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The application of an organic amendment modifies the arbuscular mycorrhizal fungal communities colonizing native seedlings grown in a heavy-metal-polluted soil

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ABSTRACT

A mesocosm experiment was conducted to investigate whether communities of arbuscular mycorrhizal (AM) fungi associated with roots of native (*Piptatherum miliaceum*, *Retama sphaerocarpa, Psoralea bituminosa, Coronilla juncea*, and *Anthyllis cytisoides*) and for comparison (*Lolium perenne)* seedlings in a heavy-metal-contaminated, semiarid soil were affected by the application of composted sugar beet waste. We also investigated whether there were relation between AMF diversity and metal concentration (Al, Cd, Cu, Fe, Mn, Pb and Zn) and total P in shoot as well as some soil parameters (total organic carbon and total N) when the SB waste was added to the soil. We analyzed a portion of approximately 795 base pairs of the small-subunit (SSU) rRNA gene by nested PCR, cloning, sequencing, and phylogenetic analyses. Twelve different AMF sequence types were distinguished: seven of these belonged to *Glomus* group A, one to *Glomus* group B, one to *Diversispora,* one to *Archaeospora*, and two to *Paraglomus*. The AM fungal populations colonizing roots in a heavy-metal-polluted soil were quite dependent on the host plant, the highest diversity values being obtained in authochtonous plants recognized as metallophytes, such as *P. bituminosa*, and in an allochtonous, invasive species (*L. perenne*). No significant correlation was found between AMF diversity and plant metal concentration and soil parameters. Excepting *P. bituminosa*, when sugar beet waste was added to soil, the populations of AM fungi in roots increased and the shoot metal concentrations decreased in all host plant species studied. Therefore, the addition of sugar beet waste can be considered a good strategy for the remediation and/or phytostabilization of mine tailing sites.
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1. Introduction

The waste materials generated by mining activities may contain great quantities of heavy metals, which can accumulate in the soil and affect microbial diversity and activity (Brooks et al., 1986; McGrath et al., 1995; Del Val et al., 1999). Among the soil microorganisms are the arbuscular mycorrhizal fungi (AMF), which belong to the phylum Glomeromycota (Schüßler et al., 2001) and form mutualistic symbioses with the majority of known terrestrial plant species (Smith and Read, 1997). Besides an improvement in the plant growth, due to enhanced water and mineral nutrient supply, and a supply of carbon compounds for the fungus, this symbiotic relationship protects plants against diverse biotic and abiotic stresses (Smith and Read, 1997), such as those produced in sites contaminated by heavy metals (Leyval and Joner, 2001). The AM fungi colonize the roots of plants growing on heavy-metal-contaminated soils and play an important role in metal tolerance, sequestration, and accumulation (Gaur and Adholeya, 2004), helping in the revegetation of mine areas or trace-element-contaminated soils (Göhre and Paszkowski, 2006; Hildebrandt et al., 2007). It has been reported that different AMF ecotypes can confer differing degrees of metal tolerance to their host plant (Del Val et al., 1999; Gaur and Adholeya, 2004; Zarei et al., 2008; Khade and Adholeya, 2009) and that AM fungal species differ in their capacity to affect heavy metal uptake by plants (Leyval et al., 1997). Thus, the establishment and survival of plants in these polluted sites might be governed by the communities of AMF. In fact, the presence, abundance, and composition of the AMF community are related strongly to the composition of the plant community (Van der Heijden et al., 1998).

Previous molecular studies have reported different AMF taxa in different plant species, showing host plant preference and/or specificity (Helgason et al., 2002; Vandenkoornhuyse et al., 2002, 2003; Alguacil et al., 2009b). Also, other environmental factors,

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such as site (Öpik et al., 2003), soil fertilization (Santos et al., 2006), or the agricultural management and ecosystem type (Hijri et al., 2006), have been found to influence the AMF community structure. Therefore, for the reclamation of heavy-metal-contaminated sites, it is very important to know the dynamics of the AMF communities in these soils and to have a good selection of tolerant plant species that can establish, grow, and survive under these conditions.

Organic amendments have been used widely to facilitate the revegetation of contaminated soils. In previous studies it has been showed that in soils affected by heavy metals, treated sugar beet (SB) waste improved the plant growth (Medina et al., 2006; Azcón et al., 2009) and structural stability (Carrasco et al., 2009). Immobilization and/or biosorption of heavy metals in soil after SB amendment have been attributed to the presence of carboxyl groups and strong complexes among its constituents (Reddad et al., 2002). Several studies have demonstrated the beneficial effect of the application of various organic amendments to soil, with regard to increasing the proliferation and development of natural AM fungal populations in crop systems (Harinikumar et al., 1990; Jacquot-Plumey et al., 2001) or degraded, semiarid soils (Alguacil et al., 2009a). However, to the best of our knowledge, no studies have been reported on the effects of organic amendments on the diversity of the AMF colonizing plant rhizospheres in a heavy-metal-polluted, Mediterranean soil.

Therefore, the objectives of this work were: 1. To assess whether the application of a composted SB waste could influence in the diversity of AM fungal populations detected in different plant species in a heavy-metal-contaminated soil. 2. To investigate whether there were relations between AMF diversity and metal concentration in shoot as well as soil parameters when the SB waste was added to the soil.

The information gained here will give us a better understanding of the interaction between plants and AMF, for future revegetation and phytoremediation studies in polluted sites.

2. Materials and methods

2.1. Study site

Soil used in this experiment comes from the La Unión mine district (Southeast Spain). The climate is semiarid Mediterranean with an annual rainfall around 250–300 mm and a mean annual temperature of 17.5 °C; the potential evapo-transpiration reaches 1000 mm year^{-1}. This zone constituted an important mining nucleus for more than 2500 years. The ore deposits of this zone have iron, lead and zinc as the main metal components. Iron is present in oxides, hydroxides, sulfides, sulfates, carbonates and silicates; lead and zinc occur in galena, sphalerite, carbonates, sulfates, and lead- or zinc-bearing (manganese, iron) oxides (Oen and Fernández, 1975). In this area a mine tailing with an age of about 50 years called “Gorguel” (UTM X687480 Y4162800 Z135, length: 200–300 m, width: 95 m, height: 25 m, volume: 750,000 m^3, IGME, 1999) was selected. Three soil samples were taken, each one consisted of a mixture of six subsamples randomly taken from the top 20 cm depth of soil. The analytical characteristics of the mine tailing are shown in the Table 1.

Table 1
Chemical, biochemical, microbiological and physical characteristics of the soil used in the experiment ($n = 3$).

pH (H_2O)	7.67 ± 0.03[a]
Electrical conductivity (1:5, dS m^{-1})	1.3 ± 0.7
Glomalin-related soil protein (μg g^{-1})	523.2 ± 24.8
Total organic C (g kg^{-1})	10.5 ± 0.1
Total N (g kg^{-1})	1.33 ± 0.05
Total Al (mg kg^{-1})	14.500 ± 300
Total Cd (mg kg^{-1})	36.8 ± 1.1
Total Cr (mg kg^{-1})	91.2 ± 2.8
Total Cu (mg kg^{-1})	163.1 ± 5.8
Total Fe (mg kg^{-1})	190.300 ± 5.100
Total Ni (mg kg^{-1})	15.3 ± 0.3
Total P (mg kg^{-1})	6.400 ± 200
P available (mg kg^{-1})	7.0 ± 0.5
Total Pb (mg kg^{-1})	6.900 ± 0
Total S (mg kg^{-1})	12.700 ± 300
Total Zn (mg kg^{-1})	12.000 ± 300

[a] Mean ± standard error.

2.2. Materials

Sugar beet residue (SB), a lignocellulosic material was dried at 60 °C and then ground to pass a 2-mm-pore sieve. Portions of 15 g of SB were mixed with 40 mL of Czapek solution (agar 15.0 g L^{-1}; di-potassium hydrogen phosphate 1.0 g L^{-1}; iron (II) sulfate heptahydrate 0.01 g L^{-1}; potassium chloride 0.5 g L^{-1}; magnesium sulfate heptahydrate 0.5 g L^{-1}; sodium nitrate 3.0 g L^{-1}; sucrose 30.0 g L^{-1}; pH = 7.3) for static fermentation in 250 mL Erlenmeyer flasks. The mixture was allowed to ferment at 30 °C for 20 days without shaking. The characteristics of the SB after fermentation were: pH, 5.3; total P, 224 μg mL^{-1}; total N, 1.2%; cellulose, 11.3%; hemicellulose, 3.1%; lignin, 4.1% and reducing sugar, 0.25 g L^{-1}.

For this study we selected five plant species which naturally grow in the nearby areas of the mine tailing, mature seeds of *Piptatherum miliaceum* (L.) Coss, *Retama sphaerocarpa* L., *Psoralea bituminosa* L., *Coronilla juncea* L. and *Anthyllis cytisoides* All plant species are drought resistant and form arbuscular mycorrhizal symbiosis, from them only *P. bituminosa* has been reported as heavy metal tolerant plant species (Poschenrieder et al., 2001; Walker et al., 2007). For comparison purposes, we selected *Lolium perenne* L. which do not naturally thrive in the area but is also mycorrhizal dependant and easy to grow. *L. perenne* has never been reported as a drought or heavy metal tolerant plant species. The seeds were surface sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 5 min and subsequently rinsed thoroughly with sterilized water prior to a wetting treatment with sterilized water for 2 h.

2.3. Experimental design

The experiment was conducted as a completely randomized two factor factorial with six replicates. The first factor was the addition or not of fermented SB residue to the soil. The second factor was the plant species with six levels (*P. miliaceum*, *R. sphaerocarpa, P. bituminosa, C. juncea*, *A. cytisoides* and *L. perenne*).

Five-hundred grams of air-dried soil were placed in 600 mL pots, where seeds of the selected plants were sowed. The fermented SB was mixed manually with the experimental soil at a rate of 2.5% (w/w). The experiment was conducted as a mesocosm assay in a greenhouse, located in the Campus of Espinardo (Murcia, Spain). During the experiment, the average maximum temperature reached 22 °C. Plants were watered regularly with sterile water to a 60% of field capacity, without any fertilizer treatment. Eight months after sowing, the plants were sampled (a total of 72 plants). Plants, including root systems, were collected and placed in polyethylene bags for transport to the laboratory, where fine roots were separated from the soil. Roots were then briefly rinsed, quickly dried on paper and used partly for morphological and partly for molecular analysis. Samples of rhizospheric soil were also collected. The soil samples were sieved through 2-mm pores to eliminate large particles and stored in plastic bags at −20 °C until processed.

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