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Microbial growth and community structure in acid mine soils after addition of different amendments for soil reclamation

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ABSTRACT

The extreme soil conditions in metalliferous mine soils have a negative influence on soil biological activity. Therefore, amendments are often used to improve soil quality and activate microbial communities. In order to elucidate some of the factors controlling microbial growth and community structure after application of amendments in acid mine soils, we performed an incubation experiment with four amendments: pig slurry (PS), pig manure (PM) and pyrogenic carbonaceous material (PCM), applied with and without marble waste (MW; $CaCO_3$). Results showed that PM and PCM (alone or together with MW) contributed to an important increase in recalcitrant organic C, C/N ratio and aggregate stability. All the treatments, except PS without MW increased soil pH above six resulting in the partial immobilization of the metals. Bacterial and fungal growths were highly dependent on pH and labile organic C. Pig slurry supported the highest microbial growth: applied alone stimulated fungal growth, whereas applied with MW stimulated bacterial growth. Pyrogenic carbonaceous material provoked the lowest microbial growth, especially for fungi, with no significant increase in fungal biomass. MW + PCM increased bacterial growth up to values similar to PM and MW + PM, suggesting that, at least in the short term, part of the PCM was degraded, and mainly by bacteria rather than fungi. PM, MW + PS and MW + PM supported the highest microbial biomass and a similar community structure, related with the presence of high organic C concentrations and high pH, with immobilization of metals and increased soil quality. PCM contributed to improved soil structure, increased recalcitrant organic C, and decreased metal mobility, with low stimulation of microbial growth. Thus, in order to activate microbial populations in reclaimed mine soils, a source of labile organic compounds should be included in the management plan of the area.

1. Introduction

Metalliferous mine soils have numerous restrictions affecting their development into natural soils, such as extremely low pH, high concentrations of metals and extremely low organic matter content (Zanuzzi et al., 2009; Martínez-Pagán et al., 2011). The importance of soil microbial communities for successful plant establishment and growth has been demonstrated by numerous studies (Kulmatiski et al., 2008; Epelde et al., 2010). However, the extreme soil conditions caused by mining activities usually have a negative influence on soil biological activity (Asensio et al., 2013; Zornoza et al., 2013). Therefore, there is a need to develop strategies to improve the soil quality of former mine tailing areas to guarantee ecosystem reclamation. One of the most common strategies involves using different amendments (Kabas et al., 2012; Asensio et al., 2013; Zornoza et al., 2013; Pardo et al., 2014). Alkaline materials are commonly used to ameliorate the acid conditions of many acid-generating mine wastes and for immobilizing metals as carbonates, mitigating metal toxicity (Kabas et al., 2012; Zornoza et al., 2012). Organic residues are also commonly used as amendments because the addition of organic matter can significantly improve the soil structure, the nutrient status, stimulate microbial populations and, in certain circumstances, reduce the availability of toxic metals through complexation (Ye et al., 2002; Senesi et al., 2007).

A correct management of the application of amendments in soil reclamation relies mainly on two aspects: efficient increase of the soil organic matter, and adequate match of the release of mineral nutrients to vegetation demand. Soil microbial community structure and activity can change in response to the quality of organic amendments. Thus, soil processes mediated by microorganisms can also change depending on the shifts in the microbial community structure and activity (e.g. Lucas et al., 2014). Furthermore, since bacteria and fungi have different pH preferences (Rousk et al., 2009), the addition of alkaline amendments, which drastically increase soil pH, is expected to alter the balance of fungal/bacterial growth and the microbial community structure, which may change the nature and magnitude of soil processes related to specific microbial groups.

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Livestock wastes have commonly been used as organic amendments (Zornoza et al., 2013; Pardo et al., 2014). Before being applied to soil, organic wastes must be properly treated to avoid possible deleterious effects on soil properties and to reduce environmental or health hazards associated with raw wastes (Fernández et al., 2012). Methods to improve the management of livestock slurries to reduce the environmental impact and carbon/nitrogen losses are gaining importance. The pyrogenic carbonaceous material (PCM) production through the pyrolysis of manures may become an interesting solution for successful waste management due to its high recalcitrant carbon content, which can increase the content of stable organic carbon in soil and contribute to carbon sequestration (Marchetti et al., 2012). Recent research shows that PCM may be more resistant to decomposition relative to other organic materials (Lehmann and Joseph, 2009; Fellet et al., 2011), and would immobilize contaminants for a much longer period, thus protecting plants and microbes against toxicity over time (Beesley et al., 2011; Park et al., 2011). Measures of the fate of the microbial community following the initiation of reclamation efforts may serve as an indicator of restoration progress and give insights into potential ways to accelerate restoration (Harris et al., 1991). The current information on the effect of amendments on microbial communities indicates that amending tends to alter microbial community composition and activity, but the effect is not univocal, with very different results obtained for different amendments, soil types and management practice (e.g. Baker et al., 2011; Lehmann et al., 2011; Epelde et al., 2014; Kelly et al., 2014).

In order to elucidate the main factors controlling microbial growth after application of amendments to mine tailing materials, we performed an experiment with four different amendments: pig slurry, pig manure and pyrogenic carbonaceous material, with and without $CaCO_3$ to raise soil pH. Our objectives were to: i) assess changes in fungal and bacterial growth and microbial community structure; and ii) investigate the extent to which microbial community composition and activity depends on physicochemical soil properties. We hypothesized that the fungal and bacterial growth would be different depending on the soil pH and quality of the organic amendment used. The addition of the amendment with the most labile organic compounds such as pig slurry would favor a faster growth and different microbial community structure than pyrogenic carbonaceous material.

2. Materials and methods

2.1. Soil and amendments used

A soil from a tailing pond at the Mining District of Cartagena-La Unión (SE Spain) (37°35′38″ N, 0°53′11″ W) was selected. It was characterized by absence of vegetation, acid conditions, high metal(loid) concentrations and low organic carbon and nutrient contents. The climate of the area is semiarid Mediterranean, with mean annual temperature of 18 °C and mean annual rainfall of 275 mm. The soil is classified as a Spolic Technosol (Toxic) (IUSS, 2014), with sandy loam texture. Soil was collected from the top 20 cm, air-dried for seven days, and sieved <2 mm for incubation experiments.

Three organic amendments with different organic carbon content and stability (raw pig slurry, pig manure and pyrogenic carbonaceous material) and an inorganic liming material (marble waste ($CaCO_3$)) were used for reclamation purposes. Pig slurry (PS) was obtained from a pig farm located in Cartagena (SE Spain). The pig manure (PM) was obtained after separation of the solid phase of the raw pig slurry from the liquid phase using a fan press screw separator of 5 m^3 h^{-1} (Westfalia, Germany). The solid fraction was outdoor air-dried under natural conditions for one month and sieved <2 mm after sampling. Pyrogenic carbonaceous material (PCM) was obtained by pyrolysis of the solid manure under oxygen-limited conditions in a muffle furnace. The temperature was gradually increased at 5 °C min^{-1} to 420 °C, and maintained for 30 min at this temperature. At termination, the PCM was cooled overnight. The marble waste (MW; formed by particles of 5–10 μm diam.) was collected from quarries in Cehegín (SE Spain). Soil and amendments characteristics are shown in Table 1.

2.2. Soil incubation

Eight different treatments were applied to soil: unamended tailing soil used as control (CT), PS, PM, PCM, MW + CT, MW + PS, MW + PM and MW + PCM. Organic amendments were thoroughly mixed with dry tailing soil at a dose of 20 g C kg^{-1} soil, which is the organic C content in the native shrubland soils of the area (Martínez-Martínez et al., 2013). The MW was added to dry soil in a rate of 50 g kg^{-1}. This rate was calculated using the method proposed by Sobek et al. (1978), which provides an indication of the quantity of $CaCO_3$ required to neutralize the acidification potential of the soil, according to the percentage of sulfides present in the mine soil, to reach a final pH of seven. Water was then added to achieve 50% of the water holding capacity of the soil.

Laboratory incubations (four replicates per treatment) were carried out in plastic pots kept in the dark, at constant humidity (50% of water holding capacity) and temperature (22 °C), and under aerobic conditions for 40 days. Soils were sampled to monitor pH, bacterial and fungal growth, respiration and ergosterol concentration at 0, 2, 5, 9, 15, 23, 30, and 40 days of incubation. Ergosterol is a fungal-specific lipid from the cell-membrane used as proxy of fungal biomass (Frostegård and Bååth, 1996). The first time-point was collected just after rewetting. Microbial community composition assessed as abundances of phospholipid fatty acids (PLFAs), organic carbon fractions, aggregate stability, total N and metal(loid) fractionation were assayed at the end of incubation.

2.3. Microbial measurements

Bacterial growth was measured with the ^{3}H-leucine (Leu) incorporation technique on bacteria extracted by homogenization/centrifugation (Bååth, 1994; Bååth et al., 2001). The relative bacterial growth was expressed as pmol Leu incorporated into extracted bacteria g^{-1} h^{-1}. Fungal growth was measured by the ^{14}C-acetate (Ac) incorporation into ergosterol method (Newell and Fallon, 1991)

Table 1
Properties and total concentration of metal (loid)s for mine tailing soil and amendments used. Values are expressed on a dry weight basis, except for pig slurry which on a volume basis.

Property	CT	PS	PM	PCM	MW
pH	3.2	6.9	7.3	8.8	8.0
Electrical conductivity (dS m^{-1})	3.84	21.9	6.81	3.71	2.23
$CaCO_3$ (%)	<d.l.	n.d.	n.d.	n.d.	99
Total organic carbon (g kg^{-1}/g L^{-1})	0.5	55	211	253	n.d.
Total N (g kg^{-1}/g L^{-1})	0.2	11.6	18.9	16.4	n.d.
Available P (g kg^{-1}/g L^{-1})	<d.l.	1.8	15.1	21.1	<d.l.
Al (g kg^{-1}/g L^{-1})	15.7	0.3	9.4	8.5	2.2
As (mg kg^{-1}/mg L^{-1})	271	0.8	4.8	5.2	<d.l.
Cd (mg kg^{-1}/mg L^{-1})	1.2	0.08	0.3	0.4	0.05
Co (mg kg^{-1}/mg L^{-1})	4.7	0.3	5.2	5.6	<d.l.
Cr (mg kg^{-1}/mg L^{-1})	19.5	3.4	16.1	19.2	0.15
Cu (mg kg^{-1}/mg L^{-1})	40.8	61.9	265	347	0.36
Fe (g kg^{-1}/g L^{-1})	149	1.5	10.4	12.4	5.9
Mn (mg kg^{-1}/mg L^{-1})	653	54	451	533	147
Ni (mg kg^{-1}/mg L^{-1})	12.5	2.4	15.8	19.2	<d.l.
Pb (mg kg^{-1}/mg L^{-1})	1225	1.2	14.3	18.6	<d.l.
Sb (mg kg^{-1}/mg L^{-1})	1.68	0.26	0.31	0.34	<d.l.
Se (mg kg^{-1}/mg L^{-1})	0.34	0.37	1.03	1.25	<d.l.
Zn (mg kg^{-1}/mg L^{-1})	1570	90	1430	1880	0.3

n.d.: not determined.
CT: control (unamended tailing soil); MW: marble waste; PS: pig slurry, PM: pig manure; PCM: pyrogenic carbonaceous material.
<d.l.: below detection limit ($CaCO_3$: 0.07%; P: 60 μg kg^{-1}; As: 7 μg kg^{-1}; Co: 3 μg kg^{-1}; Ni: 13 μg kg^{-1}; Pb: 30 μg kg^{-1}; Sb: 35 μg kg^{-1}; Se: 51 μg kg^{-1}).