



Temporal evolution of bacterial communities associated with the in situ wetland-based remediation of a marine shore porphyry copper tailings deposit



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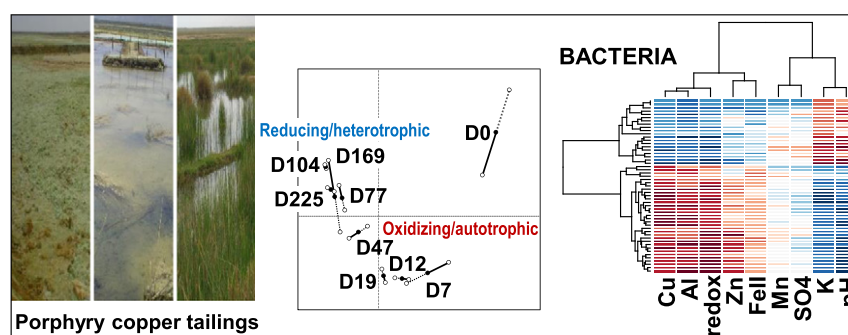
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HIGHLIGHTS

- Time-dependent sequence of major bacterial taxa during mine tailings bioremediation.
- Statistics showed strong links between diversity and ecosystem functioning.
- Populations significantly correlated with pH, redox, and K concentration.
- Strong dependency on constant water flow for the stabilization of the wetland.

GRAPHICAL ABSTRACT



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ABSTRACT

Mine tailings are a serious threat to the environment and public health. Remediation of these residues can be carried out effectively by the activation of specific microbial processes. This article presents detailed information about temporal changes in bacterial community composition during the remediation of a section of porphyry copper tailings deposited on the Bahía de Ite shoreline (Peru). An experimental remediation cell was flooded and transformed into a wetland in order to prevent oxidation processes, immobilizing metals. Initially, the top oxidation zone of the tailings deposit displayed a low pH (3.1) and high concentrations of metals, sulfate, and chloride, in a sandy grain size geological matrix. This habitat was dominated by sulfur- and iron-oxidizing bacteria, such as *Leptospirillum* spp., *Acidithiobacillus* spp., and *Sulfobacillus* spp., in a microbial community which structure resembled acid mine drainage environments. After wetland implementation, the cell was water-saturated, the acidity was consumed and metals dropped to a fraction of their initial respective concentrations. Bacterial communities analyzed by massive sequencing showed time-dependent changes both in composition and cell numbers. The final remediation stage was characterized by the highest bacterial diversity and evenness. Aside from classical sulfate reducers from the phyla δ -Proteobacteria and Firmicutes, community structure comprised taxa derived from very diverse habitats. The community was also characterized by an elevated proportion of rare phyla and unaffiliated sequences. Numerical ecology analysis confirmed that the temporal population evolution was driven by pH, redox, and K. Results of this study demonstrated the usefulness of a detailed follow-up of the

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remediation process, not only for the elucidation of the communities gradually switching from autotrophic, oxidizing to heterotrophic and reducing living conditions, but also for the long term management of the remediation wetlands.

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1. Introduction

A total of 1150 million tons of metals have been exploited since the Stone Age, generating substantial amounts of mine waste (Sheoran and Sheoran, 2006). Both composition and volume of waste minerals have changed due to the development of modern mining techniques. Nowadays, ores are crushed and milled to reduce grain size. Records showed that up to 80%–99% of the crushed ore may be set apart and dumped as tailings waste or mixed with water and transported in suspension for a final disposal in tailings impoundments (Sheoran and Sheoran, 2006). Acid mine drainage (AMD) forms when sulfide minerals are oxidized on exposure to oxygen (Baker and Banfield, 2003). While the rate of AMD formation is generally slow in massive rock assemblages, the process is accelerated as a result of grinding and mixing with chemical reagents. However, sulfide mineral oxidation is limited as long as the ground material remains water-saturated, preventing a quick access to oxygen. In unsaturated zones, microbial-enhanced oxidative dissolution of sulfide minerals is a prime cause of water pollution (Dold and Fontboté, 2001; Johnson and Hallberg, 2003). AMD formation is particularly evident in the case of copper porphyry mine tailings, which typically contain 0.4%–4% sulfur, mainly contained in pyrite (Dold and Weibel, 2013). In arid area such as the Atacama Desert (Peru), metal mobilization by sulfide-oxidizing bacteria result in the transportation toward the tailings surface by evaporation. Metal precipitation occurs in the form of secondary chloride and/or sulfate minerals (Diaby, 2009; Dold, 2006; Dold et al., 2011; Smuda et al., 2014). These salts can be dispersed through Aeolian transport and may contaminate adjacent areas (Dold, 2006).

Johnson (2012) showed that chemoautotrophic Bacteria and Archaea are often the most numerous microorganisms in low pH environments. The majority of these acidophilic organisms use oxygen as main terminal electron acceptors, with Fe(III) under oxygen-limited conditions. *Acidithiobacillus* spp., *Sulfobacillus* spp., and *Leptospirillum* spp. are well known for their abilities to use reduced forms of Fe and S and use them as sources of energy (Baker and Banfield, 2003; Johnson and Hallberg, 2003; Kimura et al., 2011). Recent advances showed that other organisms (for instance the archaean *Ferroplasma* spp. and the bacterial *Leptospirillum* Group III) were dominant in certain specific mine environments (Kuang et al., 2013; Ziegler et al., 2013). According to Ñancuqueo and Johnson (2012), these organisms display minimal growth requirements and tolerate high concentrations of dissolved metals in acidic solutions.

AMD formation can be avoided by limiting the contact between mine tailings and oxygen, for instance by submerging the tailings deposit in a reducing environment, e.g. a constructed wetland (Johnson and Hallberg, 2005). As a passive system, constructed wetlands are particularly attractive due to low operating cost (Mayes et al., 2009) and are now a technology with potential success when deep reducing conditions are attained (Sheoran and Sheoran, 2006; Ñancuqueo and Johnson, 2012). Constructed wetlands have been successfully applied to some mining environments to remove contaminants from water, especially from metal-rich effluents (Diaby and Dold, 2014). The geochemical processes involved in this technique are based on sulfur and sulfate reduction activities, mediated or accelerated by a heterotrophic microbial community fueled with organic carbon produced by the aquatic plants growing in the wetland. Sulfate reduction is a very attractive method for AMD mitigation because it combines the reduction of metals, which is essential to their immobilization, the depletion of sulfate anions as electron acceptor and finally the production of alkaline potential by the production of bicarbonate ions (Moreau et al., 2013).

Sulfate-reducing bacteria have been isolated from pyrite-rich tailings and are likely involved in sulfur cycling in these habitats (Giloteaux et al., 2013). At present, few extreme acidophilic microorganisms that grow via dissimilatory reduction of sulfate and/or elemental sulfur have been described (Johnson, 2012). Among them, most of the organisms showed high temperature preferences, for instance the Crenarchaeota *Acidianus* spp., *Stygiolobus* spp., *Sulfurisphaera* spp. or the Euryarchaeota *Thermoplasma* sp. and uncultured ARMAN (Archaeal Richmond Mine Acidophilic Nanoorganism). Among Bacteria, recent reports mentioned the presence of extremely acidophilic Firmicutes taxa, such as *Desulfosporosinus* spp. and *Desulfitobacterium* spp. (Koschorreck et al., 2010; Johnson, 2012). As pH rises above 4, the diversity of sulfate-, but also Fe(III)- and Mn(IV)-reducing acidophilic bacteria increases with guild members of the δ -Proteobacteria (*Desulfomonile* spp., *Desulfobacterium* spp., *Desulfosporosinus* spp., *Desulfitobacterium* spp., and *Geobacter* spp.) (Koschorreck et al., 2010; Burns et al., 2011; Sánchez-Andrea et al., 2013). Other lineages were also found, such as members of the phylum Nitrospirae (*Thermodesulfobrevibrio* spp. and *Thermodesulfobacterium* spp.). The presence of species traditionally belonging to neutrophilic lineages in acidified habitats could in this case be explained either by the presence of microhabitats or the existence of acidotolerant strains (Sánchez-Andrea et al., 2014).

At present, little information is available on the mechanisms with which natural wetland systems mediate the conversion of metals and acidity on the long term (Moreau et al., 2013). Relationships between wetland stability, remediation capacity and the biological diversity remain unclear (Dean et al., 2013). In this project, we present temporal changes observed during the successful remediation of a section of a marine shoreline tailings deposit, the Bahía de Ite tailings, located in the Atacama Desert (Peru). Major sections of the tailings were remediated using a constructed wetland (ca. 1000 ha) built on the oxidizing tailings (Diaby, 2009; Dold et al., 2011). For this study, a remediation cell (ca. 30 by 30 m) was constructed on a remaining oxidized section of the tailings (Diaby, 2009; Diaby and Dold, 2014). Chemical analysis and a comprehensive survey of the microbial community present in the tailings were conducted over a period of nine months, starting at the beginning of the flooding (Diaby and Dold, 2014). Goals of this study were first to assess in details the time-dependent changes observed in the bacterial populations. Secondly, the investigation aimed to assess the significance of controlling geochemical parameters affecting the populations in order to evaluate possible long-term stability of the remediated tailings section. Careful attention was paid to the long-term presence of facultative aerobic organisms (such as *Acidithiobacillus* spp.) involved in AMD formation. Elucidation of the relationships between community composition and habitat evolution are anticipated to contribute to a better understanding of the processes occurring during the rapid evolution of the remediated area. Outcomes from this detailed survey include the development of strategies required for the implementation and the long term management of constructed wetlands applied to the remediation of tailings impoundments.

2. Material and methods

2.1. Background about Bahía de Ite

Between 1960 and 1997, the Bahía de Ite (Atacama Desert, about 50 km south of Ilo, Southern Peru) received about 785 million metric tons of tailings from two porphyry copper mines, Cuajone and Toquepala, operated by the Southern Peru Copper Corporation (SPCC). Grinded mine waste were sent via the Locumba River for final shore

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