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Short communication

Arbuscular mycorrhizal fungal diversity in rhizosphere spores versus roots of an endangered endemic tree from Argentina: Is fungal diversity similar among forest disturbance types?

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ABSTRACT

The aim of this study was to compare the arbuscular mycorrhizal fungal (AMF) community of the rhizosphere and inside the roots of the perennial *Polylepis australis* tree. Three forest types differing in their structural complexity due to anthropogenic disturbances were chosen at three different sites at the high mountains of central Argentina. Rhizosphere spores and *P. australis* roots of four randomly selected trees were isolated from 36 soil samples, DNA was extracted and the 18S rDNA fragments were amplified by nested-PCR. The products were analyzed by DGGE and the bands were excised for sequencing. In total, 36 OTUs were defined from 56 DGGE bands successfully sequenced. Forest disturbance types showed similar communities of AMF, as rhizosphere spores and within the roots of *P. australis*. However, DGGE clustering showed mainly differences between rhizosphere spores and root-colonizing AMF. Members of Glomeraceae, Pacisporaceae, Acaulosporaceae and Gigasporaceae were shown in rhizosphere spore samples. Root samples showed only members of Acaulosporaceae and Gigasporaceae, which might be complementary in terms of soil resources exploration. The prevalence of the root system with their community of symbionts might explain the resilience of AMF soil communities to forests structural changes. This study presents evidence of a possible preference in the AMF–*P. australis* interaction.

1. Introduction

The high mountain forests of central Argentina are dominated by the perennial tree *Polylepis australis* Bitt, an endemic species of Argentina (Renison et al., 2013). These mountain forests have been reduced due to the effect of livestock rearing combined with fires, set to promote grass re-growth. The forests remnants are represented by patches differing in their structural complexity and their degree of conservation, depending on their disturbance history. The most preserved forest fragments are located far away from rancher houses, roads and in deep ravines where livestock and fire ignitions are less frequent; while most degraded woodlands are easily accessible (Renison et al., 2011).

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophic symbionts that colonize ~80% of the land plant species studied to date. In exchange for plants assimilated carbon, AMF promote hosts nutrition and growth, provide protection against root pathogens and improve soil structure (Smith and Read, 2008). Forest structural changes might affect native AMF communities through changes in vegetation cover, microclimatic conditions or soil physical-chemical characteristics (e.g. Zangaro et al., 2013). However, in previous studies, AMF communities (richness, abundance, diversity and evenness) of *P. australis* mountain forests were not influenced by structural changes (Soteras et al., 2015) nor by increasing livestock density (Menoyo et al., 2009), showing that AMF soil communities might be resilient to these antropogenic disturbances.

The lack of AMF host specificity has been widely acknowledged (Smith and Read, 2008). However, it is becoming evident that exists a degree of selectivity in the plant–AMF association (Davison

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et al., 2011; Helgason et al., 2002; Öpik et al., 2009). Furthermore, in nature a single root system is usually colonized simultaneously by different species of AMF (Saks et al., 2014), and controlled experiments indicated that different AMF species can work synergistically to improve plant growth (Maherali and Klironomos, 2007). There are differences in the AMF colonizing strategies based at the family level, while Gigasporaceae family produce extensive extra-radical mycelia, Glomeraceae mostly colonize inside the roots (De Souza et al., 2005a). Meanwhile, Acaulosporaceae family could be considered as an intermediate strategy, producing low biomass inside and outside the roots (Hart and Reader, 2002; Hart et al., 2001). Moreover, AMF taxa differ in their carbon and phosphorous demand (Pearson and Jakobsen, 1993). Therefore, host–AMF interaction preference could be more related to the reciprocal correspondence of both partners to the same functional group rather than by plant-symbionts identity (Chagnon et al., 2013; Öpik et al., 2009Saks et al., 2014).

The AMF spores of the rhizosphere of *P. australis* at the high mountains forests of central Argentina have been morphologically described (Menoyo et al., 2009; Soteras et al., 2015). However, molecular techniques that could help improving soil community's characterization have not yet been implemented. In addition, research in this high altitudinal forests has been focused on rhizosphere spore communities without considering root-colonizing AMF. The identification of *P. australis* symbionts will allow to state general ecological hypothesis about host-AMF preferences. But, more meaningful due to the endemic nature and current degradation of *P. australis* forests, the identification of root-colonizing symbionts will increase the knowledge about the most appropriate AMF-inocula to facilitate reforestation efforts. Molecular techniques provide an accurate approach for the identification of the AMF community of the rhizosphere soil and within the target plant roots (Helgason et al., 1998; Husband et al., 2002). The denaturing gradient gel electrophoresis (DGGE) can separate ribosomal DNA (rDNA) fragments of different sequence amplified from total community DNA (Muyzerm and Smalla, 1998). Therefore, this molecular fingerprinting technique together with the sequencing of amplified rDNA fragments has been applied for the AMF community characterization (Kowalchuk et al., 2002; Liang et al., 2008; Öpik et al., 2003).

One of the most important dimensions of AMF niche space is comprised by the root system of host plant species. Other niche axes of these obligate plant symbionts include the soil environment, as AMF allocate great part of their biomass to spores and/or extra-radical mycelia (Bever et al., 2001; Lekberg et al., 2011; Smith and Read, 2008). The resilience of the AMF community against anthropogenic disturbances might be mainly influenced by the degree that below- or aboveground changes physically disrupt the soil (Kladivko, 2001). Disturbance history in the high mountain forests of central Argentina has mainly changed vegetation cover and soil impedance but maintaining the dominant plant species of the community (Renison et al., 2004; 2011). Therefore, symbionts of the root system may have prevailed after disturbance occurrence thus restoring the soil community of the ecosystem. In this context and according to previous field evidence (Menoyo et al., 2009; Soteras et al., 2015), we hypothesized that soils of *P. australis* forests with different disturbance histories are similar in terms of AMF community composition. We expected that the different forest disturbance types (degraded, young and mature) might not strongly affect the AMF community composition thus showing similar DGGE banding patterns. However, considering that coexisting plant species might be colonized by different AMF communities (Vandenkoornhuyse et al., 2003) and that hosts-AMF preference is generally the rule, we hypothesized that *P. australis* is colonized by a subset of the soil AMF. We expected that this perennial host would show a tight relationship with AMF taxa belonging to the same functional group (i.e. AMF members of the Gigasporaceae family).

To test these predictions, the aim of this study was to compare the AMF community of the rhizosphere spores of *P. australis* trees with the root-colonizing AMF community in three forests types differing in their structural complexity, using nested-PCR DGGE analysis of the 18S rDNA followed by sequencing of excised bands.

2. Materials and methods

2.1. Field sampling of rhizosphere soil and root system

During June 2012 soil samples were collected from three *P. australis* forest disturbance types (degraded forest, young forest and mature forest) at three river basins (spatial replicates) of the high mountains of central Argentina. Lateral roots and rhizospheric soil were collected with a trowel 15 cm away from the main trunk and below the soil litter layer (0–20 cm depth) of four randomly selected *P. australis* trees separated for at least 20 m of distance. Thus rhizosphere soil samples totalized 36 replicates (4 trees × 3 forest types × 3 sites).

Forest disturbance types have been shaped by livestock and fire management and differed among each other according to their structural complexity (e.g. canopy cover, age and height of the oldest tree, exposed rock surfaces; Renison et al., 2011). Mean temperature for the coldest and warmest months are 5 °C and 11.4 °C, respectively, with no frost-free period. Mean annual precipitation is 840 mm, being concentrated in the warmest months (October–April) (Cabido et al., 1987).

2.2. Spore isolation from soil and DNA extraction

AMF spores were extracted by wet sieving and decanting of 25 cm^3 of soil, followed by centrifugation in sucrose solution (50% w/v) (Walker et al., 1982). The material on the top sieve (500 μm) was discarded and the content on the fine sieve (38 μm) was suspended in water and centrifuged for 4 min at 3000 rpm. The resulting pellet was resuspended and centrifuged in sucrose for 2 min at 2000 rpm. Finally, the suspension was transferred to a 20 ml tube and stored at 4 °C until DNA extraction.

DNA extraction from the isolated spores was performed using UltraClean™ soil DNA kit (MO BIO Laboratories, Solana beach, CA, USA) following the manufacturer's specifications. DNA extracted was checked by agarose gel electrophoresis (0.8%), stained with ethidium bromide and visualized under UV light.

2.3. DNA extraction from root system

Roots were carefully separated from rhizosphere samples and washed with tap water. Then the samples were dried for 48 h at 50 °C and stored at −5 °C until DNA extraction. Root material (60 mg) was ground in a mortar with liquid nitrogen and DNA extraction was performed following manufacturer's protocol of the Invisorb® Spin Plant Mini Kit (Invitek, Berlin, Germany).

2.4. Amplification of 18S rDNA fragments by nested-PCR

In order to characterize the AMF community of soil and roots the methodology proposed by Liang et al. (2008) with modifications was followed. Nested-PCR reactions were done in a sterile microcentrifuge 0.5 ml tube using GoTaq® Flex DNA Polymerase (Promega, Madison, USA) according to manufacturer's advices, adding elution buffer (20 mM Tris–HCl, 50 mM KCl, pH 8.4) and reaching a total reaction volume of 50 μl.

A 580 bp sequence of the SSU rRNA gene was amplified using the universal eukaryotic primer NS31 (Simon et al., 1992) and the