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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.

2.2. Experimental design

The experiments were arranged in a randomised block design, with two factors and fivefold replication. The first factor was the addition of three doses of urban organic waste compost to soil (0, 0.5 and 2.0% w/w). The second factor consisted in the inoculation or not with the *Glomus* strain.

In October 2012, the seedlings were transported to the experimental field, where planting holes 15 × 15 cm wide and 15 cm deep were dug manually. The OWC were mixed manually with the experimental soil at a rate of 0.5 and 2.0% (w:w). The arbuscular mycorrhizal inoculum was applied at a rate of 5% (v/v). The same amount of the autoclaved inoculum was added to non-mycorrhizal plants, supplemented with a filtrate (Whatman no. 1 paper) of the culture to provide the microbial populations accompanying the mycorrhizal fungi. The mycorrhizal inoculum and organic residue were manually mixed into 2 kg of soil in plastic bags and introduced in the plantation holes. The compost used contained an organic matter stabilized without phytotoxic effects and consequently we carried out the transplanting immediately after its addition into the soil. The seedlings were planted at least 1 m apart between holes, with 3 m between treatment levels, and 10 m between replication blocks. At least 25 seedlings per treatment level were planted. Eight months after sowing, the whole plants were harvested, separating the soil from the roots. Soil was sieved at 2 mm and stored at 4 °C for further analysis.

2.3. Plant analyses

Fresh and dry weight of shoots and roots (105 °C, 5 h) were recorded.

Total metal contents and foliar P and K concentrations were determined after nitric–perchloric digestion: 1 g of crushed sample was placed in a Kjeldahl flask, and 10 ml of concentrated HNO_3 plus 10 ml of concentrated $HClO_4$ were added. The mixture was heated at 210 °C for 90 min, and then left to cool down at room temperature. When cool, the content of the tubes was filtered through an Albet® 145 ashless filter paper, and the volume was made to 50 ml by washing the Kjeldahl flasks with 0.5 N HCl several times. All metals and foliar P were quantified using an ICP-MS (Thermo electron corporation Mod. IRIS intrepid II XDL). Shoot N was determined by dry combustion using a LECO Tru-Spec CN analyser (Leco Corp., St. Joseph, MI, USA).

The percentage of root length colonised by arbuscular mycorrhizal fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Phillips and Hayman, 1970).

2.4. Soil analyses

Soil total N was determined by the Kjeldahl method. Soil available P was extracted with sodium bicarbonate and determined by colorimetry (Watanabe and Olsen, 1965).

Dehydrogenase activity was determined using INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride) as an oxidising agent according to García et al. (1997). Briefly, after adjusting soil samples to 60% of its water holding capacity they were incubated with 0.4% INT for 20 h at 22 °C in the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with methanol and measured on a spectrophotometer at 490 nm. Controls were made with water instead of INT.

Acid phosphatase activity was determined using p-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate (Naseby and Lynch, 1997). Soil samples were buffered with 0.5 M sodium acetate at pH 5.5 and incubated after substrate addition shaking at 37 °C in a water bath for 90 min. The reaction was stopped by cooling to 2 °C and adding 0.5 M $CaCl_2$ and 0.5 M NaOH. After centrifugation of the samples, the p-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm. Controls were made adding the PNPP after incubation.

β-Glucosidase was determined using p-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate (Tabatabai, 1994). This assay is again based on the release and detection of PNP spectrophotometrically at 398 nm after incubation in a water bath of 0.5 g soil with 2 ml maleate buffer (0.1 M, pH 6.5) and 0.5 ml of substrate for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethane (THAM).

2.5. Statistical analysis

Percentage colonisation was arcsin-transformed, and the other parameters were log-transformed to meet ANOVA assumptions. Mycorrhizal inoculation and organic amendment and their interactions effects on measured variables were tested by a two-way analysis of variance and comparisons among means were calculated using the Tukey's HSD-test at $P < 0.05$. Statistical procedures were carried out with the software package R.

3. Results

3.1. Plant nutrient and growth

The combined use of the highest dose of OWC (2% w/w) and *Glomus* inoculation significantly increased the biomass production of shoots (dry weight) *A. cytisoides* by 64%, with respect to non-amended and non-inoculated seedlings (Fig. 1). However, the separate use of each treatment had no effect on shoot biomass. The roots biomass of *A. cytisoides* was affected neither by the OWC nor by mycorrhizal inoculation. Moreover, *Glomus* inoculation did not increase the mycorrhizal colonisation of *A. cytisoides* roots (Table 2). However, the ANOVA revealed the existence of a synergistic interaction between the AM fungus and the OWC amendment (2%) on mycorrhizal colonisation ($P = 0.033$). Only the higher dose of OWC significantly increased the contents of foliar N, P and K in relation to non-amended plants. Also, there was a significant amendment × mycorrhizal inoculation interaction for foliar N (A × M interaction, $P = 0.001$) and K (A × M interaction, $P = 0.033$), which were significantly enhanced in inoculated plants with the higher dose of OWC (Table 2).

3.2. Soil biochemical parameters

The OWC amendment at 2% increased the available P and total N concentrations in the rhizosphere of non-inoculated and *Glomus*-inoculated plants, almost doubling the values with respect to the control soils (Table 3). Dehydrogenase activity was significantly increased by the OWC amendment, alone ($P < 0.001$) or in combination with mycorrhizal inoculation ($P = 0.038$). The highest values were recorded in the treatment combining OWC addition at 2% and *Glomus* inoculation, corresponding to an increase of 63% with respect to the control soil (Table 3). Neither inoculation nor OWC amendment had any significant effect on phosphatase activity (Table 3). The β-glucosidase activity was increased by the organic amendment, the highest values occurring in soils amended with 2% OWC with non-inoculated plants.

3.3. Shoot heavy metals

The concentrations of heavy metals (except those of Mn and Zn) in the shoots of *A. cytisoides* were not significantly affected by the treatments assayed (Table 4). The concentration of foliar Zn was increased both by the organic amendment alone and in combination with mycorrhizal inoculation, whereas the concentration of foliar Mn was enhanced only by the combined treatment, with differences between the amendment doses assayed (Table 4). The highest levels of Zn were found in the shoots of inoculated plants grown in the soil amended at 2% (about 90% higher than in control plants).

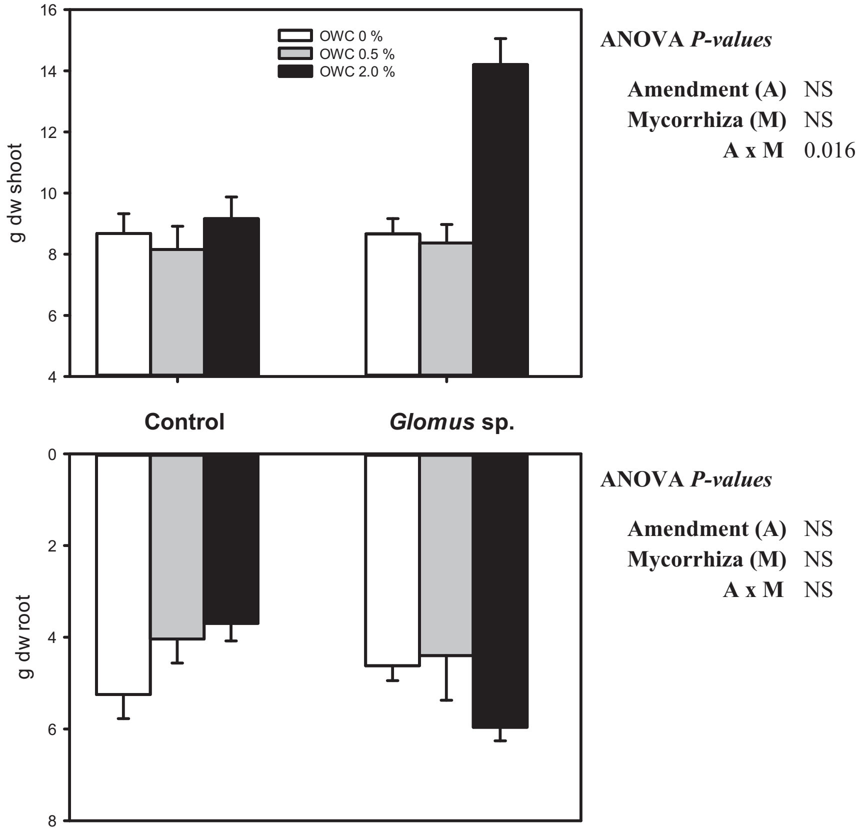

Fig. 1. Effects of organic amendment addition and mycorrhizal inoculation on shoot and root dry weight of *A. cytisoides* 8 months after planting (n = 5). Bars represent standard deviation.

4. Discussion

4.1. Effect of the amendment and mycorrhizal inoculum on plant growth

The present study confirms the synergistic effect of mycorrhizal inoculation and urban organic waste compost addition on plant growth in a semiarid mine tailing under natural conditions. Similar results were obtained by Wang et al. (2013) using AMF and cattle manure in an artificially polluted soil. It is noteworthy that the mine tailing assayed is located in a semiarid area, since few studies of phytostabilisation have been carried out under such environmental conditions. The two doses of the amendment applied to the soil promoted the growth of *A. cytisoides* seedlings, although only the higher dose increased soil nutrient levels. In dry ecosystems such as this, the success of an organic amendment with regard to plant establishment could be limited by drought (Fernández et al., 2012). As AMF are known to improve the water uptake by plants under drought conditions (Querejeta et al., 2007), they could have played a crucial role in the use of added nutrients and water by the plants. This suggests that the effects of arbuscular mycorrhizae and amendments regarding promotion of plant growth in a contaminated soil are closely interlinked and, to a certain extent, interdependent. These findings indicate that the setting-up of plant-favourable edaphic environments is not sufficient to guarantee the onset of primary succession and that the involvement of the mycorrhizal symbionts associated with pioneer plants is also necessary.

The growth advantage conferred on the host plant by the *Glomus* strain in the amended soil, compared with the uninoculated seedlings, might be related to the capacity of the fungus to acquire nutrients released from the composted residue (Hodge et al., 2001; Cavagnaro, 2014). It is worth noting that the increase of available P in the amended soil was about twice as high as in the soil treated with OWC and the AM fungus. The fact that the highest contents of P in leaves occurred for

Table 2
Effects of organic amendment addition and mycorrhizal inoculation on mycorrhizal colonisation, foliar nitrogen, phosphorus and potassium concentrations of *A. cytisoides* seedlings 8 months after planting (means ± standard deviations, n = 5).

Treatment	OWC	Colonisation (%)	Foliar N (mg plant^{-1})	Foliar P (mg plant^{-1})	Foliar K (mg plant^{-1})
Control	0	25.0 ± 4.7 a	122 ± 7 a	5.1 ± 0.7 a	90 ± 13 a
	0.5	28.3 ± 3.0 a	126 ± 3 a	5.6 ± 0.7 a	117 ± 16 ab
	2.0	32.8 ± 4.4 a	156 ± 4 b	7.2 ± 0.7 b	120 ± 11 ab
Glomus sp.	0	33.3 ± 5.1 a	122 ± 7 a	4.1 ± 0.6 a	100 ± 15 a
	0.5	32.4 ± 4.0 a	130 ± 6 a	5.7 ± 0.5 a	119 ± 20 ab
	2.0	50.4 ± 5.6 b	242 ± 12 c	9.0 ± 1.8 b	191 ± 25 b
ANOVA p-values					
Amendment (A)		NS	<0.001	0.003	0.001
Mycorrhiza (M)		NS	NS	NS	0.018
A × M		0.033	0.001	NS	0.033

For each parameter, different letters in the column indicate significant differences at the 0.05 level according to the Tukey's HSD-test. Significance of the effects of amendment and mycorrhizal inoculation on the measured variables are also shown. NS, not significant at P < 0.05.

Table 3
Effects of organic amendment addition and mycorrhizal inoculation on soil nutrient and enzymatic activities in the rhizosphere of *A. cytisoides* seedlings 8 months after planting (means ± standard deviations, n = 5).

Treatment	OWC	Available P (mg kg^{-1})	Total N (g kg^{-1})	Dehydrogenase (μg INTF g^{-1})	Phosphatase (μmol PNP g^{-1} h^{-1})	β-Glucosidase (μmol PNP g^{-1} h^{-1})
Control	0	14 ± 1 a	0.9 ± 0.1 a	12.2 ± 1.0 a	2.4 ± 0.2	0.80 ± 0.07 a
	0.5	16 ± 1 a	1.3 ± 0.2 a	12.2 ± 2.2 a	2.5 ± 0.3	0.83 ± 0.06 a
	2.0	27 ± 2 b	2.2 ± 0.2 b	16.5 ± 1.5 b	2.8 ± 0.2	1.16 ± 0.06 b
Glomus sp.	0	15 ± 1 a	1.1 ± 0.3 a	12.3 ± 0.4 a	2.5 ± 0.2	0.90 ± 0.03 a
	0.5	15 ± 1 a	1.5 ± 0.2 a	15.3 ± 1.9 a	2.6 ± 0.1	0.88 ± 0.04 a
	2.0	22 ± 2 b	2.0 ± 0.3 b	20.2 ± 1.0 b	2.4 ± 0.2	0.96 ± 0.02 b
ANOVA p-values						
Amendment (A)		<0.001	<0.001	<0.001	NS	<0.001
Mycorrhiza (M)		NS	NS	NS	NS	NS
A × M		0.048	0.045	0.038	NS	<0.001

For each parameter, different letters in the column indicate significant differences at the 0.05 level according to the Tukey's HSD-test. Significance of the effects of amendment and mycorrhizal inoculation on the measured variables are also shown. NS, not significant at P < 0.05.

seedlings grown in amended soil and inoculated with *Glomus* demonstrates higher accumulation of P as a consequence of mycorrhizal inoculation. In addition to the supply of nutrients, organic amendments can also immobilise heavy metals and reduce their phytoavailability, leading to favourable conditions for plant establishment (Santibañez et al., 2012; Kohler et al., 2014). The neutral character of the mine tailings in this study contributes to the fact that the heavy metals are present mostly in non-bioavailable forms in the soil (Kohler et al., 2014). The Cu, Cd, Mn and Pb concentrations in shoots were within the normal ranges for plants (Kabata-Pendias and Pendias, 2001). However, the OWC alone or in combination with mycorrhizal inoculation treatment provoked excessive or potentially toxic levels of Zn in the aboveground parts of this perennial forage legume, which could constitute a risk through their entry into the food chain. Moreover, neither the OWC nor the AM fungus was effective for decreasing the excessive levels of Fe recorded in shoots of control plants. It is worth noting that in our study the levels of heavy metals in the shoots of *A. cytisoides* were lower than those shown by this shrub species in a pot experiment (Kohler et al., 2014), where the growing conditions favour the availability and accessibility of soil metals and their uptake by plant roots (Conesa et al., 2007).

In our study, AM inoculation – alone or in combination with the lowest dose of the amendment – did not enhance P uptake by the host seedlings or, consequently, plant growth. Shetty et al. (1995) found that the mycorrhizal effect on plant growth in Zn and Pb contaminated soils depended on the origin of the fungal strain and the heavy metal concentrations. Both the local AMF community and the added AM fungus (native) should be adapted to the harsh conditions of a contaminated soil (Regvar et al., 2006; González-Chávez et al., 2009). The inoculated endophyte was able to colonise the roots of *A. cytisoides* and improve plant growth when the soil fertility was enhanced by the amendment at 2%. This finding could be related to the ability of compost to suppress soil microorganisms including pathogenic/competitive fungi and enhance specific groups of bacteria and fungi, which facilitate the colonisation of roots by AMF (Neeraj and Singh, 2011). So, it seems that the addition of OWC at moderate rates favours certain fungal species, in agreement with other studies that described shifts in the AMF community as a consequence of the application of different organic amendments to heavy metal contaminated, semiarid soils (Alguacil et al., 2011).

4.2. Effect of the amendment and mycorrhizal inoculum on soil quality

Dehydrogenase and hydrolase activities are valuable indicators of the functional state of soils and are very sensitive to heavy metal contamination (Hinojosa et al., 2004; Renella et al., 2006; Fernández et al., 2012). Dehydrogenase activity, in particular, has been widely used to estimate the metabolic activity of soil microbial communities (Lee et al., 2002; Pérez-de-Mora et al., 2006) and has been shown to be a suitable parameter for soil ecotoxicological studies (Oliveira and Pampulha, 2006). In general, an increase in the available concentration of metals diminishes enzymatic activity, by suppression of the microbial population producing the enzymes and/or because of direct inactivation by metals (Nannipieri, 1994; Kandeler et al., 2000). Assays of soil enzyme activity are important for estimating the effects of metal pollution on the soil environment. The input of microbial biomass and bioavailable carbon compounds from organic amendments, which are used as carbon and energy sources by soil microflora, may promote soil microbial activity (Caravaca et al., 2003). In addition, a metal fraction might be adsorbed on the organic colloids added with the organic residue, which would prevent potential toxic effects of heavy metals on microbial populations. However, only the combined treatment of amendment and AM fungus inoculation was effective for promoting the overall soil microbial activity, estimated by the dehydrogenase activity. In addition, soil hydrolases can provide early indications of the soil fertility status, since these enzymes are related to the mineralisation of important

Table 4
Effects of organic amendment addition and mycorrhizal inoculation on heavy metals uptake by *A. cytisoides* seedlings 8 months after planting (means ± standard deviations, n = 5).

Treatment	OWC	Cu (mg kg^{-1})	Cd (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Pb (mg kg^{-1})	Al (mg kg^{-1})
Control	0	8 ± 1	0.18 ± 0.06	43 ± 3 a	170 ± 19	112 ± 7 a	11 ± 1	212 ± 38
	0.5	10 ± 0	0.20 ± 0.06	66 ± 9 a	270 ± 75	195 ± 26 b	16 ± 4	284 ± 87
	2.0	10 ± 1	0.17 ± 0.04	47 ± 5 a	229 ± 36	159 ± 7 b	16 ± 3	243 ± 65
Glomus sp.	0	10 ± 1	0.19 ± 0.04	51 ± 7 a	304 ± 44	168 ± 14 b	20 ± 4	298 ± 60
	0.5	9 ± 0	0.16 ± 0.03	60 ± 8 a	232 ± 32	166 ± 21 b	15 ± 3	216 ± 45
	2.0	10 ± 1	0.20 ± 0.11	89 ± 20 b	267 ± 52	213 ± 20 b	19 ± 4	260 ± 85
ANOVA p-values								
Amendment (A)		NS	NS	NS	NS	0.024	NS	NS
Mycorrhiza (M)		NS	NS	NS	NS	NS	NS	NS
A × M		NS	NS	0.050	NS	0.029	NS	NS

For each parameter, different letters in the column indicate significant differences at the 0.05 level according to the Tukey's HSD-test. Significance of the effects of amendment and mycorrhizal inoculation on the measured variables are also shown. NS, not significant at P < 0.05.

nutrient elements such as N, P and C. Acid phosphatase activity is involved in P cycling. This enzyme is secreted predominantly by plant roots and associated mycorrhizae and other fungi (Tarafdar and Marschner, 1994). The AM hyphae are known to hydrolyse organic P by producing phosphatase, so that they can transport the resultant inorganic P to the host plant (Wang et al., 2013; Koide and Kabir, 2000). Nevertheless, inoculation of the AM fungus had no effect on phosphatase activity, probably due to the scarce colonisation by the introduced endophyte. As a hydrolase, β-glucosidase is responsible for cellulose degradation and, thus, is involved in the carbon cycle (Nannipieri et al., 2002). The increased β-glucosidase activity in the soil amended with the higher dose of OWC can therefore be attributed to the addition of a considerable amount of organic matter to soil, which is degraded by microbes (Alef and Nannipieri, 1995).

It can be concluded that the urban waste compost applied at a moderate dose (2%) in combination with the inoculation of a native AM fungus synergistically promoted the growth of *A. cytisoides* in a neutral mine tailing under field conditions. The organic amendment improved soil nutrient status and stimulated mycorrhiza formation and soil microbial function, thereby influencing nutrient turnover and plant performance. Meanwhile, the introduced endophyte promoted the transfer of nutrients from the amended soil to the host plant, thus leading to enhanced plant growth. However, the potentially toxic levels of Zn reached in the shoots of this forage legume could increase the risk of entry of metals into the food chain and their accumulation therein. These findings suggest the need to search for alternative organic amendments that do not favour the excessive accumulation of such heavy metal in the edible parts of plants.

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References

Alef, K., Nannipieri, P., 1995. Enzyme activities. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, UK, pp. 311–373.

Alguacil, M.M., Torrecillas, E., Caravaca, F., Fernández, D.A., Azcón, R., Roldán, A., 2011. The application of an organic amendment modifies the arbuscular mycorrhizal fungal communities colonizing native seedlings grown in a heavy-metal-polluted soil. Soil Biol. Biochem. 43, 1498–1508.

Alvarenga, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., 2009. Organic residues as immobilizing agents in aided phytostabilization: effects on soil chemical characteristics. Chemosphere 74, 1292–1300.

Anjum, N.A., Umar, S., Iqbal, M., 2014. Assessment of cadmium accumulation, toxicity, and tolerance in Brassicaceae and Fabaceae plants—implications for phytoremediation. Environ. Sci. Pollut. Res. 21, 10286–10293.

Azcón, R., Medina, A., Roldán, A., Biró, B., Vivas, A., 2009. Significance of treated agrowaste residue and autochthonous inoculates (arbuscular mycorrhizal fungi and *Bacillus cereus*) on bacterial community structure and phytoextraction to remediate soils contaminated with heavy metals. Chemosphere 75, 327–334.

B.O.E., 2013. Real Decreto 506/2013, de 28 de junio, sobre productos fertilizantes.

Bernal, M.P., Alburquerque, J.A., Moral, R., 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresour. Technol. 100, 5444–5453.

Bolan, N.S., Park, J.H., Robinson, B., Naidu, R., Huh, K.Y., 2011. Phytostabilization: a green approach to contaminant containment. Adv. Agron. 112, 145–204.

Caravaca, F., Figueroa, D., Alguacil, M.M., Roldán, A., 2003. Application of composted urban residue enhanced the performance of afforested shrub species in a degraded semiarid land. Bioresour. Technol. 90, 65–70.

Carrasco, L., Gattinger, A., Fließbach, A., Roldán, A., Schloter, M., Caravaca, F., 2010. Estimation by PLFA of microbial community structure associated with the rhizosphere of *Lygeum spartum* and *Piptatherum miliaceum* growing in semiarid mine tailings. Microb. Ecol. 60, 265–271.

Cavagnaro, T.R., 2014. Impacts of compost application on the formation and functioning of arbuscular mycorrhizas. Soil Biol. Biochem. 78, 38–44.

Conesa, H.M., Robinson, B.H., Schulin, R., Nowack, B., 2007. Growth of *Lygeum spartum* in acid mine tailings: response of plants developed from seedlings, rhizomes and at field conditions. Environ. Pollut. 145, 700–707.

Curaqueo, G., Schoebitz, M., Borie, F., Caravaca, F., Roldán, A., 2014. Inoculation with arbuscular mycorrhizal fungi and addition of composted olive-mill waste enhance plant establishment and soil properties in the regeneration of a heavy metal-polluted environment. Environ. Sci. Pollut. Res. Int. 21, 7403–7412.

de la Fuente, C., Clemente, R., Martínez-Alcalá, I., Tortosa, G., Bernal, M.P., 2011. Impact of fresh and composted solid olive husk and their water-soluble fractions on soil heavy metal fractionation; microbial biomass and plant uptake. J. Hazard. Mater. 186, 1283–1289.

Diaz, G., Azcón-Aguilar, C., Honrubia, M., 1996. Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. Plant Soil 180, 241–249.

Fernández, D.A., Roldán, A., Azcón, R., Caravaca, F., Bååth, E., 2012. Effects of water stress, organic amendment and mycorrhizal inoculation on soil microbial community structure and activity during the establishment of two heavy metal-tolerant native plant species. Microb. Ecol. 63, 794–803.

García, C., Hernández, T., Costa, F., 1990. The influence of composting and maturation processes on the heavy-metal extractability from some organic wastes. Biol. Waste. 31, 291–301.

García, C., Hernández, M.T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil Sci. Plant Nutr. 28, 123–134.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.

González-Chávez, M.C., Carrillo-González, R., Gutiérrez-Castorena, M.C., 2009. Natural attenuation in a slag heap contaminated with cadmium: the role of plants and arbuscular mycorrhizal fungi. J. Hazard. Mater. 161, 1288–1298.

González-Guerrero, M., Benabdellah, K., Azcón-Aguilar, C., Ferrol, N., 2009. Heavy metal tolerance in arbuscular mycorrhizal fungi. In: Azcón-Aguilar, C., Barea, J.M., Gianinazzi, S., Gianinazzi-Pearson, V. (Eds.), Mycorrhizas: Functional Processes and Ecological Impact. Springer, Heidelberg, pp. 107–122.

Hammer, E.C., Nasr, H., Wallander, H., 2011. Effects of different organic materials and mineral nutrients on arbuscular mycorrhizal fungal growth in a Mediterranean saline dryland. Soil Biol. Biochem. 43, 2332–2337.

Hetrick, B.A.D., Wilson, G.W.T., Fige, D.A.H., 1994. The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine soil. Environ. Pollut. 86, 171–179.

Hildebrandt, U., Regvar, M., Bothe, H., 2007. Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68, 139–146.

Hinojosa, M.B., Carreira, J.A., García-Ruíz, R., Dick, R.P., 2004. Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils. Soil Biol. Biochem. 36, 1559–1568.

Hodge, A., Campbell, C.D., Fitter, A.H., 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413, 297–299.

Instituto Geológico y Minero de España (IGME), 1999. Inventario Nacional de Balsas y Escombreras. Comunidad Autónoma de la Región de Murcia. Technical Report. Instituto Geológico y Minero de España, Madrid, Spain.

Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants. third ed. CRC Press, Boca Raton, Florida.

Kandeler, E., Tscherko, D., Bruce, K.D., Stemmer, M., Hobbs, P.J., Bardgett, R.D., Amelung, W., 2000. Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. Biol. Fertil. Soils 32, 390–400.

Kohler, J., Caravaca, F., Azcón, R., Díaz, G., Roldán, A., 2014. Selection of plant species-organic amendment combinations to assure plant establishment and soil microbial function recovery in the phytostabilization of a metal-contaminated soil. Water Air Soil Pollut. 225, 1930.

Koide, R.T., Kabir, Z., 2000. Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. New Phytol. 148, 511–517.

Lee, I.S., Kim, O.K., Chang, Y.Y., Bae, B., Kim, H.H., Baek, K.H., 2002. Heavy metal concentrations and enzyme activities in soil from a contaminated Korean shooting range. J. Biosci. Bioeng. 94, 406–411.

Meier, S., Borie, F., Bolan, N., Cornejo, P., 2012. Phytoremediation of metal-polluted soils by arbuscular mycorrhizal fungi. Crit. Rev. Environ. Sci. Technol. 42, 741–775.

Mendez, M.O., Maier, R.M., 2008. Phytostabilization of mine tailings in arid and semiarid environments — an emerging remediation technology. Environ. Health Perspect. 116, 278–283.

Nannipieri, P., 1994. The potential use of soil enzymes as indicators of productivity, sustainability and pollution. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., Grace, P.R. (Eds.), Soil Biota: Management in Sustainable Farming Systems. CSIRO, Australia, pp. 238–244.

Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical processes in soil. In: Burns, R.G., Dick, R.P. (Eds.), Enzymes in the Environment. Marcel Dekker, New York, pp. 1–34.

Naseby, D.C., Lynch, J.M., 1997. Rhizosphere soil enzymes as indicators of perturbations caused by enzyme substrate addition and inoculation of a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. Soil Biol. Biochem. 29, 1353–1362.

Neeraj, Singh, K., 2011. Organic amendments to soil inoculated arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* treatments reduce the development of root-rot disease and enhance the yield of *Phaseolus vulgaris* L. Eur. J. Soil Biol. 47, 288–295.

Oliveira, A., Pampulha, M.E., 2006. Effects of long-term heavy metal contamination on soil microbial characteristics. J. Biosci. Bioeng. 102, 157–161.

Pardo, T., Clemente, R., Bernal, M.P., 2011. Effects of compost, pig slurry and lime on trace element solubility and toxicity in two soils differently affected by mining activities. Chemosphere 84, 642–650.

Park, J.H., Panneerselvam, P., Lamb, D.T., Choppala, G., Bolan, N.S., Chung, J.W., 2011. Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils. J. Hazard. Mater. 185, 549–574.

Parraga-Aguado, I., Querejeta, J.I., González-Alcaraz, M.N., Jiménez-Cárceles, F.J., Conesa, H.M., 2014. Usefulness of pioneer vegetation for the phytomanagement of metal(loid)s enriched tailings: grasses vs. shrubs vs. trees. J. Environ. Manag. 133, 51–58.

Pascual, J.A., García, C., Hernández, T., 1999. Comparison of fresh and composted organic waste in their efficacy for the improvement of arid soil quality. Bioresour. Technol. 68, 255–264.

Pérez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaeckel, P., Schloter, M., 2006. Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. Soil Biol. Biochem. 38, 327–341.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55, 158–161.

Querejeta, J.I., Egerton-Warburton, L.M., Allen, M.F., 2007. Hydraulic lift may buffer rhizosphere hyphae against the negative effects of severe soil drying in a California Oak savanna. Soil Biol. Biochem. 39, 409–417.

Regvar, M., Vogel-Mikuš, K., Kugonič, N., Turk, B., Batič, F., 2006. Vegetational and mycorrhizal successions at a metal polluted site: indications for the direction of phytostabilisation? Environ. Pollut. 144, 976–984.

Renella, G., Egamberdiyeva, D., Landi, L., Mench, M., Nannipieri, P., 2006. Microbial activity and hydrolase activities during decomposition of root exudates released by an artificial root surface in Cd-contaminated soils. Soil Biol. Biochem. 38, 702–708.

Santibañez, C., de la Fuente, L.M., Bustamante, E., Silva, S., León-Lobos, P., Ginocchio, R., 2012. Potential use of organic- and hard-rock mine wastes on aided phytostabilization of large-scale mine tailings under semiarid Mediterranean climatic conditions: short-term field study. Appl. Environ. Soil Sci. http://dx.doi.org/10.1155/2012/895817.

Shetty, K.G., Hetrick, B.A.D., Schwab, A.P., 1995. Effects of mycorrhizae and fertilizer amendments on zinc tolerance of plants. Environ. Pollut. 88, 307–314.

Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis. 3rd edn. Academic Press, Amsterdam.

Tabatabai, M.A., 1994. Soil enzymes. In: Mickelson, S.H., Bigham, J.M. (Eds.), Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. SSSA, Madison, pp. 775–833.

Tarafdar, J.C., Marschner, H., 1994. Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biol. Biochem. 26, 387–395.

Tejada, M., Moreno, J.L., Hernández, M.T., García, C., 2007. Application of two beet vinasse forms in soil restoration: effects on soil properties in an arid environment in southern Spain. Agric. Ecosyst. Environ. 119, 289–298.

Turnau, K., Anielska, T., Ryszka, P., Gawronski, S., Ostachowicz, B., Jurkiewicz, A., 2008. Establishment of arbuscular mycorrhizal plants originating from xero-thermic grasslands on heavy metal rich industrial wastes—new solution for waste revegetation. Plant Soil 305, 267–280.

Walker, D.J., Clemente, R., Roig, A., Bernal, M.P., 2003. The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. Environ. Pollut. 122, 303–312.

Wang, B., Qiu, Y.L., 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16, 299–363.

Wang, F.Y., Shi, Z.Y., Xu, X.F., Wang, X.G., Li, Y.J., 2013. Contribution of AM inoculation and cattle manure to lead and cadmium phytoremediation by tobacco plants. Environ. Sci. Process Impacts 15, 794–801.

Watanabe, F.S., Olsen, S.R., 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proc. 29, 677–678.

Watts-Williams, S.J., Cavagnaro, T.R., 2012. Arbuscular mycorrhizas modify tomato responses to soil zinc and phosphorus addition. Biol. Fertil. Soils 48, 285–294.

Zarei, M., Hempel, S., Wubet, T., Schäfer, T., Savaghebi, G., Jouzani, G.S., Nekoueie, M.K., Buscot, F., 2010. Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. Environ. Pollut. 158, 2757–2765.