Journal of Environmental Management 169 (2016) 236–246

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Suitability of the microbial community composition and function in a semiarid mine soil for assessing phytomanagement practices based on mycorrhizal inoculation and amendment addition

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ARTICLE INFO

Article history:
Received 9 June 2015
Received in revised form
22 December 2015
Accepted 30 December 2015
Available online 12 January 2016

Keywords:
Arbuscular mycorrhizal fungi
Enzyme activity
Heavy metals
PLFAs
Semiarid climate
Sugar beet residue

ABSTRACT

The recovery of species composition and functions of soil microbial community of degraded lands is crucial in order to guarantee the long-term self-sustainability of the ecosystems. A field experiment was carried out to test the influence of combining fermented sugar beet residue (SBR) addition and inoculation with the arbuscular mycorrhizal (AM) fungus *Funneliformis mosseae* on the plant growth parameters and microbial community composition and function in the rhizosphere of two autochthonous plant species (*Dorycnium pentaphyllum* L. and *Asteriscus maritimus* L.) growing in a semiarid soil contaminated by heavy metals. We analysed the phospholipid fatty acids (PLFAs), neutral lipids fatty acids (NLFAs) and enzyme activities to study the soil microbial community composition and function, respectively. The combined treatment was not effective for increasing plant growth. The SBR promoted the growth of both plant species, whilst the AM fungus was effective only for *D. pentaphyllum*. The effect of the treatments on plant growth was linked to shifts in the rhizosphere microbial community composition and function. The highest increase in dehydrogenase and β-glucosidase activities was recorded in SBR-amended soil. The SBR increased the abundance of marker PLFAs for saprophytic fungi, Gram+ and Gram− bacteria and actinobacteria, whereas the AM fungus enhanced the abundance of AM fungi-related NLFA and marker PLFAs for Gram− bacteria. Measurement of the soil microbial community composition and function was useful to assess the success of phytomanagement technologies in a semiarid, contaminated soil.

1. Introduction

Phytostabilization of abandoned mine lands based on the use of native metal-tolerant plant species with different functional traits, such as shrubs and grasses, has been recommended for the establishment of a self-sustaining plant community, allowing the further recovery of such degraded sites (Parraga-Aguado et al., 2014). Among the technologies for implanting a permanent vegetation cover in semiarid mine tailings, inoculation with plant-growth-promoting microorganisms and the application of newly-developed organic amendments to the soil can be a suitable approach (Gamalero et al., 2009; Fernández et al., 2012). Arbuscular mycorrhizal fungi (AMF) may protect their host plants from the toxicity of excessive metal concentrations through direct hyphal sequestration and accumulation of metals or by indirectly improving P nutrition under such harmful conditions (Giasson et al., 2008; Meier et al., 2012). The AMF are able to colonise heavy-metal-contaminated soils, although their diversity and abundance usually decrease with increasing heavy metal content, and some strains are more heavy-metal-resistant than others (Zarei et al., 2010).

The establishment of plants on mine tailings generally requires the input of an organic residue, to alleviate the toxicity of the tailings and improve soil fertility (Mendez and Maier, 2008). Organic amendments are known to increase the metal complexation and adsorption, decreasing the availability of heavy metals to

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http://dx.doi.org/10.1016/j.jenvman.2015.12.037
0301-4797/

plants (de la Fuente et al., 2011). In addition, organic residues such as *Aspergillus niger*-treated sugar beet agrowaste (SBR) are able to improve the structural stability of mine tailings in semiarid ecosystems as well as plant growth (Carrasco et al., 2009; Kohler et al., 2014). The efficacy of organic amendments has been proven in both mesocosms (Pérez-de-Mora et al., 2006) and field assays (Pérez-de-Mora et al., 2011; Pardo et al., 2014). The combination of AM fungal inoculation and organic waste amendment as a phytomanagement technology in heavy-metal-polluted soils has been researched only scarcely, but there are some indications that demonstrate its effectiveness with regard to maximising the success of revegetation (Wang et al., 2013). However, the efficacy of combining these two technologies has not been demonstrated under field conditions.

Excessive levels of heavy metals may constitute a serious hazard for soil microorganisms – affecting their growth, activity and composition (Giller et al., 2009; Mandal et al., 2014). Considering that soil microbial populations are essential to nutrient cycling and plant nutrient availability, the recovery of the composition and activity of microbial communities may be key to the sustainability of mine ecosystems. Meanwhile, the composition and activity of soil microbial populations have been proposed as useful indicators of the improvement of soils contaminated by heavy metals following the implementation of phytomanagement technologies (Pérez-de-Mora et al., 2006). We have previously shown, in a greenhouse pot assay, that the populations of AMF change in response to the addition of an organic amendment to a contaminated soil (Alguacil et al., 2011). Changes in the bacterial community composition have been reported also in mine soils subjected to different amendments (Pérez-de-Mora et al., 2006; Zornoza et al., 2015), which may also be modulated by the soil moisture regime (Fernández et al., 2012). However, there are relatively few studies that demonstrate the usefulness of measuring such microbiological properties during phytomanagement tasks under semiarid conditions.

Therefore, we hypothesised that the fermented SBR and/or the inoculation with a native AM fungus may alter the microbial community composition, resulting in the establishment of a fully-functional population that could favour plant growth. The objective of this study was to evaluate in a field experiment the influence of combining the addition of fermented SBR and the inoculation with AM fungus *Funneliformis mosseae* on the plant growth parameters and on composition and functions of microbial communities in the rhizosphere of two Mediterranean plant species (*Dorycnium pentaphyllum* and *Asteriscus maritimus*) growing in a semiarid soil contaminated by heavy metals. The soil microbial community composition and functions were assessed by measuring phospholipid fatty acids (PLFAs) and AM fungi-related neutral lipid fatty acid (NLFA) and enzyme activities involved in the cycling of carbon and phosphorus, respectively. The information obtained will permit determining whether such microbiological properties, related to the functioning and maintenance of ecosystems, can be used as indicators to monitor and judge the suitability of phytomanagement practices.

2. Materials and methods

2.1. Study site

This research was conducted on a mine tailing mound (37º35′33.2″ N, 0º52′35.5″ W, length: 200–300 m, width: 95 m, height: 25 m, volume: 750,000 m^3) at The Cartagena–La Unión mining district "Sierra Minera" (SE Spain). The ore deposits of the mine tailings contain Fe, Pb and Zn as main heavy metal components. The climate is semiarid Mediterranean with a mean annual precipitation of 275 mm, a mean annual temperature of 17.5 °C and a mean potential evapo-transpiration of 1000 mm. For soil characterization, we randomly took three soil samples from 0 to 20 cm depth each consisting of a mixture of six subsamples. Initial characteristics of mine tailing soil are shown in Table 1.

2.2. Materials

The plants used were *D. pentaphyllum* Scop. and *A. maritimus* (L.) Less. The woody legume *D. pentaphyllum* is highly mycorrhizal and responded well to organic amendment (Caravaca et al., 2004). Its use for phytostabilization of heavy metal-contaminated areas was proposed by Lefèvre et al. (2009) due to its resistance to Cd and Zn. *A. maritimus* (synonym *Pallenis maritima*) is an herbaceous perennial halophyte with a high dependence of AM fungi (Estrada et al., 2013). It is a representative plant species in arid and saline Mediterranean ecosystems, found generally in rocks and stony slopes. Prior to the experimental procedures, *D. pentaphyllum* and *A. maritimus* seedlings were grown for 1 year in nursery conditions with peat as substrate. At planting, *D. pentaphyllum* and *A. maritimus* were 51.2 ± 12.2 and 17.5 ± 1.3 cm high, respectively with a shoot dry mass of 1.50 ± 0.29 and 1.69 ± 0.36 g, respectively ($n = 5$).

The mycorrhizal inoculum was a *F. mosseae* (former *Glomus mosseae*) strain, being the most abundant AMF in the mine tailing (Azcón et al., 2009). The mycorrhizal inoculum was multiplied using trap cultures of *Sorghum bicolor* (L.) Moench, and consisted of rhizospheric soil, spores, hyphae and infected root fragments.

Sugar beet residue (SBR) was inoculated with *A. niger* strain NB2 and rock phosphate (Morocco fluorapatite, 12.8% P, 1 mm mesh) by solid state fermentation (Kohler et al., 2014). The concentration of P was determined after nitric-perchloric acid digestion using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo electron corporation Mod. IRIS intrepid II XDL). Total N was determined by dry combustion using a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA). The main characteristics of fermented SBR are described in Kohler et al. (2014).

2.3. Experimental design and layout

The experiment was conducted as a complete randomised factorial design with two factors. The first factor had two levels: non-addition or addition of fermented SBR; and the second had two levels: non-inoculation or inoculation with *F. mosseae*. In the experimental area, planting holes 10 × 10 cm wide and 20 cm deep

Table 1
Physico-chemical and chemical characteristics of the soil used in the experiment ($N = 3$).

pH (H_20)	7.7 ± 0.3
EC (1:5, dS m^{-1})	2.5 ± 0.4
$CaCO_3$ (%)	<5
Total organic C (g kg^{-1})	4.3 ± 0.6
Total N (g kg^{-1})	0.21 ± 0.03
Clay (%)	5 ± 2
Silt (%)	24 ± 5
Sand (%)	71 ± 6
Aggregate stability (%)	24.7 ± 1.6
Fe_2O_3 (%)	16 ± 1
Al_2O_3 (%)	8 ± 1
Total Zn (mg kg^{-1})	12100 ± 900
Soluble Zn (mg kg^{-1})	192 ± 62
Total Pb (mg kg^{-1})	8950 ± 300
Soluble Pb (mg kg^{-1})	3 ± 1
Total Cu (mg kg^{-1})	221 ± 20
Soluble Cu (mg kg^{-1})	<0.01
Total Cd (mg kg^{-1})	61 ± 11
Soluble Cd (mg kg^{-1})	1 ± 0