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Diversity of arbuscular mycorrhizal fungi along an environmental gradient in the Brazilian semiarid

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ABSTRACT

Plants host, environmental characteristics and dispersal limitation are the main factors affecting the diversity of arbuscular mycorrhizal fungi (AMF) in global scale, whereas at the local scale other factors might also influence the composition of the AMF community. Therefore, the objective of this study was to assess the diversity of AMF and to relate it with the richness of plant species, season (dry or rainy) and soil parameters along an environmental gradient to know which are the drivers of the spatial distribution of AMF in the Brazilian semiarid. The gradient was composed of a dry forest (DF), a transitional zone (TZ) and a moist forest (MF). Ten soil samples were collected during the rainy (August 2011) and dry (February 2012) seasons in each site. The soil properties were determined, as well as AMF diversity, the latter based on morphological spore identification. There were significant differences between the DF and the other two areas in most soil chemical parameters, whereas the majority of soil attributes in the MF and TZ were similar. Altogether, 50 AMF species were identified, and the genera *Acaulospora* and *Glomus* were predominant. The AMF community structure in DF was significantly different from the other two areas by ordination (NMDS) and statistical method (PERMANOVA). However, the highest species diversity, based on the Shannon index, occurred in the TZ. The AMF community structure differed between seasons, with greater spore abundance in the dry season. Spatial AMF distribution was influenced by plants host, season, but the soil was the main factor. Four edaphic attributes showed approximately 60% of correlation with AMF community composition (Zn, Mg, base saturation and clay) based on the BIO-ENV analysis. We conclude that vegetation, seasonal variations and soil type affect the AMF diversity, and that the latter is a key factor for the similarity/dissimilarity of AMF communities between areas in the Brazilian semiarid.

1. Introduction

The soil is a complex system that supports plants, harbors microorganisms and is, where ecological associations are shaped by the environment (Lavelle, 2009; Paul, 2007). One of the most important ecological soil associations involves the arbuscular mycorrhiza, formed by representatives of the phylum Glomeromycota and more than 70% of Angiosperm species (Brundrett, 2009; Smith and Read, 2008). This association is the oldest type of mycorrhiza in nature (Das and Varma, 2009), widely distributed on the planet (Brundrett, 2009; Öpik et al., 2010, 2013), and is important for supporting the ecosystem services which maintain terrestrial environmental stability.

Arbuscular mycorrhizal fungi (AMF) benefit their host plants nutritionally (Smith and Smith, 2012), improve their defense against pathogens (Sikes et al., 2009) and increase their resistance to environmental stress (Yano-Melo et al., 2003). AMF also contribute to edaphic stability because they promote the aggregation of soil

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particles, water retention via the mycelial network and glomalin production (Gianinazzi et al., 2010). Furthermore, AMF diversity contributes to the coexistence, productivity and maintenance of plant diversity under different environmental conditions (van der Heijden et al., 1998; Wagg et al., 2011a, 2011b).

On a global scale, dispersal limitation, host plant communities and environmental variables shape AMF community structure (Öpik et al., 2010, 2013; Kivlin et al., 2011), whereas, at a local level soil properties, dispersal, interspecific competition and climatic differences are the main factors affecting AMF diversity (Ji et al., 2012; Lekberg et al., 2007).

This study was designed to assess AMF species richness, diversity and community composition along a vegetation gradient composed of a dry forest, a transitional zone and a moist forest. The following hypotheses were tested: (1) AMF diversity and community structure differ at sites with different vegetation types; (2) the area with the greatest plant species richness harbors the greatest AMF richness and diversity; (3) fungal richness and diversity are higher in the dry season; (4) the soil properties are important factors that influence the AMF community in semiarid areas. We assumed that by studying the diversity of these mycobionts along an environmental gradient (Mangan et al., 2004) we would arrive at a better understanding of the factors determining AMF diversity.

2. Materials and methods

2.1. Study site

The study area was located in the municipality of Triunfo, Pernambuco, Brazil. This municipality is located in the semiarid region of Pernambuco and has a hot and humid climate. The vegetation consists mainly of semi-deciduous forests (IBGE, 2011). The topography of the site is strongly undulating and hilly, and soil type is oxic Cambisols according to the Brazilian classification (CPRM, 2005).

The mean annual temperature was 21 and 22 °C, and the rainfall was 1471 and 284 mm for the years 2011 and 2012, respectively (AGRITEMPO, 2013). Triunfo has a historical average annual rainfall of around 1000 mm (CPRM, 2005; AGRITEMPO, 2013), but 2012 was atypical due to a period of extreme drought.

Sampling was conducted on a mountainside located in 'Carro Quebrado' in the district of Canaã (07°52′36.30″S and 38°6′18.40″W). The corresponding vegetation gradient consisted of a dry forest (DF – 7°52′45.29″S and 38°06′13.64″W) at 570 m.a.s.l., a transitional zone (TZ – 7°52′37.01″S and 38°06′16.59″W) at around 620 m.a.s.l. and a moist forest (MF – 7°52′29.42″S and 38°06′12.07″W) above 670 m.a.s.l. At altitudes over 600 m these environments are characterized by moist forests with milder weather ranging from humid to sub-humid, greater water availability and higher clay content in the soil. Together, these factors support a variety of shrubs and epiphytes (Rodal and Nascimento, 2002; Souza et al., 2010). The lower altitudes are characterized by shallow soils with low water and higher sand content. The vegetation, typically 4–7 m height, is adapted to tolerate the dry season via a variety of mechanisms such as the loss of leaves, presence of spines, water storage bodies, small leaf area and deep roots (Giulietti et al., 2006).

The following plant families are common to the three areas: Boraginaceae, Euphorbiaceae, Fabaceae, Rhamnaceae and Schoepfiaceae. More detailed information about the plant species and families found in the three areas is shown in Table 1.

2.2. Sampling

Soil sampling was conducted in August 2011 (rainy season) and in February 2012 (dry season). In each area, ten composite soil samples (each comprising five sub-samples) were randomly collected at a depth between 0 and 20 cm. Each sample contained approximately 5 kg of rhizospheric soil. Samples were packed in plastic bags, transported to the Mycorrhiza Laboratory at the Federal University of Pernambuco (UFPE), and kept at room temperature (26 °C) for direct evaluation of the AMF community and for the assembly of trap cultures. To avoid pseudo-replication we used 10 composite samples per site, each of which was considered independent. It was not possible to use true repetitions due to the lack of independent sites with similar characteristics.

2.3. Chemical and physical analyses

For soil analysis, the ten samples from each area were mixed in pairs, producing five composite samples of soil from each area. The determination of the chemical and physical attributes of the samples was carried out at the Universidade Federal Rural de Pernambuco – the 'Estação Experimental de Cana-de-açúcar' in Carpina.

For nitrogen determination, samples were digested and quantified by titration according to method of Kjeldahl (Thomas et al., 1967). For the evaluation of other soil chemical properties we followed the methodology described in the 'Manual de Análises Químicas para a Avaliação da Fertilidade do Solo' (Silva et al., 1999). Soil pH was measured in water (1:2.5 (v:v) soil:water suspension). Ca^{2+} and Mg^{2+} were extracted with 1 M KCl and determined by atomic absorption; K^+, Na^+, P, Cu, Zn and Mn were extracted using the Mehlich 1 reagent (HCl 0.05 M + H_2SO_4 0.0125 M) at dilutions of 1:5 and 1:10 (w:v) soil:solution for micro and macronutrients, respectively; K^+ and Na^+ were determined by flame photometry; P, Cu, Zn and Mn by colorimetry. The organic matter (OM) and C content were determined by potassium dichromate oxidation and titration with ferrous ammonium sulphate. The cation exchange capacity (CEC) was determined as the total exchangeable base and aluminum content. The base saturation (BS) was calculated by dividing total exchangeable bases by CEC and multiplying by 100.

Soil moisture was determined gravimetrically after drying 2 g of soil in an oven (105 °C/24 h) and the values expressed as percentage (Debosz et al., 1999). For evaluation of the proportions of sand, silt and clay, the pipette method was used (Embrapa, 1997).

2.4. Trap cultures

Trap cultures were established to obtain healthy looking spores and detect possible AMF species not recovered from field soil samples. Traps were set up in the Mycology Department greenhouse using composite samples from each collection site. One kg of sterilized sand and one kg of field soil (composed of 500 g of soil from every two sample units as described above). The five pots were grouped according to their origin along the gradient. The sterilized sand was used to increase soil aeration of trap cultures and provide the necessary conditions for plant growth and survival, mycorrhizal colonization and consequent sporulation of AMF species (Saif, 1981; Gaur and Adholeya, 2000).

Maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench) and beans (*Phaseolus vulgaris* L.) were used as host plants because they grew well in the greenhouse. Furthermore, maize and sorghum produce large root biomasses. The beans on the other hand represented the Fabaceae, one of the main nitrogen-fixing plant families found in the study areas.

The cultures remained in the greenhouse for two cycles of four months each, and were irrigated daily. They were fertilized every 15 days following the method of Hoagland and Arnon (1950) modified by Jarstfer and Sylvia (1992). The irrigation was suspended at the end of each cycle and 100 g of soil were taken from each culture for spore extraction and subsequent identification of AMF species. The average temperature in the greenhouse