ferroplasma* and *Aplasma*, two archaeal genera involved in tailings acidification, existed in UT communities at high relative abundances (up to 52%), which were 14–913 times greater than those in ULRT and ALRT communities. The overall microbial community composition of ULRT was different greatly from that of UT, but tended to shift towards that of ALRT as the phytostabilization progressed. Redox potential was among the major determinants of microbial community composition of the tailings. These findings have implications for further development of effective phytostabilization schemes for extremely acidic mine tailings.

1. Introduction

Mine tailings represent a major worldwide environmental problem (Mendez and Maier, 2008). Acidification is widespread among mine tailings containing considerable amounts of metal sulfides and always leads to increased pollution loads by generating metal-rich acid mine drainage (AMD; Evangelou, 1995). Tailings acidification can be ascribed largely to the oxidation of sulphide minerals (Evangelou, 1995). At circumneutral pH, the oxidation rate of sulphide minerals is chemically controlled and usually very slow; at low pH, however, the oxidation rate is microbiologically controlled and can be accelerated by iron- and/or sulfur-oxidizing microbes by up to six orders of magnitude (Evangelou, 1995). In agreement with this, there is evidence that the microbial community composition of acidified mine tailings often contrasts greatly with that of circumneutral mine tailings. More specifically, members of the genera (such as *Ferroplasma*, *Leptospirillum* and *Acidithiobacillus*) characterized by their iron- and/or sulfur-oxidizing capacities have been always found to dominate microbial communities of acidified mine tailings, compared to their low abundance or even absence in those of circumneutral mine tailings (Schippers et al., 2000; Diaby et al., 2007; Mendez et al., 2008; Chen et al., 2013).

Phytostabilization has long been considered as an attractive approach for remediation of mine wastelands (Salt et al., 1995). This approach, indeed, is being widely practiced around the world to revegetate mine tailings (Li, 2006; Mendez and Maier, 2008;

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http://dx.doi.org/10.1016/j.soilbio.2017.07.004

Vangronsveld et al., 2009). Note, however, that phytostabilization of an extremely acidic mine tailings via direct revegetation remains a challenge. To our knowledge, there are only 3 published studies on practical phytostabilization of extremely acidic ($pH < 3$) mine wastelands (Clemente et al., 2006; Gil-Loaiza et al., 2016; Yang et al., 2016). Moreover, only one of these three studies investigated the effects of phytostabilization practice on microbes in mine wastelands. Specifically, in a field trial, Gil-Loaiza et al. (2016) showed that neutrophilic heterotrophic bacterial counts were 1.5–4 orders of magnitude higher in revegetated plots than in the unrevegetated plots. It is therefore apparent that little is known about the effects of phytostabilization practice on microbial community composition (especially iron- and/or sulfur-oxidizing microbes) in extremely acidic mine tailings.

Recently, there have been few efforts to examine the responses of microbial communities in circumneutral or moderately acidic mine tailings to practical phytostabilization (Li et al., 2015, 2016a, b). In a small-scale field experiment conducted at a circumneutral mine tailings impoundment, Li et al. (2015) found that after 3 years of phytostabilization the biomass and frequency of organotrophic genera were significantly higher in revegetated treatment than in the un-revegetated treatment. In another recent study, it was observed that the abundances of iron- and sulfur-oxidizing bacteria (especially *Leptospirillum* and *Acidithiobacillus*) in an artificial vegetation area of a moderately acidic (pH 3.8) tailings were significantly lower than those of the bare tailings (Li et al., 2016a, b), but the phytostabilization settings were unclear. These previous findings may have important implications for understanding of shifts in microbial community composition following practical phytostabilization of circumneutral or moderately acidic mine tailings. However, it is unknown whether they can be applicable to extremely acidic mine tailings, considering the reported differences in microbial community composition among mine tailings of varying pHs (Schippers et al., 2000; Mendez et al., 2008; Chen et al., 2013).

In this study, 4000 m^2 of vegetation was successfully established on an extremely acidic (pH 2.5) Cu mine tailings located in southern China (Fig. S1 in Supporting Information) by implementing a phytostabilization program, wherein direct revegetation was used. To explore the mechanism by which amending materials employed in the program help to improve the growth conditions in the reconstructed root zone and thereby facilitate the initial establishment of vegetation in the tailings, changes in microbial community composition following the phytostabilization were examined using Illumina MiSeq high-throughput sequencing technique. We hypothesized that amending materials could suppress the dominance of iron- and/or sulfur-oxidizing microbes and favor the colonization of heterotrophic microbes in the reconstructed root zone (Huang et al., 2012). It has been proposed that the presence of soil microbial communities consisting mainly of heterotrophic microbes may be viewed as one of the most important indicators of a transition in root zone development from a hydro-geochemical dynamic state to a relatively stable state (Huang et al., 2012). We also attempted to identify factors affecting microbial community composition in the reconstructed root zone, which has implications for further development of effective phytostabilization schemes.

2. Materials and methods

2.1. Site description and field experiment

This study was carried out at the Chengmenshan Cu mine tailings impoundment (29°40′52″N, 115°49′21″E), located approximately 22 km southwest of Jiujiang City, Jiangxi Province, China (Fig. S1). The study site has a subtropical climate with a mean annual precipitation of 1426 mm and a mean annual temperature of 17.0 °C. The tailings impoundment has been abandoned for over 8 years and the surface tailings was highly acidified (pH 2.5, Table 1). We executed a phytostabilization program in an area of 4000 m^2 located at the center of the tailings impoundment. A direct revegetation strategy was employed in the phytostabilization program. Briefly, the procedure of phytostabilization could be described as follows: 1) turning over the surface tailings (0–30 cm depth); 2) constructing planting strips by digging ditches (30 cm width, 20 cm depth, and 50-cm intervals); 3) adding lime (20 t ha^{-1}) and chicken manure (40 t ha^{-1}) to the ditches at a depth of 10 cm; 4) growing plants (from seedlings and seeds) and nurturing afterward; 5) covering the planted tailings with a layer of straw mulch to maintain a warm and damp environment. The plant species selected for the phytostabilization program included *Festuca arundinacea*, *Bermuda grass*, *Paspalum natatum*, *Artemisia capillaris*, *Panicum repens*, *Sesbania cannabina*, *Boehmeria nivea*, and *Robinia pseudoacacia*.

2.2. Sample collection

The phytostabilization trial was sampled twice in 2014, i.e. at six and twelve months after phytostabilization treatment implementation, respectively. At each sampling date, 30 tailings samples were collected randomly from amended layer (0–10 cm) of the reclaimed tailings (ALRT) and unamended layer (11–20 cm) of the reclaimed tailings (ULRT), respectively. Meanwhile, six tailings samples were also collected from the nearby unreclaimed tailings (UT) at a depth of 0–10 cm and were considered as controls. Sampling was conducted using a stainless steel trowel. Each sample consisted of three subsamples, which were collected from three randomly distributed spots covering an area of about 4 m^2 in each treatment plot. That is, at each sampling date, 90 and 18 spots of reclaimed and unreclaimed tailings were sampled, respectively. All samples were kept under refrigeration until arrival at laboratory where they were stored at 4 °C before processing.

2.3. Geochemical analysis

Moisture content was measured by determining the changes in the weight of a tailings sample before and after drying at 105 °C in an oven for 12 h. Colorimetric method was employed to determine ferric and ferrous iron extracted by 0.5 M HCl, using 1, 10-phenanthroline at 530 nm wavelength (Hill et al., 1978). Redox potential (Eh) was measured with an Eh meter plus Ag/AgCl reference electrode. pH and electronic conductivity (EC) were measured in a 1:2.5 (w/v) aqueous solution using a pH meter and EC meter, respectively. Net acid generation capacity (NAG) and NAG-pH were measured as described previously (Shu et al., 2001). Sulfate was determined by barium sulfate turbidimetric method. Total concentrations of heavy metals (including Pb, Zn, Cu and Cd) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 2100DV, PerkinElmer, Wellesley, MA, USA) after sample digestion (EPA 3052 method). Diethylenetriaminepentaacetic acid (DTPA) was used to extract the bioavailable fraction of the heavy metals, which were also measured by ICP-OES. Total C (TOC-VCPH; Shimadzu, Columbia, MD), total P (SmartChem; Westco Scientific Instruments Inc., Brookfield, CT, USA), total N and NH_4^+-N (SmartChem) were measured according to standard methods. Organic carbon (OC) and water-soluble organic carbon (WSOC) were determined according to Ghani et al. (2003). Available P extracted with 0.5 M $NaHCO_3$ was measured using the ascorbic acid method at 700 nm wavelength. Additionally, X-ray diffraction was used to determine the mineralogical composition of the tailings sampled from UT at six month according to Chen et al.