



Diversity of arbuscular mycorrhizal fungi in Atlantic forest areas under different land uses



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ABSTRACT

Agricultural land use compromises the viability of Atlantic forest remnants and may permanently alter the structure of the biological soil community. Soil micro-organisms such as arbuscular mycorrhizal fungi (AMF) which participate in symbiotic associations with plant roots are of particular importance. In order to assess the impact of cultivation we measured the diversity of AMF in six areas in Goiana, PE, Brazil: a sapodilla plantation, a rubber tree plantation, a mahogany plantation, a eucalyptus plantation, a crop rotation area currently being used to cultivate cassava, and an area of Atlantic forest. A total of 96 samples of rhizospheric soil were collected in the wet (June 2011) and dry (March 2012) seasons. Glomerospores were extracted from the soil, counted and used for AMF species identification. A total of 50 species belonging to 15 genera were recorded. *Acaulospora* spp. and *Glomus* spp. predominated, accounting for 52% of total species. The low value found in non-metric multidimensional scaling (NMS) multivariate analyses (33.2%) indicated that AMF community composition was more affected by different land uses than by physical and chemical characteristics of the soil. Diversity, evenness and richness indices were higher for the environment under greater stress (crop rotation), indicating that mycorrhizal symbiosis could be a strategy by which fungi and plants overcome biotic and abiotic stresses that occur in the soil. Diversity, evenness and richness indices tended to be lower in communities established in climax environments, such as in the Atlantic forest, rather than in the ones established in cultivation areas.

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1. Introduction

The Brazilian Atlantic forest is one of 25 world biodiversity hotspots (Myers et al., 2000) and contains more species' diversity than most Amazonian forest formations (Morellato and Haddad, 2000). The Atlantic forest extends from Rio Grande do Norte to Rio Grande do Sul and encompasses a variety of different land formations, landscapes and climates (Silva and Casteleti, 2003). It is considered one of the two most threatened biomes on Earth. Only about 7% of the original vegetation remains today and what is left has a highly fragmented distribution along the coast (SOS Mata Atlântica, 2013). Two processes that have led to massive destruction of the forest, particularly in the northeast of Brazil are the cultivation of extensive areas and the process of intense

urbanization, resulting in very small forest fragments widely spaced one from another (Tabarelli et al., 2005; Wright and Muller-Landau, 2006; Ribeiro et al., 2009).

Compared to plants and animals relatively little is known about micro-organisms diversity in Atlantic forest soils and the functional roles they play in this biome. Among these organisms, arbuscular mycorrhizal fungi (AMF, Glomeromycota) form a mutualistic symbiosis (Bonfante and Genre, 2010) in which the plant provides the fungus with energy for growth and maintenance through its photosynthetic products, while the fungus provides water and nutrients such as phosphorus to the plant (Smith and Read, 2008).

The conversion of natural vegetation into agricultural fields triggers serious damage to the soil, such as negative influences on the energy and biogeochemical cycles and changes in particle aggregation, as well as exposing it to insolation, erosion and nutrient leaching (Islam and Weil, 2000). These changes in vegetation cover have a global impact on biodiversity, soil degradation, and the ability of biological systems to support human needs (Lambin et al., 2003).

These new land uses can also alter the structure of mycorrhizal communities in the soil, affecting their functions and hence

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ecosystem sustainability. In this regard AMF may be used as biological soil quality indicators (Oehl et al., 2004, 2010; Lamb et al., 2005). Due to the agricultural and ecological importance of AMF, taxonomic studies on the group have been intensified (Caruso et al., 2012; van der Heijden et al., 1996; Oehl et al., 2011; Redecker et al., 2013). In this context, the study of morphological and functional diversity of AMF in Atlantic forest areas can contribute to increased knowledge on the distribution of these fungi, as well as provide useful information about their role in edaphic dynamics.

Assessing the impact of land use changes on AMF communities is important for their management and for understanding the effects caused by this human action on the environment in order to generate effective options for creating recovery strategies and/or biodiversity conservation. AMF are a key functional group in terrestrial ecosystems but more studies are needed to qualitatively evaluate their performance in the environment and understand the effects of conversion of natural areas to cultivation.

This study was designed to determine AMF diversity in Atlantic forest areas under different land uses and specifically to test whether agricultural practices negatively affect these microorganisms and to see whether different systems of land use compromise AMF species diversity, evenness and richness.

2. Material and methods

2.1. Study areas

The study was conducted at the Itapirema Experimental Station, Agronomic Institute of Pernambuco – IPA, located in the city of Goiana, Pernambuco, Brazil (07°38'20"S, 034°57'10"W, altitude 13 m). The climate in the area is Ams' (Köppen) type-rainy tropical monsoon with dry summer, with average annual precipitation and temperature of 24°C and 2000 mm, respectively. The soil type is Ultisol. Six areas under different soil use were selected (Table 1): sapodilla plantation (SA), rubber tree plantation (RT), mahogany plantation (MA), eucalyptus plantation (EU), a crop rotation area currently producing cassava (CA), and an Atlantic forest area (AF). These areas were divided in three classes of intensity of use according to the ongoing pressure factors such as plant utilization, proximity of roads, soil disturbance and frequency of fertilization (Table 1).

2.2. Sampling

Rhizosphere soil samples (0–20 cm deep) were collected in June 2011 and March 2012. In each of the five areas under different vegetation cover (SA, RT, MA, EU, CA) and also in the reference area (AF), a plot of 1000 m² and eight composite samples (five sub-samples at equidistant points) were collected around the host plants, packed in plastic bags and transported to the Mycorrhizae Laboratory/UFPE at ambient temperature. The soil collected was divided into portions intended for: mounting trap cultures, AMF community assessment, and physicochemical soil characterization. This last analysis was performed at the Federal Rural University of Pernambuco, at the Sugarcane and Sugar Experimental Station of Carpina according to EMBRAPA (1997). The results indicated that the soils had low levels of phosphorus and organic matter (Table 2).

2.3. Glomerospore analysis

Glomerospores were extracted from 50 g of field soil from each sample by wet sieving followed by sucrose centrifugation (Gerdemann and Nicolson, 1963; Jenkins, 1964), and quantified using a stereomicroscope (40×). In order to assist taxonomic analysis, trap cultures were set with soil collected from the field, in 2 L plastic pots with millet (*Panicum miliaceum* L.), corn (*Zea*

mays L.) and sunflower (*Helianthus annuus* L.) as hosts. The trap cultures were maintained in a greenhouse for three multiplication cycles (four months each). At the end of each cycle, the plants were allowed to dry and cut off and aliquots of 50 g of soil were collected; after that, the soil was reseeded for the subsequent cycle. The aliquots of the soil collected at the end of each cycle were used for glomerospore extraction and subsequent AMF identification. For the taxonomic study, after counting, the glomerospores were mounted on microscope slides with PVLG (polyvinyl alcohol and lactoglycerol) and PVLG + Melzer's reagent (1:1, v/v). Species identification was performed with the aid of a defined bibliography (Schenck and Perez, 1990), publications with descriptions of new species and by consulting the international culture collection of arbuscular mycorrhizal fungi database-INVAM (<http://invam.caf.wvu.edu>) and the on-line AMF collection of the Department of Plant Pathology, University of Agriculture in Szczecin, Poland (<http://www.agro.ar.szczecin.pl/~jblaszkowski/>). In this work we adopted the classification proposed by Oehl et al. (2011), including recently described new taxa (e.g. Goto et al., 2012; Błaszkowski and Chwat, 2013).

2.4. AMF diversity analysis

AMF communities from field samples were evaluated both quantitatively and qualitatively from population data (occurrence and distribution frequency) and had their structure analyzed using ecological indices to measure species richness and diversity. The frequency of occurrence (FO) of the species was estimated according to the equation: $F_i = J_i/k$, where F_i = occurrence frequency of species i , J_i = number of samples in which species i occurred and k = total number of soil samples. The species were classified as dominant (FO > 0.50), very common (FO between 0.31 and 0.50), common (FO between 0.10 and 0.30) and rare (FO < 0.10) (Zhang et al., 2004). The species were also classified as generalists (present in all six areas), intermediate (present in two to five areas) or exclusive (present in one area) (Stürmer and Siqueira, 2011). Species richness was measured as the ratio between the number of species observed and the sample size, and the estimated number of species was calculated using the jackknife first-order index (Jackknife1). For the calculation of diversity in the study areas, the Shannon index was used on a logarithmic base: $H' = \sum p_i \ln p_i$, where p_i = glomerospore number of each species/total glomerospores. The species evenness was calculated using the Pielou evenness (J') index, where: $R = H'/\log S$, where H' = value obtained using Shannon and S = total number of AMF species present in the sample. The Simpson dominance index (C) was calculated according to the formula $C = \sum (n_i(n_i - 1)/N(N - 1))$ where n_i = the abundance of species i and N = total abundance. Similarity between AMF communities was assessed with the Sørensen index (Brower et al., 1990).

2.5. Statistical analyses

The data from soil physicochemical attributes were submitted to ANOVA and the means compared by the LSD test ($p < 0.05$) using the STATISTICA program (Statsoft, 1997).

To assess the impact of changes in land use on the AMF community, the NMS multivariate ordination method (non-metric multidimensional scaling) with Sørensen distance was used to explore the relationship between soil properties and AMF distribution. The analysis of indicator species was randomized using the Monte Carlo test to determine which AMF species were sensitive to land use. The indication value (VI) and the significance (p) value are products of the relative abundance and occurrence frequency of species in each area (Dufrière and Legendre, 1997). The values adopted in this work to consider a species as an indicator were $VI > 50$ and $p < 0.001$. NMS analysis and the identification of indicator species were calculated

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