Science of the Total Environment 409 (2011) 1009–1016

Contents lists available at ScienceDirect

Science of the Total Environment

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

# Effect of arbuscular mycorrhizal fungi on plant biomass and the rhizosphere microbial community structure of mesquite grown in acidic lead/zinc mine tailings

Fernando A. Solís-Domínguez, Alexis Valentín-Vargas, Jon Chorover, Raina M. Maier *

*Department of Soil, Water and Environmental Science, The University of Arizona, 429 Shantz Bldg., Tucson, AZ 85721, USA*

ARTICLE INFO

*Article history:*
Received 16 July 2010
Received in revised form 16 November 2010
Accepted 17 November 2010
Available online 5 January 2011

*Keywords:*
*Prosopis juliflora*
Phytostabilization
DGGE
Mycorrhizal fungi
Mine tailing

ABSTRACT

Mine tailings in arid and semi-arid environments are barren of vegetation and subject to eolian dispersion and water erosion. Revegetation is a cost-effective strategy to reduce erosion processes and has wide public acceptance. A major cost of revegetation is the addition of amendments, such as compost, to allow plant establishment. In this paper we explore whether arbuscular mycorrhizal fungi (AMF) can help support plant growth in tailings at a reduced compost concentration. A greenhouse experiment was performed to determine the effects of three AMF inocula on biomass, shoot accumulation of heavy metals, and changes in the rhizosphere microbial community structure of the native plant *Prosopis juliflora* (mesquite). Plants were grown in an acidic lead/zinc mine tailings amended with 10% (w/w) compost amendment, which is slightly sub-optimal for plant growth in these tailings. After two months, AMF-inoculated plants showed increased dry biomass and root length ($p<0.05$) and effective AMF colonization compared to controls grown in uninoculated compost-amended tailings. Mesquite shoot tissue lead and zinc concentrations did not exceed domestic animal toxicity limits regardless of whether AMF inoculation was used. The rhizosphere microbial community structure was assessed using denaturing gradient gel electrophoresis (DGGE) profiles of the small subunit RNA gene for bacteria and fungi. Canonical correspondence analysis (CCA) of DGGE profiles showed that the rhizosphere fungal community structure at the end of the experiment was significantly different from the community structure in the tailings, compost, and AMF inocula prior to planting. Further, CCA showed that AMF inoculation significantly influenced the development of both the fungal and bacterial rhizosphere community structures after two months. The changes observed in the rhizosphere microbial community structure may be either a direct effect of the AMF inocula, caused by changes in plant physiology induced by AMF, or a combination of both mechanisms.


## 1. Introduction

Natural plant colonization of mine tailings is affected not only by metal toxicity but also by the physical–chemical and microbial properties of the mine tailings (Mendez and Maier, 2008). Successful establishment of a vegetation cover on mine tailings requires selection of plants that are tolerant to heavy metals and adapted to the environmental conditions of the site. This research is focused on desert mine tailings and in particular on the ability of arbuscular mycorrhizal fungal (AMF) inocula to aid in establishment of mesquite (*Prosopis juliflora*) in acidic mine metalliferous tailings. The AMF were evaluated both as plant growth promoting microorganisms and as a contributor to the re-establishment of microbial communities in the mine tailings. Criteria used to evaluate the AMF tested included plant biomass production, metal uptake by plant shoots, and the impact of the AMF on both bacterial and fungal community structure in the tailings.

Mesquite was selected for this study because it has shown potential for growth in metal-contaminated soils from urban industrial sites (Senthilkumar et al., 2005) and tolerance to Pb, As and Cr under hydroponic conditions and on agar media (Aldrich et al., 2004; Arias et al., 2010). This native desert plant is a salt- and drought-tolerant deciduous, large crowned and deep rooted bush or tree with the ability to establish symbiosis with nitrogen-fixing bacteria and to form AMF associations. Mesquite has been widely valued in the desert regions of northwest Mexico and the United States southwest for its use as fuel wood and in the manufacture of hardwood products and also for use in habitat restoration for eco-tourism (Stanton et al., 2001).

AMF have previously been examined for establishment of vegetation on polluted sites such as heavy metal-contaminated soils or mine tailings including taconite iron ore tailings and copper smelter factory fly ash (Dueck et al., 1986; Khan, 2005; Noyd et al., 1996). AMF are known for their ability to protect plants against heavy metal toxicity by mediating the interaction between metals and plant roots. For example, AMF can bind heavy metals in their cell wall, compartmentalize them in the vacuole or chelate them into the cytoplasm restricting the influx of heavy metals into the plant (Leyval et al., 1997). Plants colonized by AMF also have greater ability to absorb

* Corresponding author. Dept. of Soil, Water and Environmental Science, 429 Shantz Bldg. #38, University of Arizona, Tucson, AZ 85721-0038, USA. Tel.: +1 520 621 7231; fax: +1 520 621 1647.
*E-mail address:* rmaier@ag.arizona.edu (R.M. Maier).

0048-9697/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2010.11.020

nutrients like P, N, K, Ca, Mg, and water which results in better survival under stressed conditions (Auge and Stodola, 1990). AMF have also been shown to interact with different groups of soil bacteria and modify the rhizosphere microbial community. For example, Albertsen et al. (2006) showed that both bacterial and saprotrophic fungal biomass increased in the presence of *Glomus intraradices* in a root-free sand environment. Wamberg et al. (2003) examined the effect of AMF inoculation on community structure in the pea rhizosphere using denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA amplicons from community DNA extracts. Results showed that while DGGE profiles were quite similar between AMF-inoculated and uninoculated treatments, there were four to five specific bright bands in uninoculated treatments that were not present in *G. intraradices* inoculated treatments. These types of changes have yet to be studied in the extreme conditions characteristic of mine tailings.

There is currently only one published study on AMF–metal–mesquite interactions. In this hydroponic study Arias et al. (2010) studied chromium accumulation into mesquite stems and leaves. Chromium was accumulated at greater than 2-fold levels in stems and leaves of mesquite plants inoculated with *Glomus deserticola* (5000 and 2600 mg $kg^{-1}$, respectively) than in uninoculated plants (2300 mg $kg^{-1}$ and 990 mg $kg^{-1}$ respectively).

The desert mine tailings used in this study were obtained from the Klondyke mine tailings site, an Arizona State Superfund site located in the southeast corner of Arizona (Grandlic et al., 2008). The Klondyke tailings are acidic and contain high levels of heavy metals including lead and zinc (4620 and 1400 mg $kg^{-1}$, respectively). Plant establishment in these tailings has been shown to require 15% w/w compost to achieve growth similar to that in a soil collected adjacent to the tailings (Mendez et al., 2007). We used these tailings to test the hypothesis that inoculation with AMF would enhance biomass production and influence the development of the rhizosphere community structure found in plants grown in the tailings at a sub-optimal level (10%) of compost amendment. The specific goals of this study were: 1) to evaluate the effects of three different AMF on the establishment and growth of mesquite (*P. juliflora*), a representative species of the Sonoran-Arizona desert ecosystem, in mine tailings, 2) to characterize metal uptake into mesquite shoot tissue in the presence and absence of AMF, and 3) to determine the impact of the AMF on both bacterial and fungal community structure in the tailings following plant establishment.

## 2. Materials and methods

### 2.1. Tailings and compost

The Klondyke mine tailings were previously characterized (Grandlic et al., 2008). Briefly the tailings have a pH of 4.54 ± 0.02, total organic carbon and total nitrogen of 0.36 ± 0.068 and 0.067 ± 0.012 g $kg^{-1}$, respectively, and electrical conductivity of 3.0 ± 0.12 dS $m^{-1}$. The tailings texture is 51.9% sand, 26.4% silt and 21.7% clay. Neutrophilic heterotrophic counts in the tailings range from 10 to 75 CFU $g^{-1}$ dry tailings, while iron- and sulfur-oxidizer counts range from $10^5$ to $10^6$ MPN $g^{-1}$ dry tailings. In comparison, a soil collected immediately adjacent to the tailings had a heterotrophic count of $8 \times 10^4$ CFU $g^{-1}$ dry tailings and undetectable iron- and sulf-oxidizer counts. Total and bioavailable metals in the tailings are shown in Table 1. The compost used in this study is a mixture of dairy manure and green waste and was obtained from the University of Arizona Controlled Environment Agricultural Center, Tucson AZ. The compost is characterized by a total carbon, organic carbon, and total nitrogen in the compost of 135.5 ± 10.02 g $kg^{-1}$, 123.3 g $kg^{-1}$ and 2.7 ± 0.44 g $kg^{-1}$ respectively.

### 2.2. AMF inocula

Three AMF inocula were used in this study including two from a commercial source: *G. intraradices* and a mix of *G. intraradices* and *G. deserticola* (desert inoculum) (Reforestation Technologies International, Salinas CA, USA) and a native inoculum (described later). Inocula were added to the tailings as mixtures of sand and mycorrhizal propagules (spores, mycelia and colonized root segments). For the commercial inocula, 2400 AMF propagules were mixed with 20 g sand for each pot (0.8 propagules gram dry mine $tailings^{-1}$). The native inoculum (2400 propagules) was placed into 40 g sand for each pot (0.8 propagules gram dry mine $tailings^{-1}$).

#### 2.2.1. Isolation and identification of the native inoculum

The native inoculum was generated from a soil sample obtained from underneath a mesquite tree in Tucson, Arizona. A primary inoculum was created by mixing the sample with sand ≤ 1 mm particle size (1:3 w/w) using sorghum as a host plant. The primary inoculum was subcultured once into sand after three months. Spores were isolated by wet sieving and decanting (Gerdemann and Nicholson, 1963) and examination with a dissection microscope yielded three unique morphotypes. Spores of each morphotype were disinfected in 2% NaOCl for 10 min, soaked in gentamycin (100 mg $L^{-1}$) three times for 10 min, rinsed four times with sterile distilled water, and then crushed in 0.25 M NaOH. DNA was extracted as described by Redecker et al. (1997) and the supernatant was collected and extracted with an equal volume of 1:1 phenol to chloroform/isoamyl alcohol (24:1) followed by an equal volume of the aqueous phase with chloroform/isoamyl alcohol. The aqueous phase sample was stored at −20 °C.

Nested-PCR was used to amplify the DNA. First round primers were NS1(f) and ITS4(r) (Rodriguez-Echeverria and Freitas, 2006). Each 20 μL reaction contained 2 μL of 10× buffer containing 15 mM

**Table 1**
Effect of AMF on accumulation of metal(loid)s and phosphorus in mesquite shoot tissues after 60 days of growth in Klondyke mine tailings. Values are means ± SD ($n = 5$). Values with different letters are significantly different at $p < 0.05$ (one-way ANOVA, Tukey's test) for each row.

| | Total[a] | Plant available[b] | DATL[c] | Accumulation of metals in the shoot (mg $kg^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|
| Metal | mg $kg^{-1}$ | | | Control uninoculated | Desert inoculum | *Glomus intraradices* | Native inoculum | Average AF[d] |
| As | 91 | 0.03 | ≤30 | 0.16 ± 0.05a | 0.15 ± 0.02a | 0.14 ± 0.07a | 0.14 ± 0.02a | 0.001 |
| Cd | 2.4 | 1.17 | ≤10 | 0.13 ± 0.04a | 0.18 ± 0.06a | 0.17 ± 0.07a | 0.20 ± 0.06a | 0.07 |
| Cr | 36 | 0.04 | ≤5 | 0.11 ± 0.04a | 0.05 ± 0.04ab | 0.02 ± 0.02b | 0.01 ± 0.01b | 0.001 |
| Cu | 653 | 96.89 | ≤40 | 11.4 ± 4.07a | 11.7 ± 3.82a | 11.8 ± 1.63a | 21.5 ± 2.99b | 0.02 |
| Mn | 2811 | 171.99 | ≤2000 | 115 ± 55.4a | 152 ± 45.1a | 117 ± 55.3a | 116 ± 8.41a | 0.04 |
| Pb | 4620 | 149.34 | ≤100 | 3.83 ± 1.80a | 3.89 ± 0.83a | 2.88 ± 1.11a | 3.48 ± 1.71a | 0.0008 |
| Zn | 1400 | 442.80 | ≤500 | 46.1 ± 21.0a | 63.3 ± 17.6a | 73.4 ± 18.5a | 111 ± 50.8a | 0.05 |
| P | 131 | 0.21 | – | 2003 ± 842.8a | 1775 ± 166.5a | 1973 ± 222.3a | 4024 ± 1065b | 19 |

[a] Total metal(loid) concentration in the Klondyke tailings before planting (Grandlic et al., 2008).
[b] Plant available metal concentrations in Klondyke tailings before planting. Determined by diethylenetriaminepentaacetic acid (DTPA) extraction (Hayes et al., 2009).
[c] DATL = domestic animal toxicity limit. Values listed are maximum tolerable levels for cattle (National Research Council, 2005).
[d] AF = accumulation factor which is the total element concentration in the shoot tissue/total element concentration in the mine tailings (Brooks, 1998). The AF presented is the average of the four treatments.