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Arbuscular mycorrhiza (AM) status of rehabilitation plants of mine wastes in South Africa and determination of AM fungal diversity by analysis of the small subunit rRNA gene sequences

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ABSTRACT

The aim of this study was to assess the arbuscular mycorrhizal (AM) status of trees currently being used for phytoremediation of mining contaminated sites in South Africa, and to determine the AM fungal diversity of these sites. The trees, *Tamarix usneoides*, *Searsia lancea* and *Searsia pendulina* planted on waste sites associated with gold and uranium and zinc and platinum mining were assessed in late summer and the AM fungi were identified by molecular analysis of the small subunit rRNA gene sequences from spore DNA. All trees on all sites showed moderate to high mycorrhizal colonisation levels including those from wild populations of *T. usneoides* growing in uncontaminated sites. The AM fungi identified fell within the *Claroideoglomus*, *Diversispora*, *Glomus*, *Acaulospora* and *Sclerocystis* taxa and although their species diversity was relatively low there were distinct trends in their association with the three plant species sampled. The study represents a first report of the mycorrhizal status of *T. usneoides* and of the use of molecular techniques for the identification of AM fungi associated with mine wastes in South Africa. The results will assist in making decisions about the application of AM fungal inoculum in phytoremediation programmes for mine waste rehabilitation.

1. Introduction

1.1. Arbuscular mycorrhizal fungi and plant host interactions

Arbuscular mycorrhizal (AM) fungi are a monophyletic group of obligate root symbionts that comprise the entirety of the phylum Glomeromycota (Audet and Charest, 2007; Hildebrandt et al., 2007; Schüßler et al., 2001; Smith and Read, 2008). They are a ubiquitous feature of terrestrial environments and are associated with more than 80% of extant plant species (Krüger et al., 2012; Smith and Read, 2008). The principal characteristic of this association is an exchange of nutrients centred on the provision of a carbon source for the fungus in exchange for a supply of phosphorus to the plant (Smith and Read, 2008). Beyond this, the symbiosis may affect several other factors that influence the plant's ecology; such as water availability, access to other nutrients (e.g. nitrogen and micronutrients), grazing resistance, and tolerance to soil pathogens and pollutants (Hildebrandt et al., 2007; Rai and Bridge, 2009; Smith and Read, 2008).

AM fungi typically have low levels of host specificity (Johnson et al., 2005; Smith and Read, 2008). There is, however, significant functional diversity within the Glomeromycota, so that multiple mycorrhizal species colonising a single individual host need not be functionally redundant in the symbiosis (Antunes et al., 2011; Klironomos, 2000). Certain groups, for example, may more significantly improve phosphorus availability; while others may be better suited in providing protection from pathogens, and the relative benefit of these traits depends on the conditions of the environment and the vigour of the host plant (Antunes et al., 2011; Klironomos, 2000). Additionally, some AM fungi have exhibited host preference when presented with the opportunity to colonise different host species while, reciprocally, different plant species may vary in the degree to which they form mycorrhizal associations (Antunes et al., 2011; Johnson et al., 2005; Klironomos, 2000). The community structures of both plants and AM fungi in a given environment are influenced by each other as a result of these factors.

1.2. Phytoremediation and the role of AM fungi in mine waste site rehabilitation

Phytoremediation encompasses a subset of bioremedial techniques that entail the use of plants for the detoxification and stabilisation of polluted environments (Audet and Charest, 2007; Hildebrandt et al., 2007). Heavy metal (HM) pollution is a prominent feature of mine waste sites (Straker et al., 2007, 2008), and phytoremedial efforts have shown promise for the rehabilitation of such sites through the use of

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metallophyte plants which are able to tolerate exceedingly high HM concentrations by either stabilising the HM in the soil (through the secretion of chelating agents), or by hyperaccumulating HM and storing them in subcellular compartments (Audet and Charest, 2007; Hildebrandt et al., 2007). It has been established that the plant–AM fungal symbiosis can potentially enhance the HM tolerance and accumulative capacity of phytoremedial plants, though the mycorrhizal status of many metallophyte plants is unknown (Alford et al., 2010; Audet and Charest, 2007; Hildebrandt et al., 2007). The potential benefit of the AM fungal association is attributable to the increased surface area that the fungus effectively affords the plant, through which HM can be reached, and the ability of AM fungi to immobilise HM in the soil by means independent of those used by plants, such as through the secretion of glomalin (a soil aggregating glycoprotein) into the soil (Audet and Charest, 2007; Hildebrandt et al., 2007). There is significant evidence to suggest that mycorrhizal plants are more effective than non-mycorrhizal plants for the remediation of polluted soil, and are better able to survive as a result of the mycorrhiza, from which it can be inferred that mycorrhizal colonisation of susceptible metallophyte plants can drastically improve the phytoremediation of HM polluted soils (Hildebrandt et al., 2007; Leyval et al., 2002). AM fungal species that are best suited for the rehabilitation of a polluted site are typically those that are indigenous, probably as they are better adapted to the presence of the pollutants and are more likely to occupy a functional niche that promotes survival in the conditions of the site (Hildebrandt et al., 2007; Takács, 2012). As such, identification of the native AM fungal species of a polluted site is a necessary step in a phytoremedial strategy incorporating mycorrhiza (mycorrhizoremediation) (Takács, 2012).

1.3. Molecular identification of AM fungi

Traditionally, AM fungi have been identified according to morphological characteristics, especially those of spores (Krüger et al., 2012). This process is both arduous and problematic because of the limited variation of spore morphotypes between species, and the propensity of some species to produce spores of different morphotypes (Krüger et al., 2012). With the development of molecular tools for the identification of AM fungi, the AM fungal community composition of an environment can now feasibly be investigated. In particular, the nested AML primer pair targeting a portion of the SSU rRNA region (Lee et al., 2008) allows for the reliable identification of AM fungi to the genus level while screening out non-AM fungal organisms (Krüger et al., 2012). Furthermore, the recent revisions and consolidations in the phylotaxonomy of AMF have provided the requisite foundation for comprehensive investigation into the diversity of AM fungi from environmental samples (Krüger et al., 2012; Redecker et al., 2013; Schüßler and Walker, 2010).

1.4. AM fungi and mine tailings rehabilitation in South Africa

Phytoremedial strategies have been implemented for the rehabilitation of gold and uranium mine tailings (or tailings storage facilities — TSFs); for example the Mine Woodlands Project in the Witwatersrand Basin (Dye and Weiersbye, 2010). The AM status of these rehabilitated and non-rehabilitated tailings in North West and Free State provinces had been previously surveyed (Straker et al., 2007, 2008), and the present work constitutes a further effort to establish the AM status of phytoremedial metallophytes used in such rehabilitation programmes. The plants sampled were *Tamarix usneoides* E.Mey. ex Bunge, *Searsia lancea* (L.f.) F.A. Barkley and *Searsia pendulina* (Jacq.) Moffett, comb. nov., all of which are trees indigenous to southern Africa and now being planted to create artificial woodlands to rehabilitate TSFs, or their footprints, and other HM-contaminated mine sites in South Africa (Dye and Weiersbye, 2010). Moreover, since no molecular data on the identity of AM fungi on mine waste sites in South Africa exist, the community composition of AM fungi associated with these plants on these sites has been explored using nested PCR amplification of spore DNA. This work represents a composite of three different studies done over three years.

2. Materials and methods

2.1. Mining sites sampled

Soil and root samples were gathered from artificial woodland sites around TSFs at West Complex (S 26°55.950′ E 26°41.654′) located in North West Province and Mispah (S 26°59.365′ E 26°46.552′) located in Free State Province for one *T. usneoides* sample set (2010 study), and for the *S. lancea* and *S. pendulina* sample set (2012 study). Both tailings are found on the Vaal Reefs mining complex owned by Anglogold Ashanti Limited. Soil and root samples for another *T. usneoides* sample set (2011 study) were gathered from the heavy metal contaminated sites, ABB Zinc (S 26°25.438′ E 28°26.110′) and Impala Platinum (S 26°13.054′ E 28°26.545′) and from wild populations in uncontaminated locations in the Northern Cape (S 28°37.381′ E 20°20.849′; S 30°40.458′ E 18°25.727′; S 27°18.766′ E 20°06.561′). The Impala Platinum site is located in Springs, Gauteng and the ABB Zinc site is located in Nigel, Gauteng. Impala Platinum samples were collected from the area immediately around four evaporation dams used by the platinum refinery, and ABB Zinc samples were collected from a grassland area just south of a galvanizing plant. Although the soil chemistry of all the sites is not available, preliminary unpublished data (iThemba LABS, Cape Town) is available for the West Complex (WC) site and a similar site to the Impala Platinum site. These data show the platinum site to be higher in the following metals: extractable Mg (10-fold) and total Mg (4-fold); extractable Cr and total Cr (both 2-fold); extractable Ni (3.5-fold) and total Ni (3-fold); and extractable Al (3-fold) and total Al (2.5-fold). On the other hand, the platinum site was found to be lower in the following metals: extractable Zn (9-fold) and total Zn (2-fold); extractable Pb (5-fold) and total Pb (6-fold); extractable Ti (5-fold) and total Ti (2-fold); and extractable Au and total Au (3.5-fold). Extractable Cu was higher in the platinum site (2-fold) but the two sites are similar in total Cu, whereas extractable Fe is lower in the platinum site (4-fold) but the two sites are similar in total Fe. The platinum soils also have a higher pH (6.4) than the WC soils (3.7).

At each site at least three trees of similar age were selected. Three sub-samples of soil and fine root material from different points around each tree within a 20–30 cm radius were collected and bulked to form a single replicate sample. Root samples were separated from the soil and excess debris was removed before being rinsed and placed in 50% ethanol solution for storage until further processing. Sampling for the three studies was done from mid-April to early May in 2010, 2011 and 2012.

2.2. Root colonisation determination

The roots were cleared and stained using the method first described by Kormanik and McGraw (1982) and modified by Koske and Gemma (1989) and analysed for extent of AM fungal colonisation using the magnified intersections method (McGonigle et al., 1990).

2.3. Spore count analysis

Spores were isolated from 100 g soil using a modified version of the sieving protocol developed by Gerdeman and Nicolson (1963), which was improved upon by Pacioni and Rosa (1985). Sieve sizes were 1000 μm, 212 μm, 125 μm and 45 μm. Isolated spores were caught in pre-wetted 9 cm filter discs in a Büchner funnel using suction filtration. Grids of roughly 1 cm resolution were drawn on the filter discs to separate microscope fields for spore counting.