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Treatment impacts on temporal microbial community dynamics during phytostabilization of acid-generating mine tailings in semiarid regions

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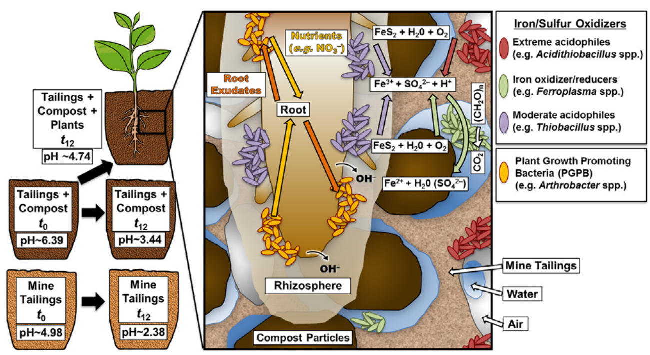
HIGHLIGHTS

- Semiarid, oxidative weathering drives acidification of pyrite-rich mine tailings.
- Oxidative weathering is associated with a temporal evolution of Fe/S-oxidizers.
- Acidification inhibits phytostabilization of pyritic, metalliferous tailings.
- Plant establishment controlled the proliferation and activity of Fe/S-oxidizers.
- Abundant plant growth promoting bacteria associated with quailbush establishment.

GRAPHICAL ABSTRACT



Treatment effects on pyritic mine tailings biogeochemistry

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ABSTRACT

Direct revegetation, or phytostabilization, is a containment strategy for contaminant metals associated with mine tailings in semiarid regions. The weathering of sulfide ore-derived tailings frequently drives acidification that inhibits plant establishment resulting in materials prone to wind and water dispersal. The specific objective of this study was to associate pyritic mine waste acidification, characterized through pore-water chemistry analysis, with dynamic changes in microbial community diversity and phylogenetic composition, and to evaluate the influence of different treatment strategies on the control of acidification dynamics. Samples were collected from a highly instrumented one-year mesocosm study that included the following treatments: 1) unamended tailings control; 2) tailings amended with 15% compost; and 3) the 15% compost-amended tailings planted with *Atriplex lentiformis*. Tailings samples were collected at 0, 3, 6 and 12 months and pore water chemistry was monitored as an indicator of acidification and weathering processes. Results confirmed that the acidification process for pyritic mine tailings is associated with a temporal progression of bacterial and archaeal phylotypes from pH sensitive *Thiobacillus* and *Thiomonas* to communities dominated by *Leptospirillum* and *Ferroplasma*. Pore-water chemistry indicated that weathering rates were highest when *Leptospirillum* was most abundant. The planted treatment was most successful in disrupting the successional evolution of the Fe/S-oxidizing community. Plant establishment stimulated growth of plant-growth-promoting heterotrophic phylotypes and controlled the proliferation of lithoautotrophic Fe/S-oxidizers. The results suggest the potential for eco-engineering a microbial inoculum to stimulate plant establishment and inhibit proliferation of the most efficient Fe/S-oxidizing phylotypes.

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1. Introduction

Hardrock mining is a major source of environmental contamination in global arid and semiarid regions (*e.g.* western North and South America, southwestern Europe, central Asia, South Africa, central Australia) where tailings, that are the residuals left after grinding and extraction of the ore, are a prominent legacy of mining activity. Surface layers of mine waste at many legacy sites contain weathered and oxidized sulfide-rich tailings characterized by elevated quantities of metal(loid)s. Further, oxidative weathering of tailings originating from sulfide-rich deposits frequently results in highly acidic conditions in the absence of significant carbonate neutralization potential. In contrast, tailings that remain neutral after extended weathering typically originated with low pyrite concentrations or high carbonate neutralizing potential (Dold and Fontboté, 2001). For sulfide-rich ore tailings, conditions created by acidification, combined with poor nutrient availability and poor water-holding capacity, severely limit natural plant colonization making the tailings highly prone to eolian dispersion and water erosion (Mendez and Maier, 2008; Zornoza et al., 2017). These conditions exacerbate the potential for these sites to negatively impact neighboring communities and ecosystems.

Direct planting or phytostabilization is a reclamation technology designed to facilitate *in situ* containment of dust and metal contaminants and initiate ecosystem regeneration (Gil-Loaiza et al., 2016; Valentín-Vargas et al., 2014; Zornoza et al., 2017). Current research suggests that development of the nutrient cycling and plant growth promoting capacity of the diversity-poor, below-ground microbial communities of mine waste is critical to revegetation success (Li et al., 2016a; Li et al., 2015; Mendez et al., 2008; Mendez et al., 2007; Solís-Dominguez et al., 2012). However, strategies designed to engineer this process remain poorly understood despite the consensus that improved knowledge of the microbial ecology of mine waste is essential to sustainable plant establishment (Garris et al., 2016). Here we investigate the temporal microbial community dynamics associated with compost-assisted phytostabilization of sulfidic mine tailings with high acid-generating potential.

Freshly deposited sulfidic tailings are typically neutral to alkaline, however *in situ* weathering in semiarid regions of pyrite (FeS_2) and other iron-sulfide minerals in the tailings (Schippers et al., 2010) generally results in highly acidic conditions in near surface layers due to $O_{2(g)}$ saturated water through-flux and episodic wetting and drying cycles (Hayes et al., 2009; Hayes et al., 2014). Near complete sulfide-species depletion to depths of 50 cm has been observed on legacy sites over time frames shorter than 50 years (Hayes et al., 2009; Hayes et al., 2014), resulting in pH values ranging from 2.3 to 5.4. Research has shown that plant growth is inhibited at these acidic pH levels unless amendments are added that help buffer the pH (Mendez et al., 2007; Solís-Dominguez et al., 2012; Shu et al., 2005; Gil-Loaiza et al., 2016). As abiotic FeS_2 oxidation drives the pH below 5, tailings oxidation becomes increasingly dominated by microbially mediated catalysis, which accelerates acidification. The model for FeS_2 oxidation shows that the main precursor of acid in pyritic mine tailings is, indeed, a microbially catalyzed secondary oxidation reaction where ferric iron, rather than oxygen, serves as main oxidant (Nordstrom and Southam, 1997; Ma and Lin, 2013). This reaction is favored at pH values below 4, where ferric iron becomes soluble and, therefore, bioavailable for microorganisms to employ as an oxidant (Kirby and Brady, 1998; Johnson and Hallberg, 2005; Akcil and Koldas, 2006; Ziegler et al., 2013).

Studies focused on understanding the microbial dynamics of pyritic mine-tailing oxidation have observed correlations between microbial community structure, substrate pH and the mineralogy of the tailings (Chen et al., 2014; Chen et al., 2013; Korehi et al., 2014; Li et al., 2016a; Liu et al., 2014; Mendez et al., 2008). Highly acidic tailings (pH 2.5–3.6) support communities dominated by autotrophic Fe- and S-oxidizing populations several orders of magnitude more abundant than co-occurring heterotrophic populations, whereas less acidic tailings (pH 5.7–6.5) located at the same site harbor communities with more abundant and diverse heterotrophic populations (Korehi et al., 2014; Mendez et al., 2008; Solís-Dominguez et al., 2012). Several studies observed distinct microbial communities associated with pyritic tailings of different pH levels and variable oxidation states. It was hypothesized that the oxidation process is driven by distinct microbial assemblages at different stages of acidification (Chen et al., 2014; Chen et al., 2013; Korehi et al., 2014). In addition, phytostabilization greenhouse- and field-scale studies in highly acidic tailings have observed that plant establishment is associated with quantitative increases in neutrophilic heterotrophic populations and concurrent decreases in Fe/S oxidizing microbial taxa (Gil-Loaiza et al., 2016; Li et al., 2016a; Mendez et al., 2007; Solís-Dominguez et al., 2012).

Here we report on a highly instrumented, long-term greenhouse experiment conducted to track the biogeochemical weathering processes associated with the acidification of pyritic mine tailings and to evaluate the impact of different reclamation treatments on those dynamics. Oxidized and unoxidized pyritic tailings collected from the Iron King Mine and Humboldt Smelter Superfund (IKMHSS) site (NPL, 2008) in Dewey-Humboldt, AZ, USA (Valentín-Vargas et al., 2014; Nelson et al., 2015) were combined in a ratio (3:1) designed to simulate the surface conditions (top 40 cm) of a parallel field study at the IKMHSS site (Gil-Loaiza et al., 2016). Treatments included an unamended control, tailings amended with 15% compost, and the 15%-compost-amended tailings planted with *Atriplex lentiformis* (quailbush). Previously published results from this study (Valentín-Vargas et al., 2014) used denaturing gradient gel electrophoresis (DGGE) to profile 16S rRNA bacterial and archaeal genes and documented treatment-specific, dynamic changes in microbial community structure during the 12 month experiment. CCA analysis revealed that the most significant environmental variables influencing microbial community structure were pH and compost for bacteria, and pH and electrical conductivity (EC) for archaea. The specific objective of the present study was to employ high throughput DNA sequencing of 16S rRNA gene amplicons combined with pore water chemistry analysis to: (*i*) define transitions in diversity and phylogenetic composition of the bacterial and archaeal communities that explain the previously published temporal changes in community structure, (*ii*) characterize the effect of reclamation treatment and pore-water chemistry on the temporal dynamics of Fe/S-oxidizing microbial communities during the biogeochemical weathering of pyritic tailings, and (*iii*) advance our understanding of microbial treatments that could be developed to eco-engineer controls on the *in situ* pyrite oxidation processes. This study is the first temporal evaluation of the capacity of specific reclamation treatments to control microbial community dynamics during oxidation of pyritic mine tailings.

2. Materials and methods

2.1. Collection of mine tailings and design of greenhouse, mesocosm experiment

The source of the mine tailings used for the greenhouse experiment was the IKMHSS tailings pile; part of a highly-contaminated former mine site (NPL, 2008) located in the high desert of Northern Arizona, USA in the town of Dewey-Humboldt (34°30′02.11″N, 112°15′08.75″ W) (Hayes et al., 2014; Root et al., 2015). The IKMHSS tailings pile has an oxidized surface layer (0–20 cm depth) rich in sulfate minerals (*e.g.* jarosite, gypsum), overlying the unoxidized subsurface material (>35 cm depth) rich in iron sulfides (*e.g.* pyrite). For this study, oxidized surface tailings were collected from three locations on the pile. Unoxidized sulfide-stable subsurface tailings were collected from one location on the pile. Tailings from each sampling location were screened to 2 cm and homogenized on-site. The tailings were transported to a greenhouse facility in Tucson (AZ), where they were mixed in a 3:1 mass ratio of oxidized surface tailings to unoxidized subsurface tailings using a cement mixer. The tailings mixture was designed to create a substrate representative of the variable top 40 cm of the IKMHSS tailings pile in which