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The molecular diversity of arbuscular mycorrhizal fungi in the arsenic mining impacted sites in Hunan Province of China

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) can establish a mutualistic association with most terrestrial plants even in heavy metal contaminated environments. It has been documented that high concentrations of toxic metals, such as arsenic (As) in soil could adversely affect the diversity and function of AMF. However, there are still gaps in understanding the community composition of AMF under long-term As contaminations. In the present study, six sampling sites with different As concentrations were selected in the Realgar mining area in Hunan Province of China. The AMF biodiversity in the rhizosphere soils of the dominant plant species was investigated by sequencing the nuclear small subunit ribosomal RNA (SSU rRNA) gene fragments using 454-pyrosequencing technique. A total of 11 AMF genera were identified, namely *Rhizophagus*, *Glomus*, *Funneliformis*, *Acaulospora*, *Diversispora*, *Claroideoglomus*, *Scutellopora*, *Gigaspora*, *Ambispora*, *Praglomus*, and *Archaeospora*, among which *Glomus*, *Rhizophagus*, and *Claroideoglomus clarodeum* were detected in all sampling sites, and *Glomus* was the dominant AMF genus in the Realgar mining area. Redundancy analysis indicated that soil pH, total As and Cd concentrations were the main factors influencing AMF community structure. There was a negative correlation between the AMF species richness and the total As concentration in the soil, but no significant correlation between the Shannon–Wiener index of the AMF and plants. Our study showed that high As concentrations can exert a selective effect on the AMF populations.

Introduction

Arsenic (As) is one of the most hazardous metalloid that can be released into the environment due to geochemical processes, *e.g.*, rock weathering or volcanic eruptions (Fitz and Wenzel, 2002), or through anthropogenic activities, such as mining, application of biocides, and fossil fuel combustion, urban wastes discharging *etc.* (Mukhopadhyay et al., 2002; Beesley and Dickinson, 2010). Arsenic contamination could potentially cause global environmental problems and health risks (Smith et al., 1998; Meharg and Hartley-Whitaker, 2002). As a result, As has been included in the list of 20 most hazardous substances by the Agency for Toxic Substances and Disease Registry (Dhuldhaj et al., 2013).

There are more than 300 As-bearing minerals, among which, realgar mine (As_4S_4) is less common (Hudson-Edwards and Santini, 2013). The Shimen Realgar Mine of Hunan Province in southern China, which had been mined for over 1500 years, had the largest source of realgar (As_4S_4) ore in Asia. Since 1958, large-scale mining activities, arsenic product processing with discharge of As-containing drainage, improper storage of tailings, and deposition of metallurgical fume,

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gradually led to severe As contamination of the soil-water systems in the mining area. Arsenic can be taken up by plants and enter the food chain at excessive levels, thus possessing significant health risks to local people. Obviously, there is an urgent need to monitor the ecological impacts of As contamination and take effective measures to restore As-contaminated environments.

Many studies on As eco-toxicity focus on agricultural ecosystem and crops, but few on soil microbial communities in the natural ecosystems. As known, soil microorganisms play important roles in As biogeochemistry (Zhu et al., 2014; Wang et al., 2014), while soil contamination with As would greatly influence the biodiversity and function of soil microbial communities (Lorenz et al., 2006), and subsequently lead to a decrease of soil fertility. Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil fungi in natural and agricultural ecosystems, and can form symbiotic associations with the majority of higher plants (Smith and Read, 2008). The AM (arbuscular mycorrhizal) association can essentially improve plant mineral nutrition (Willis et al., 2013), plant water relations (Li et al., 2013) and enhance plant resistance to As contaminations (Chen et al., 2007; Zhang et al., 2015). The ecological significance of AMF in stabilizing ecosystem structure and function has been widely accepted (van der Heijden et al., 1998; Rillig, 2004), and there is also a potential role of AMF in assisting bioremediation of As contaminated environments (Liu et al., 2005; Dong et al., 2008). Recent studies showed that AMF naturally occur in As-contaminated sites (Meharg and Cairney, 1999; Smith et al., 2010a). High As concentrations can exert a selective effect on the AMF populations, and reduce AMF species richness (Smith et al., 2010a; Schneider et al., 2012). AMF species are generally resistant to short-term As toxicity (Smith et al., 2010b), but may be changed under severe As contaminations. So far, little information is available about the AMF biodiversity in natural As-contaminated sites.

In the present study, we collected soil samples from the mining sites severely polluted by As in the Realgar mining area to detect the biodiversity of AMF by using 454-pyrosequencing technique. The study was aimed to test the impacts of As contamination on the AMF biodiversity, and to explore key factors influencing AMF community structure. It was expected that results from present study would deepen our understanding of the ecological impacts of As contamination, and also unravel the feasibility of isolating and introducing tolerant AMF in future ecological restoration programs.

1. Materials and methods

1.1. Study area and sampling

Soil samples were collected in October 2012 from Shimen Realgar Mine (N 29°38′11″–29°38′43″, E111°2′06″–111°2′23″) in Hunan Province of China. Based on the different pollution sources, six representative sites were selected and coded as REF, S1, S2, S3, S4, and S5, according to the total As concentration from low to high (Fig. 1). S1 and S3 located in the riverbank of Lishui River that was contaminated by the wastewater discharged from the tailing pool. S2 was situated in the As product processing factory. S4 was a slagheap, and S5 lied in the tailing pooled with mineral residue sediments. The site REF on another river bank that was almost parallel with the Lishui River, was chosen as an uncontaminated reference site.

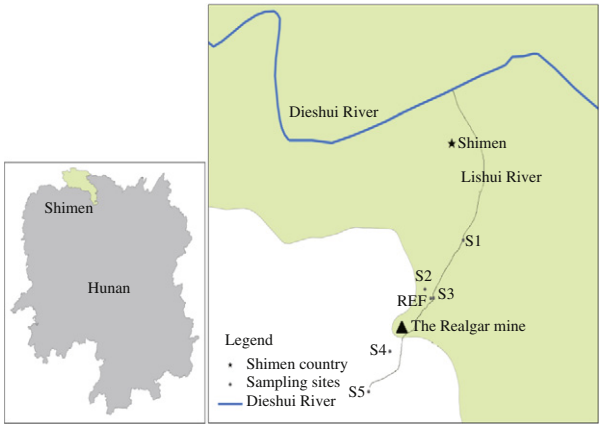

Fig. 1 – Map of the study area. REF, S1, S2, S3, S4 and S5 represent the six sampling sites.

Plant and soil samples were collected according to the methods described by Xiang et al. (2014). At each sampling site, four 1 × 1 m^2 quadrats were designated for vegetation survey. All plant species present in each quadrat were identified. The total number of individuals per plant species was counted. Ten soil cores (3 cm in diameter and 15 cm in depth) from each quadrat were taken, then mixed and stored in polyethylene bags at 4°C in a refrigerated box. After being transported to the laboratory, the composite soil samples were passed through a 2 mm sieve and divided into two subsamples. One subsample was kept at −80°C for molecular analysis and the other was air-dried for analysis of soil physicochemical properties. The mixed roots were manually collected from the soil samples for measuring mycorrhizal colonization rates.

Soil chemo-physical properties

Soil pH was measured in a 1:2.5 (V/V) soil:water suspension. Soil available phosphorus (AP) was assayed according to the method described by Olsen (1954). Bioavailable As in the soil was extracted by 0.1 mol/L HCl (soil:solution = 1:10, V/W). Calculation of the soil C/N ratio was based on the total C and total N contents that were analyzed using Element Analyzer (Vario EL III, Germany) (Xiang et al., 2014). Soil organic carbon (SOC) was assayed according to the Walkley-Black dichromate oxidation procedure (Nelson and Sommers, 1996).

Air dried soil samples were further passed through 0.15 mm sieve. Approximately 0.2 g sample was soaked in 10 mL HNO_3 + 2 mL HF for 12 hr, then digested by CEM Mars 5.0 (CEM Co. Ltd., USA). The digested samples were analyzed for Mg, Mn, Ni, Sb, Sn, Ti, Zn, Al, Ba, Cd, Fe, Bi, Co by inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 2000 DV, Perkin-Elmer, USA) in 3% HNO_3, while the As concentration was measured by inductively coupled plasma mass spectrometry (ICP-MS; Agilent Technologies, 7500, USA) Blanks and internal standards of soil (GSS-6, China Standard Research Center) were used to ensure the accuracy of chemical analysis. All reagents were of analytical grade.