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Glomeromycota communities survive extreme levels of metal toxicity in an orphan mining site

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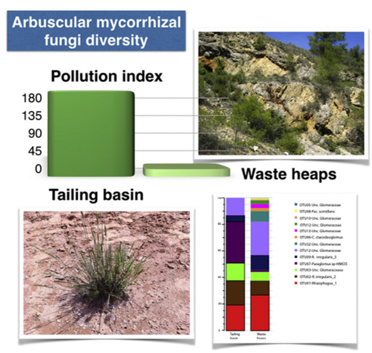
HIGHLIGHTS

- Heavy metal reduces mycorrhizal diversity.
- Mycorrhizal fungi survive extreme levels of heavy metal.
- Detection of heavy metal adapted mycorrhizal fungi.

GRAPHICAL ABSTRACT



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ABSTRACT

Abandoned tailing basins and waste heaps of orphan mining sites are of great concern since extreme metal contamination makes soil improper for any human activity and is a permanent threat for nearby surroundings. Although spontaneous revegetation can occur, the process is slow or unsuccessful and rhizostabilisation strategies to reduce dispersal of contaminated dust represent an option to rehabilitate such sites. This requires selection of plants tolerant to such conditions, and optimization of their fitness and growth. Arbuscular mycorrhizal fungi (AMF) can enhance metal tolerance in moderately polluted soils, but their ability to survive extreme levels of metal contamination has not been reported. This question was addressed in the tailing basin and nearby waste heaps of an orphan mining site in southern France, reaching in the tailing basin exceptionally high contents of zinc (ppm: 97,333 total) and lead (ppm: 31,333 total). In order to contribute to a better understanding of AMF ecology under severe abiotic stress and to identify AMF associated with plants growing under such conditions, that may be considered in future revegetation and rhizostabilisation of highly polluted areas, nine plant species were sampled at different growing seasons and AMF root colonization was determined. Glomeromycota diversity was monitored in mycorrhizal roots by sequencing of the ribosomal LSU. This first survey of AMF in such highly contaminated soils revealed the presence of several AMF ribotypes, belonging mainly to the Glomerales, with some examples from the Paraglomerales and Diversisporales. AMF diversity and root colonization in the tailing basin were lower than in the less-contaminated waste heaps. A *Paraglomus* species previously identified in a polish mining site was common in roots of different plants. Presence of active AMF in such an environment is an outstanding finding, which should be clearly considered for the design of efficient rhizostabilisation processes.

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1. Introduction

Mining sites are often abandoned once their economic value becomes less interesting, leading to orphan mining sites. The closure of such sites with surrounding wastes containing high levels of heavy metals (HM) is often the cause of severe environmental disturbances over extensive areas that can persist for centuries (Younger, 1997) becoming a serious global problem (Glick, 2010; Wu et al., 2016). When the HM concentrations become excessive, plant growth is strongly inhibited, if not completely absent, resulting in a significant risk of off-site migration of metals by erosion of the contaminated dust or leaching of the contaminants into the groundwater (Chen et al., 2008; Komárek et al., 2010). So far, numerous remediation technologies, such as HM mechanical removal or plant-mediated strategies, have been proposed to reduce the toxicity of orphan mining sites and the associated polluted environment (Khan, 2006; Dary et al., 2010; Miransari, 2011). Among them, phytoextraction or rhizostabilisation represent promising technologies with a low disruptive impact in nature. Rhizostabilisation consists in the promotion of a cover crop, which through the development of an extensive root system reduces leaking of HM into the groundwater as well as erosion (Gosh and Singh, 2005; Turnau et al., 2006; Ryszka and Turnau, 2007). However, this requires the use of metallicolous plants adapted to such conditions (Bothe, 2011), and optimization of their fitness and growth for example in combination with symbiotic microorganisms such as rhizobia for leguminous plants (Vidal et al., 2009), and arbuscular mycorrhizal fungi (AMF) belonging to the Glomeromycota (Turnau et al., 2001; He et al., 2014; Yang et al., 2015b). This group of fungi has been shown to increase the tolerance of plants to the presence of HM (Pawlowska et al., 1996; Weissenhorn et al., 1995; Hildebrandt et al., 2007; Azcón et al., 2010) which can be trapped by the fungal hypha or spores, reducing the availability for the plant and consequently their phytotoxicity (González-Chávez et al., 2009; Azcón et al., 2010; Cornejo et al., 2013). AMF are not host specific and are ubiquitous in a wide range of soils including those contaminated by diverse HM (Turnau et al., 2001; Vogel-Mikuš et al., 2006; Regvar et al., 2006; Hassan et al., 2011; Ban et al., 2015; Yang et al., 2015b), although fungal diversity is highly reduced by the presence of the HM (del Val et al., 1999b; Turnau et al., 2001; Zarei et al., 2008b). Persistence of the mycorrhizal symbiosis under such adverse conditions is considered a key factor in enhancing plant HM tolerance, and consequently an increase in the plant survival rate (Joner and Leyval, 2001). Moreover, distinct AMF ecotypes can induce differing degrees of HM tolerance in their host plant (Weissenhorn et al., 1995; Leyval et al., 1997; del Val et al., 1999a; Gaur and Adholeya, 2004; Zarei et al., 2008b; Khade and Alok, 2009), suggesting the existence of an AMF functional diversity. Therefore, knowledge of the AMF diversity occurring in situ becomes an essential first step for the effective application of AMF-based bioremediation (Sudová et al., 2008; Wu et al., 2009; Yang et al., 2015a).

Although AMF have been proven to enhance plant metal tolerance in polluted soils, their ability to survive in such high levels of metal contamination as those reported here (97,333 ppm total Zn and 31,333 ppm total Pb) has, to the best of our knowledge, not yet been demonstrated. This question was addressed in the present study by analyzing the presence and molecular diversity of AMF in roots of spontaneous and non-spontaneous vegetation at particularly high pollution levels never studied before. Such conditions are characteristic of the Les Avinières orphan mining site in the Languedoc region of southern France. After nearly 100 years of exploitation, soils in this area present very high levels of HM, mainly Zn and Pb (Escarré et al., 2011). The aim of the study is to contribute to a better understanding of the ecology of AMF under severe abiotic stress and to identify AMF that may be considered in future revegetation and rhizostabilisation of so highly polluted areas.

2. Materials and methods

2.1. Description of the study site and sampling procedures

The study was carried out on the ancient mining site of Les Avinières, located in the mining district Les Malines within the region of Saint-Laurent-le-Minier, Gard (43° 55′ 97″ North, 3° 39′ 97″ East), 40 km north of Montpellier (France). The site is characterized by a temperate Mediterranean climate with monthly mean temperatures between 5.7 °C (January) and 25 °C (July). The average annual rainfall is 1500 mm (2007–2011).

The mine Les Avinières was active from 1870 to 1914 and was one of the most important sources of lead and zinc in France (Vincent, 2006). After closure of the mine, a contaminated area covering 15 ha remained and was abandoned. Previous analyses indicated that pollution was highest in two major plots of approximately 2 and 3 ha contaminated with Zn, Pb, Cd and other HM (Escarré et al., 2011). The first of these is a tailing basin along the Vis River valley, used during the 20th century for processing ore from Les Malines mine. The second is composed by the waste heaps located in the slope of the hill, next to the mine entry. In order to confirm the extreme HM contents, the total, EDTA and DTPA-extractable concentrations of Zn, Pb and Cd were measured in the soil associated with root samples of selected plants (see below) from sampling areas in the tailing basin and waste heaps, as described by Escarré et al. (2011).

The soil in this type of environment is sandy and typically presents low organic matter content with a paucity of major nutrients that together with the high metal toxicity make these soils very unfertile. Consequently, both plots are mainly dominated by bare soil, especially in the tailing basin, but sparsely vegetated spots occur where metal-tolerant ecotypes (*Festuca arvernensis*, *Koeleria vallesiana* and *Armeria arenaria*) and HM hyperaccumulators (*Anthyllis vulneraria*, *Thlaspi caerulescens* and *Silene latifolia*) have thrived despite the limiting conditions. The localization of the sampling sites and sampled plants is indicated in Fig. S1. In order to have a broad picture of the mycorrhizal fungi present in these polluted areas, a total of 52 roots and rhizosphere soil samples from partial root systems of 9 different plant species (*A. vulneraria*, *F. arvernensis*, *K. vallesiana*, *A. arenaria*, *T. caerulescens*, *Biscutella laevigata*, *Arenaria aggregata*, *Silene vulgaris* and *Thymus vulgaris*) were collected within the tailing basin (41 samples) and waste heaps (11 samples), and over three sampling times corresponding to April (18 samples), May (19 samples) and September (15 samples) in 2011 (Table S1). April corresponded to the beginning of the growth season, May during the growth season, and September at the end of summer. Some of the plants belonged to previous phytoremediation experiments conducted at the same site (Frérot et al., 2006; Mahieu et al., 2011). All root samples were placed in labeled polyethylene bags and transported to the laboratory for further processing. Here, fine roots were separated from the soil, washed with tap water and rinsed with distilled water, cut into 1-cm segments and homogenized. A portion of the root sample was used to measure AMF colonization. The rest of the root sample was dried at 50 °C overnight as previously described (Farmer et al., 2007) and stored at room temperature until molecular analyses were performed. Rhizospheric soil attached to representative root samples from both sampling sites (tagged as 31, 34, 35, 37, 38, 39, 40 and 41 in Table S1) was used for HM content analyses.

2.2. Determination of AMF root colonization

Roots from all samples were stained with trypan blue in glycerol after clearing with potassium hydroxide (Phillips and Hayman, 1970). AMF root colonization was estimated by light microscopy as described by Trouvelot et al. (1986). Overall frequency of mycorrhization (F%), percentage of root cortex colonization (M%), overall arbuscule abundance (A%) and arbuscule abundance in colonized regions (a%) were