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Main factors controlling microbial community structure and function after reclamation of a tailing pond with aided phytostabilization

R. Zornoza [a,*], J.A. Acosta [a], S. Martínez-Martínez [a], A. Faz [a], E. Bååth [b]

[a] *Sustainable Use, Management, and Reclamation of Soil and Water Research Group, Department of Agrarian Science and Technology, Universidad Politécnica de Cartagena, Paseo Alfonso XIII, 48, 30203 Cartagena, Spain*

[b] *Microbial Ecology, Department of Biology, Ecology Building, Lund University, SE-223 62 Lund, Sweden*

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ABSTRACT

Reclamation on bare tailing ponds has the potential to represent soil genesis in Technosols favoring the understanding of the changes of microbial communities and function. In this study we used phytostabilization aided with calcium carbonate and pig slurry/manure to reclaim an acidic bare tailing pond with the aim of investigating the effect of amending and different species on microbial community structure and function. We sampled after two years of amending and planting: unamended tailing soil (UTS), non-rhizospheric amended tailing soil (ATS), rhizospheric soil from four species, and non-rhizospheric native forest soil (NS), which acted as reference. The application of amendments increased pH up to neutrality, organic carbon (Corg), C/N and aggregate stability, while decreased salinity and heavy metals availability. No effect of rhizosphere was observed on physicochemical properties, metals immobilization and microbial community structure and function. To account for confounding effects due to soil organic matter, microbial properties were expressed per Corg. The high increments in pH and Corg have been the main factors driving changes in microbial community structure and function. Bacterial biomass was higher in UTS, without significant differences among the rest of soils. Fungal biomass followed the trend UTS < ATS = rhizospheric soils < NS. Bacterial growth increased and fungal growth decreased with increasing pH, despite the high availability of metals at low pH. Enzyme activities were lower in UTS, with β-glucosidase and β-glucosaminidase activities highly correlated with bacterial growth. Microbial activities were not correlated with the exchangeable fraction of heavy metals, indicating that microbial function is not strongly affected by these metals, likely due to the efficiency of the reclamation procedure to reduce metals toxicity. Changes in microbial community composition were largely explained by changes in pH, heavy metals availability and Corg, with increments in fungal and actinobacterial proportions with soil amending.

1. Introduction

Metalliferous mine tailing ponds have numerous restrictions affecting their future development into natural soils, such as extreme low pH, high concentrations of metals and low organic matter content. They are also of environmental concern because of the potential hazards of surface or groundwater pollution by heavy metals and metalloids, offsite contamination by wind-transported materials or runoff, and the uptake by vegetation and bioaccumulation in food chains (Zanuzzi et al., 2009). Therefore, it is necessary to take actions towards remediation of this pollution. Aided phytostabilization takes its place as a reclamation technique in the stabilization of soil metals by the use of vegetation and soil amendments. Alkaline materials such as calcium carbonate are commonly used as an amendment for ameliorating the acidic conditions of many acid-generating mine wastes and for immobilizing metals in the form of (metal)carbonates, mitigating metal toxicity (Zornoza et al., 2013). Organic wastes such as sewage sludge, compost or animal manure can be used as nutrient and organic matter sources which stimulate soil formation (Zanuzzi et al., 2009).

Soil microbial communities are of critical importance to the ecological functioning of an ecosystem (Mummey et al., 2002). Soil microorganisms control ecosystem functioning as mediators of organic matter decomposition, C stabilization and nutrient cycling (Coleman and Whitman, 2005). At present, our understanding of soil microbial composition and function during ecosystem development is limited in comparison to our understanding of the succession processes of plant communities. Since bacterial and fungal communities have different pH preferences (Rousk et al., 2010a), the addition of alkaline amendments, which drastically increases soil pH, will alter the microbial community structure. So, measures of the fate of the microbial community following the initiation of reclamation efforts could serve as an indicator of restoration progress and may give insights into potential ways to accelerate a restoration (Harris et al., 1991).

We used phospholipid fatty acid (PLFA) analysis to measure microbial community composition. PLFA analysis uses the lipids of the microbial

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* Corresponding author.
E-mail address: raul.zornoza@hotmail.com (R. Zornoza).

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membranes as biomarkers for specific groups of microorganisms, besides creating a profile or finger print of the community structure. As a consequence, rapid changes in soil microbial community structure can be detected by changes in the PLFAs pattern (Zelles, 1999). Bacterial growth was measured using ^{3}H-leucine incorporation into extracted bacteria, which uses an estimate for protein synthesis as an index for in situ bacterial growth rate (Bååth et al., 2001). Fungal growth was measured using ^{14}C-acetate incorporation into ergosterol, which uses an estimate of ergosterol synthesis as an index for in situ fungal growth rate (Bååth, 2001).

As a general rule, for the establishment of a self-sustaining vegetation cover by the help of aided phytostabilization, native species that are adapted to the specific conditions of the area are a preferred option, since they have usually higher ameliorate capacity on the mine soil (Mukhopadhyay et al., 2013). Hence, plant screening is a prerequisite for successful reclamation. However, little information is available on the combined effect of wastes amendment and plant species on soil microbial community composition and function during the reclamation of mine areas. The rhizosphere can enhance microbial activity because plants roots release large amounts of the photosynthate captured, most of which is available to the microbial community (Söderberg et al., 2002). Since root exudates and debris can differ between plant species, microbial growth and activity may also be dependent of plant species (Söderberg et al., 2002), and changes in plants composition can alter belowground microbial diversity (Warembourg et al., 2003; Garcia et al., 2005). Chodak and Niklińska (2010) reported that soil microbial biomass, respiration and enzyme activities highly depended on the specific plant species, but the community level physiological profiles depended on soil texture.

Most of the studies on the reclamation of metal polluted soils through aided phytostabilization have been performed under laboratory conditions or focused on changes in chemical properties or microbial biomass and enzyme activities. In this study we aimed at investigating: (i) the effect of amending and plant species on compositional and functional attributes of the microbial community under field conditions on a mine tailing pond; and (ii) the extent to which the composition of the microbial community depends on physicochemical soil properties. We hypothesized that even if there may be a link between microbial community structure and function and plant species, the main variability must be driven by differences in physicochemical properties such as organic C, pH, nutrients and available heavy metals.

2. Materials and methods

2.1. Study site

The experiment was established in a tailing pond at Cartagena-La Unión Mining District (SE Spain; 37° 35′ 38″N, 0° 53′ 11″W). The climate of the area is semiarid Mediterranean with mean annual temperature of 18 °C and mean annual precipitation of 275 mm. The potential evapotranspiration rate surpasses 900 mm year^{-1}. Soil is classified as a Spolic Technosol (Toxic) (IUSS, 2014), with sandy loam texture. The tailing pond has an area of 14,095 m^{2}, and the soil remained bare, with pH = 3.35, 0.08% soil organic matter (SOM) and high levels of heavy metals. The concentrations of total metals were 13.1 g kg^{-1} for Al, 120 g kg^{-1} for Fe, 653 mg kg^{-1} for Mn, 12.5 mg kg^{-1} for Ni, 40.8 mg kg^{-1} for Cu, 1570 mg kg^{-1} for Zn, 1.43 mg kg^{-1} for Cd and 1220 mg kg^{-1} for Pb.

2.2. Experimental design

The tailing pond was plowed and leveled to create uniform surface soil conditions prior to conducting the experiment. A reclamation strategy was carried out in ~2/3 of the total surface based on the use of three different amendments (raw pig slurry (PS), pig manure (M) and marble waste (MW)), in order to increase soil organic matter and soil nutrients, decrease heavy metals availability, ameliorate soil structure, neutralize potential acidity generated by sulfides, and facilitate microbial and vegetation growth. The characteristics of the amendments are given in Table 1.

The marble waste (6.7 kg m^{-2}) was formed by particles of 5–10 μm diameter, and was applied in July 2011. This rate was calculated to establish the quantity of calcium carbonate required to neutralize all the potential acid according to the percentage of sulfides present in the mine soil (Sobek et al., 1978). We applied the organic amendments in three different episodes to favor a suitable stabilization of organic matter in soil before introduction of vegetation. We applied 1.7 L m^{-2} PS in July, 2.6 L m^{-2} PS in September and 7 kg m^{-2} M in November 2011. The PS dose was based on the agronomic rate established by Spanish legislation RD 261/1996 (framed within the European Directive 91/676/CEE), to avoid contamination of groundwater by nitrates. The M dose was calculated on the basis of its organic carbon content to increase soil organic carbon to >5 g kg^{-1}. This solid manure was obtained after separation of the solid phase of the PS from the liquid phase using a physical phase separator. The solid fraction was air-dried outdoor under environmental conditions for four weeks. After the mechanical application of the amendments, all materials were mixed to a depth of 0–50 cm to incorporate the amendments into the soil.

Twelve Mediterranean native species were introduced in March 2012 for phytostabilization. Seedlings (15–20 cm in height) of *Atriplex halimus* L., *Cistus albidus* L., *Helichrysum decumbens Cambess.*, *Hyparrhenia hirta* (L.) Stapf, *Lavandula dentata* L., *Lygeum spartum* (L.) Kunth., *Rosmarinus officinalis* L. and *Phagnalon saxatile* (L.) Cass. were manually and randomly planted at a density of 1 plant per m^{2} to create a mosaic landscape. The species *Dittrichia viscosa* (L.) Greuter, *Piptatherum miliaceum* Beauv., *Limonium caesium* (Girard) Kuntze and *Cynodon dactylon* (L.) Pers. were homogenously sown covering the surface of the amended area. Three irrigations were carried out during summer 2012 owing to the extreme drought to avoid high mortality; after that, no water was added and plants were exposed to the semiarid climatic conditions of the study area.

Approximately a third of the tailing pond surface was kept unamended and unplanted, acting as control. An adjacent area under natural vegetation was used as a standard for local native soil, acting as reference. The reference soil is classified as a Leptic Calcaric Regosol (IUSS, 2014), with sandy clay loam texture, 66% calcium carbonate, 4.58% SOM and pH = 8.45. This natural area has higher altitude than the tailing pond, therefore no pollution of metals by water transport is expected. The vegetation cover in this area is 80%, with the most dominant plant species being *Calicotome infesta* (C. Presl) Guss., *Asparagus horridus* L., *Brachypodium retusum* (Pers.) Beauv., *Chamaerops humilis* L., *Cistus lavandulifolius* Lam., *H. decumbens Cambess.*, *Pinus halepensis* Mill., *R. officinalis* L. and *Stipa tenacissima* L. In each of the three different study areas (unamended tailing pond, amended tailing pond and native soil), we established four plots (10 m × 10 m) to monitor soil and

Table 1
Main characteristics of the amendments used for reclamation purposes.

Parameters	Pig slurry	Pig manure	Marble waste
pH	7.8	9.1	8.0
Electrical conductivity (dS m^{-1})	39.1	10.2	2.2
Calcium carbonate (%)	–	–	99
Moisture (%)	96	10	1
Total organic carbon (g L^{-1}/g kg^{-1})	17.8	171	–
Total nitrogen (g L^{-1}/g kg^{-1})	5.1	13.6	–
C/N	3.5	12.5	–
Copper (mg L^{-1}/mg kg^{-1})	19.3	157	0.36
Zinc (mg L^{-1}/mg kg^{-1})	28.0	732	0.26
Available phosphorus (mg L^{-1}/mg kg^{-1})	623	9.64	<d.l.
Calcium (mg L^{-1}/mg kg^{-1})	249	855	2190
Magnesium (mg L^{-1}/mg kg^{-1})	14.4	802	347
Sodium (mg L^{-1}/mg kg^{-1})	459	4280	69
Potassium (mg L^{-1}/mg kg^{-1})	1059	15,700	59

<d.l.: below detection limit.