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The combination of compost addition and arbuscular mycorrhizal inoculation produced positive and synergistic effects on the phytomanagement of a semiarid mine tailing

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HIGHLIGHTS

- Phytomanagement using combination of compost and AMF was tested in field conditions.
- The efficacy of combined treatment to increase shrub growth depended on compost dose.
- Amendment promoted soil microbial function and AM formation.
- The urban waste compost induced potentially toxic levels of Zn in shoots.

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ABSTRACT

A field experiment was carried out to assess the effectiveness of combining mycorrhizal inoculation with a native AM fungus (*Glomus* sp.) and the addition of an urban organic waste compost (OWC) applied at two rates (0.5 and 2.0% (w:w)), with regard to promoting the establishment of *Anthyllis cytisoides* L. seedlings in a heavy metal polluted mine tailing, as well as stimulating soil microbial functions. The results showed that the combined use of the highest dose of OWC and AM inoculation significantly increased shoot biomass – by 64% – compared to the control value. However, the separate use of each treatment had no effect on the shoot biomass of this shrub species. At the 2% rate, OWC enhanced root colonisation by the introduced fungus as well as soil nutrient content and soil dehydrogenase and ß-glucosidase activities. The combined treatment increased the uptake of Zn and Mn in shoots, although only Zn reached excessive or potentially toxic levels. This study demonstrates that the combination of organic amendment and an AM fungus is a suitable tool for the phytomanagement of degraded mine tailings, although its effectiveness is dependent on the dose of the amendment.

1. Introduction

The phytostabilisation of soils contaminated by heavy metals is an environmentally friendly and relatively cheap method in comparison to conventional remedial techniques, which achieves contaminant containment thereby mitigating offsite contamination (Mendez and Maier, 2008; Bolan et al., 2011; Meier et al., 2012). The vegetation and its microbial rhizosphere flora may promote the uptake and accumulation of heavy metals by roots and their precipitation within the roots, avoiding their translocation from root to shoot. Likewise, the established plant cover provides physical protection against soil erosion by wind and water and the subsequent dispersal of inorganic contaminants in runoff. Pioneer plants that naturally colonise these habitats are considered appropriate for phytostabilisation as they are adapted to the prevailing environmental conditions and would tolerate, but not accumulate, high levels of metals (Anjum et al., 2014). In semiarid regions, the scarce availability of water and nutrients to support plant growth is an additional constraint to the phytostabilisation of mine tailings (Carrasco et al., 2010). In such environments, shrub species form part of the primary plant succession and are considered suitable candidates to be employed in the phytostabilisation of semiarid mine tailings

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(Parraga-Aguado et al., 2014). Organic residues are commonly used as a plant nutrient source and as soil amendments in order to promote the phytoremediation process in such contaminated environments (Walker et al., 2003; Tejada et al., 2007; Alvarenga et al., 2009; Pardo et al., 2011; Park et al., 2011). Meanwhile, organic matter (OM) addition may decrease metal availability by increasing the soil pH and cation exchange capacity and/or through complexation with the reactive fraction of OM (Hetrick et al., 1994). On the other hand, OM additions can increase the amount of soluble organic ligands and eventually lead to the opposite effect. The primary concerns about the prolonged use of organic residues are the heavy metal content, pathogen levels and salts — which cause harmful effects to the environment. In this respect, the use of composted residues rather than non-composted residues is advisable because some toxic substances are eliminated during the composting process (Pascual et al., 1999; Tejada et al., 2007; de la Fuente et al., 2011).

For the successful phytostabilisation of metal polluted soils in semiarid environments, inoculation with suitable arbuscular mycorrhizal fungi (AMF) has been proposed to overcome unfavourable conditions for plant establishment such as low required nutrient content, lack of soil structure, high salinity and low water retention (Hildebrandt et al., 2007; Turnau et al., 2008; González-Guerrero et al., 2009; Meier et al., 2012). The AMF are obligate symbionts with the vast majority of terrestrial plants, colonising their roots and developing an extraradical mycelium (Smith and Read, 2008). Inoculation with strains of AMF has been shown to increase the survival and growth of woody seedlings (Fernández et al., 2012; Curaqueo et al., 2014) and grasses (Azcón et al., 2009) in soils derived from tailings in semiarid areas. The AMF are known to colonise heavy metal contaminated soils, showing a decreased diversity and abundance with increasing heavy metal content, although some strains seem to be specialised on highly contaminated sites (Zarei et al., 2010; Alguacil et al., 2011). Mycorrhizal plant species are also known to replace non-mycorrhizal species during the normal succession of the vegetation of post-mining landscapes, indicating their role in allowing plants to cope with heavy metal stress (Regvar et al., 2006), while pioneer plants are colonised by AMF in the plant establishment phase (González-Chávez et al., 2009).

Organic matter constitutes an important source of carbon and energy for soil microflora, although the AMF represent one exception to this because they are obligate biotrophs and rely on their host plant for proliferation and survival. However, the positive effect of OM on the growth of the external mycelium of AMF is well known (Hammer et al., 2011; Hodge et al., 2001). The addition of high rates of nutrients to soil may affect the mycorrhizal colonisation of roots adversely due to plant feedback mechanisms (Watts-Williams and Cavagnaro, 2012). There are also some indications that AMF and organic amendments may make a synergistic contribution to the phytostabilisation of the soil polluted by heavy metals (Wang et al., 2013; Curaqueo et al., 2014), although these studies were carried out in mesocosms under controlled conditions. Thus, this phytomanagement technology needs to be validated under field conditions, because metal uptake by plants is greatly affected by the growing conditions — such as water availability and soil structure (Conesa et al., 2007). We hypothesised that organic amendments stimulate the formation of arbuscular mycorrhizas, in turn enhancing the performance of AMF on host plant and soil conditions. Also, we hypothesised that the effectiveness of the combination of organic amendment addition and AMF inoculation for successful phytostabilization can depend on the amount of organic amendment added. To assess these hypotheses, we performed a field experiment with *Anthyllis cytisoides* seedlings, inoculated or not with a native AM fungus. The seedlings were grown under semiarid conditions, in a heavy metal polluted soil to which an urban organic waste compost was added at two rates. The formation of arbuscular mycorrhizas was measured as the percentage mycorrhizal colonisation of roots, whereas the performance of the AM fungus was assessed through plant nutrient acquisition and growth.

2. Material and methods

2.1. Study site

The experimental area was situated in "El Gorguel" mine tailing (37°35′33.2" N, 0°52′35.5" W, length: 200–300 m, width: 95 m, height: 25 m, volume: 750,000 m^3, IGME, 1999), within the Cartagena–La Unión mining district "Sierra Minera" (SE Spain). The climate is typically Mediterranean with an annual rainfall around 250–300 mm and an annual average temperature of 18 °C. For soil characterization, three samples were taken from the top 20 cm depth of soil, consisting of a mixture of six subsamples. The analytical characteristics of the mine tailing are indicated in Table 1.

The plant used was *A. cytisoides* L., a legume shrub used for afforestation and reclamation of degraded Mediterranean areas, which is highly mycorrhizal (Wang and Qiu, 2006) and also known as a heavy metal- and drought-tolerant species (Diaz et al., 1996). Plants were grown in a nursery with peat as substrate for 10 months prior to experimental procedures. At planting, *A. cytisoides* was 28.5 ± 1.8 cm high, with a shoot dry weight of 2.13 ± 0.15 g (n = 3).

The urban organic waste compost (OWC) used was the organic fraction of a municipal solid waste obtained from a treatment plant in Murcia, Spain. The composting process lasted 2 months during which the open-air heaps were turned regularly (Indore system). The maximum temperature reached was 65–68 °C and humidity was maintained at 50–65% throughout the process. The compost was left undisturbed for 4 months for organic matter stabilization (García et al., 1990). Based on phytotoxicity bioassays for compost maturity assessment (Bernal et al., 2009), the compost obtained can be considered as a mature and non-phytotoxic product because its germination index was higher than 80% (Table 1). The composted residue was ground and sieved to 0.5 mm particles and air-dried for analysis. Total heavy metal contents of OWC used were below the limits imposed by the Spanish legislation for use of an organic residue as fertiliser in agricultural soils (B.O.E., 2013).

The mycorrhizal inoculum was a *Glomus* strain (GenBank accession number LN610456) isolated from a semiarid soil close to the experimental site. The AM inoculum consisted of a mixture of rhizospheric soil from trap cultures (*Sorghum bicolor*) containing spores, hyphae and mycorrhizal root fragments.

Table 1
Characteristics of the soil and urban organic waste compost used in the experiment.

	Soil	Compost
pH (H_2O)	7.7	6.7
EC (dS m^{-1})	2.5	4.7
$CaCO_3$ (%)	<5	nd
Total organic C (g kg^{-1})	4	276
Total N (g kg^{-1})	0.2	14.5
Total P (g kg^{-1})	6.4	3.8
Clay (%)	5	nd
Silt (%)	24	nd
Sand (%)	71	nd
Water-soluble C (mg kg^{-1})	41	1,950
Water-soluble carbohydrates (mg kg^{-1})	10	nd
Dehydrogenase (μg INTF g^{-1})	6.9	nd
Aggregate stability (%)	24.7	nd
Fe_2O_3 (%)	16	nd
Al_2O_3 (%)	8	nd
Total Zn (mg kg^{-1})	12,100	261
Total Pb (mg kg^{-1})	8,950	98
Total Cu (mg kg^{-1})	221	146
Total Cr (mg kg^{-1})	91	63
Total Cd (mg kg^{-1})	61	3
Total Ni (mg kg^{-1})	26	25
Germination index (%)	nd	88.3

nd: not determined.