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Environmental factors influencing the structural dynamics of soil microbial communities during assisted phytostabilization of acid-generating mine tailings: A mesocosm experiment

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HIGHLIGHTS

- Bacterial, fungal and archaeal communities respond differently to revegetation.
- The three microbial groups responded differently to environmental fluctuations.
- Revegetation and compost mitigated tailings acidification and metal solubilization.
- Cobalt and pH were major drivers of change in microbial community structure.

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ABSTRACT

Compost-assisted phytostabilization has recently emerged as a robust alternative for reclamation of metalliferous mine tailings. Previous studies suggest that root-associated microbes may be important for facilitating plant establishment on the tailings, yet little is known about the long-term dynamics of microbial communities during reclamation. A mechanistic understanding of microbial community dynamics in tailings ecosystems undergoing remediation is critical because these dynamics profoundly influence both the biogeochemical weathering of tailings and the sustainability of a plant cover. Here we monitor the dynamics of soil microbial communities (*i.e.* bacteria, fungi, archaea) during a 12-month mesocosm study that included 4 treatments: 2 unplanted controls (unamended and compost-amended tailings) and 2 compost-amended seeded tailings treatments. Bacterial, fungal and archaeal communities responded distinctively to the revegetation process and concurrent changes in environmental conditions and pore water chemistry. Compost addition significantly increased microbial diversity and had an immediate and relatively long-lasting buffering-effect on pH, allowing plants to germinate and thrive during the early stages of the experiment. However, the compost buffering capacity diminished after six months and acidification took over as the major factor affecting plant survival and microbial community structure. Immediate changes in bacterial communities were observed following plant establishment, whereas fungal communities showed a delayed response that apparently correlated with the pH decline. Fluctuations in cobalt pore water concentrations, in particular, had a significant effect on the structure of all three microbial groups, which may be linked to the role of cobalt in metal detoxification pathways. The present study represents, to our knowledge, the first documentation of the dynamics of the three major microbial groups during revegetation of compost-amended, metalliferous mine tailings.

1. Introduction

Mine tailings are a primary component of mine wastes produced during ore processing for metal extraction. Legacy mine tailings are a major source of environmental contamination due to historical inefficiencies of mining technologies, which left relatively high concentrations of toxic metal(loid)s in the tailings (Dybowska et al., 2006). Due to small particle size, limited quantities of essential nutrients and organic matter, high metal(loid) content, acidic pH, and the lack of a normal soil structure, these mine tailings generally do not support plant growth or a normal soil microbial community (Alvarenga et al., 2008; Mendez and Maier, 2008b). The inability to support plants is exacerbated in arid environments due to climatic conditions and high levels of salinity (Mendez and Maier, 2008a). Thus, mine tailings in arid environments

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are especially susceptible to wind dispersion and water erosion (Kabas et al., 2011; Meza-Figueroa et al., 2009).

Several methods for the assisted growth of plants on mine tailings piles and other metal-contaminated sites in arid environments have been proposed to minimize contaminant dispersion through revegetation (Alvarenga et al., 2008; Mendez et al., 2007; Mendez and Maier, 2008a,b, Tordoff et al., 2000; Wong, 2003). Phytostabilization is one such method that employs plants to control dust emissions, stabilize tailings materials against water erosion, and reduce water percolation through contaminated materials by enhanced evapotranspiration. The use of plants to control dust emissions and minimize wind and water erosion of bare soils, especially in arid environments, is well documented (Gyssels et al., 2005; Grantz et al., 1998; Kort et al., 1998). In the case of phytostabilization, the plants used are specifically selected for their capacity to immobilize metal contaminants in the root zone rather than accumulating the metals in the shoot tissues (Mendez and Maier, 2008a,b; Solís-Domínguez et al., 2012). Solís-Domínguez et al. (2012) demonstrated decreases in tailings aqueous extractable metals following 60 days of plant growth in compost-amended tailings.

Several studies have suggested that root-associated microbes may be important for phytostabilization as microbes can facilitate plant establishment on tailings, enhance plant biomass production and potentially serve as bioindicators of revegetation status (Grandlic et al., 2008; Ma et al., 2011; Mendez et al., 2008; Solís-Domínguez et al., 2011). Still, little is known about the long-term dynamics of microbial communities during the assisted revegetation of mine tailings and, specifically, how the microbial communities may: 1) respond to the remediation treatment, 2) influence the stabilization of the metal contaminants, and 3) influence the success of plant establishment.

Previous work has shown that tailings amendment and plant growth during the revegetation of mine wastes have a significant impact on the composition, abundance and stability of portions of the soil microbial communities (Pérez-de-Mora et al., 2006; Li et al., 2013; Mummey et al., 2002; Rosario et al., 2007). Pérez-de-Mora et al. (2006) and Li et al. (2013) independently showed that plant selection for the revegetation of metal-contaminated land had a stronger effect on microbial community composition and diversity than the type of soil amendment used. Additional studies have shown that plant growth and addition of soil amendments can significantly increase the abundance of heterotrophs and decrease the abundance of chemolithotrophs in mine tailings, thus improving soil health (Mendez et al., 2007; Solís-Domínguez et al., 2012).

An important limitation of previous revegetation-research efforts is the focus on a single microbial group, usually bacteria, rather than simultaneously analyzing multiple microbial groups (*e.g.* fungi, archaea) (Gremion et al., 2004; Pérez-de-Mora et al., 2005; Solís-Domínguez et al., 2011). Numerous studies have demonstrated that the dominant soil microbial groups, bacteria, fungi, and archaea, behave differently in their response to environmental stressors, and in their influence on the functioning of an ecosystem (Hayden et al., 2012; Navarrete et al., 2010; Pereira-e-Silva et al., 2012; Reed and Martiny, 2007). Therefore, it is crucial that a comprehensive study of the dynamics of microbial communities in mine tailings during revegetation monitors multiple microbial groups simultaneously, as each group may respond differently to various aspects of the remediation treatment.

The objective of this study was to evaluate the relationship between microbial community structure and phytostabilization outcomes in a well-instrumented mesocosm experiment using metalliferous acid mine tailings. We hypothesize that changes in key environmental parameters resulting from compost amendment and plant establishment will drive group-specific changes in the structure of root-associated bacterial, fungal and archaeal communities during revegetation of acidic mine tailings. To test this hypothesis we employed community DNA fingerprinting analysis of small subunit bacterial, archaeal and fungal RNA genes in combination with multivariate analysis of the influence of environmental parameters on community structure in a one year greenhouse experiment.

2. Materials and methods

2.1. Iron King Mine tailings

Tailings used for this study were collected and homogenized from both the oxidized surface layer and the underlying reduced zone at the Iron King Mine and Humboldt Smelter Superfund Site (IKMHSS), in Dewey-Humboldt, Arizona (34°30′02.11″N, 112°15′08.75″W). The IKMHSS was added to the National Priorities List in 2008 due to the exposure risks associated with high levels of arsenic (2590 mg kg^{-1}) and lead (2200 mg kg^{-1}) in the surface layer of the tailings pile (EA-EST, 2010). The oxidized surface layer (0 to 25 cm) of the tailings is highly acidic (pH 2.3 to 3.7) while the reduced zone (>25 cm) has a higher pH (5.5 to 6.3). The IKMHSS tailings also have high levels of salinity, low organic carbon (0.014%) and nitrogen (0.043%) contents, and a stressed microbial community dominated by chemolithoautotrophic microbial populations (Solís-Domínguez et al., 2012). These microbial populations are primarily iron- and sulfur-oxidizers, which diverge substantially from the heterotrophic populations commonly found in healthy soil ecosystems (Mendez et al., 2008; Tan et al., 2008). Chemolithoautotrophic microbial activity is supported by the pyritic composition of the tailings and is the driving force behind the low pH of the surface tailings layer, a condition that influences metal(loid)s solubility and limits plant growth on the tailings (Mendez et al., 2007; Schippers et al., 2010; Solís-Domínguez et al., 2012). The net acid producing potential (NAPP) was determined for the tailings used in this mesocosm study as described by Solís-Domínguez et al. (2011). NAPP = acid potential (AP)-acid neutralizing capacity (ANC).

2.2. Iron King Mine tailings and setup of greenhouse experiment

A 12-month greenhouse mesocosm study was conducted to evaluate changes in physical, chemical and biological parameters during plant establishment in compost-amended IKMHSS mine tailings. The experiment consisted of 4 treatments that were monitored for 1 year under controlled conditions at the Controlled Environment Agricultural Center (CEAC) at the University of Arizona (Tucson, AZ). Twelve large polypropylene containers (ProPlastics, Chandler, AZ, USA), measuring 1 m in diameter and 0.5 m in depth, were custom-built to serve as mesocosms (Fig. 1). Each mesocosm was equipped with pore water samplers that operated under constant tension (5–15 kPa), with a pore size of 2 μm and a sampling area of 33 cm^2. Samplers were placed at 10 cm depth intervals from 5 cm to 35 cm. The following four treatments were examined in triplicate and arranged in a spatially randomized design (Fig. 1): (*i*) Tailings only (TO); (*ii*) Tailings mixed with 15% dry-weight/dry-weight (w/w) compost (TC); (*iii*) Tailings mixed with 15% (w/w) compost and seeded with *Buchloe dactyloides* (buffalo grass) (BG); and (*iv*) Tailings mixed with 15% (w/w) compost and seeded with *Atriplex lentiformis* (quail bush) (QB). These native plant species were selected based on their salinity tolerance and ability to grow in IKMHSS tailings amended with 15% (w/w) compost without accumulating elevated levels of metals in their shoots (Solís-Domínguez et al., 2012).

Surface and subsurface tailings were homogenized using a rotating cement mixer in a 3:1 ratio of oxidized surface tailings (collected from 0–20 cm depth) and reduced subsurface tailings (collected from >35 cm depth) in order to create a substrate representative of the variable top 40 cm of the IKMHSS tailings pile. Amended treatments contained the tailings mixture homogenized with 15% (w/w) compost made from a mixture of composted cattle manure and green waste (Arizona Dairy Compost LLC, Anthem, AZ) and composted steer manure (El Toro De-Odorized Steer Manure, Tempe, AZ). All materials were sieved to 0.5 cm. The unamended tailings were packed to a depth of