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Arbuscular mycorrhizal fungi in Mimosa tenuiflora (Willd.) Poir from Brazilian semi-arid

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A B S T R A C T
Many plant species from Brazilian semi-arid present arbuscular mycorrhizal fungi (AMF) in their rhizosphere. These microorganisms play a key role in the establishment, growth, survival of plants and protection against drought, pathogenic fungi and nematodes. This study presents a quantitative analysis of the AMF species associated with Mimosa tenuiflora, an important native plant of the Caatinga flora. AMF diversity, spore abundance and root colonization were estimated in seven sampling locations in the Ceará and Paraíba States, during September of 2012. There were significant differences in soil properties, spore abundance, percentage of root colonization, and AMF diversity among sites. Altogether, 18 AMF species were identified, and spores of the genera Acaulospora, Claroideoglomus, Denticutata, Entrophospora, Funneliformis, Gigaspora, Glomus, Racocetra, Rhizoglomus and Scutellospora were observed. AMF species diversity and their spore abundance found in M. tenuiflora rhizosphere shown that this native plant species is an important host plant to AMF communities from Brazilian semi-arid region. We concluded that: (a) during the dry period and in semi-arid conditions, there is a high spore production in M. tenuiflora root zone; and (b) soil properties, as soil pH and available phosphorous, affect AMF species diversity, thus constituting key factors for the similarity/dissimilarity of AMF communities in the M. tenuiflora root zone among sites.

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Introduction

The Brazilian semi-arid region is an important Caatinga ecoregion that provides the required biotic and abiotic conditions for many endemic species to inhabit; thus, having high species diversity when compared with other semi-arid regions of the World. This ecoregion covers approximately 12% of the country, and is located in the innermost region of northeastern Brazil. It is characterized by frequent dry periods, and by a unique biota, with thousands of endemic species and over 1000 species of vascular plant.1,2 However,
the establishment, growth and dispersal of native vegetation are often restricted by long dry periods anthropogenic activities, and exotic species introduction. In this context, plant mutualistic interactions with other organisms, such as fungi, might play a significant role during their establishment and growth. Mutualistic symbiosis between arbuscular mycorrhizal fungi (AMF) and plants are advantageous for both organisms involved, because the plant provides the AMF with energetic products resulting from photosynthesis, allowing AMF growth and maintenance, while AMF provides the plant with water and mineral nutrients, such as phosphorus and nitrogen. By enhancing plant’s mineral nutrients uptake, AMF play a key role in the establishment and survival of native plants in this biome, promote plant growth and offer protection against drought and soil pathogens.

*Mimosa tenuiflora* (Willd.) Poir. (Fabaceae) is a perennial native plant from northeastern Brazil. Similarly to other Fabaceae, *M. tenuiflora* is able to fix nitrogen because its roots form associations with bacteria from the genus *Rhizobium*. This plant species is considered to be a prolific pioneer plant, which is very tolerant to dry periods, fires and other major ecological disturbances. During highly stress periods, its leaves start to fall, representing a mechanism for the plant to save energy. It develops extensive root systems, which are colonized by mutualistic organisms, as bacteria and fungi. Indeed, previous studies have shown the association between AMF and *M. tenuiflora* root system. The primarily objective of most studies conducted have been to verify the presence of AMF, but more recently, they have focused on the AMF species diversity commonly found associated with native species of the Brazilian semi-arid region. Those studies have shown that species from the genera *Archaeospora*, *Acaulospora*, *Gluomus*, *Gigaspora* and *Scutellospora* frequently form mutualistic symbiosis with native plant species.

Soil fertility is often reported as an important factor conditioning AMF species diversity, having effects at the community level, but also on the biological activity of these microorganisms. Thus, the objective of the present study was to provide a quantitative assessment of the AMF species associated with *M. tenuiflora* in the Brazilian xeric shrublands sampled from different locations across the Ceará and Paraíba States. To accomplish this objective, we selected seven independent sites where we sampled *M. tenuiflora* root zone and we hypothesized that (1) the root zone of this native plant species has high AMF spore abundance and root colonization during the dry period; and (2) soil properties are an important factor that influence the AMF community in different *M. tenuiflora* root zones.

### Materials and methods

#### Site description and sampling

Sampling of the study species root zone area was performed in locations with similar importance value index (IVI, see table below) of *M. tenuiflora* in Ceará and Paraíba States, Brazil (Table 1). During the dry season, the average annual temperature is of 30°C, while during the rainy season, the average annual temperature is around 23°C. Rainfall in the greater part of this region is scanty, unpredictable and irregular. The flowering season for *M. tenuiflora* starts on August in Ceará State, one month earlier than Paraíba State. The flowering starts after the rainy season (August) and during the dry season that the plant growth is very limited.

In each site, we established 40 plots of 100 m², accordingly to the methodology proposed by Gregg-Smith and Fortin and Dale. Before sampling *M. tenuiflora* root zone, the following criteria were established: (a) each plant should be larger than 3 cm in diameter near soil surface; (b) its height should vary between 1 and 3 m; and (c) no other individuals from a different plant species should occur at a distance lower than 3 m from the sampling point. In total, twenty-five root zone samples per plot including soil and root fragments were collected at a depth ranging between 0 and 20 cm. Soil was kept in plastic bags and root fragments were stored in 50% ethanol until laboratory analysis. Because fungal sporulation is expected to be higher during the dry period in semiarid environments, the sampling was conducted during September of 2012.

#### Soil analyses

For each plot, soil and roots samples were pooled resulting in 40 samples per site. In each of these samples, the following variables were measured: pH, carbon, total nitrogen and available phosphorus. Soil pH was determined in a suspension of soil and distilled water; soil organic carbon was estimated accordingly to the methodology described by Okalebo; total soil nitrogen content was estimated following the Kjeldahl method; and available phosphorus was determined spectrophotometrically following the molybdenum blue method and using stannous chloride as the reducing agent.

#### Spores assessment

Spores were extracted from field soil samples (*n* = 40) by wet sieving followed by sucrose centrifugation. Initially, the extracted spores were examined in aqueous solution under a dissecting microscope and separated based on morphological characteristics. Then, polyvinyl alcohol lactoglycerol (PVLG) mixed or not with Melzer’s reagent was used as permanent mounting medium. Species identification was based on the descriptions provided by Schenck and Perez, publications with descriptions of new genera and families, and by consulting the international culture collection of arbuscular mycorrhizal fungi database – INVAM (http://invam.caf.wvu.edu). For the purpose of this work, we adopted the classification proposed by Oehl et al., including recently described new taxa.

#### Mycorrhizal root colonization assessment

To assess mycorrhizal root colonization, roots were first cleared in 2% KOH for 1h at 90°C, and subsequently they were acidified in 1% HCl overnight. Blue ink (Parker Quink) was used as staining agent for 30 min at 60°C, followed by a destaining treatment with lactoglycerol. The extent of mycorrhizal root colonization was estimated by using a grid-intersect method with the examination of 100 intersects under...
a compound microscope at 200× magnification.\textsuperscript{39,40} Prior to microscopy examinations, we decided to score hyphae as "mycorrhizal", based on the associated presence of vesicles, arbuscules, spores, and the morphology of the mycelium. Thus, if any of the structures referred were found in one of the 100 microscope intersections per sample per study site (n = 400 intersections per study site), they were scored as "mycorrhizal". To avoid error associated with the observer, all microscopic examinations were carried out by the same individual.

**Statistical analysis**

One-way ANOVA was used to compare spore abundance and root colonization among the seven locations sampled. The relationship between the AMF community structure and soil properties was examined by calculating the Pearson correlation coefficient. To explore the variability and similarities among soils and AMF communities composition among sites, principal component analysis (PCA) was used.

The following ecological indexes were calculated after AMF species identification: average species richness of AMF, diversity index\textsuperscript{41} and dominance index\textsuperscript{42} for each studied site. One-way ANOVA was used to compare species richness of AMF, the diversity index and the dominance index among sites. In addition, the Zhang's frequency of occurrence classification\textsuperscript{42} was used to analyze (a) total spore abundance (total number of spores), (b) spore abundance of a given AMF species (number of spores of a particular AMF species), and (c) AMF species occurrence frequency (percentage of samples containing a particular AMF species). This procedure allowed AMF species to be classified as dominant (FO > 50%), most common (31 ≤ FO ≤ 50%), common (10 ≤ FO ≤ 30%) and rare (FO < 10%).\textsuperscript{43} Additionally, AMF species were also classified as generalists (present in six areas), intermediate (present in two to five areas) or exclusive (restricted to only one area).\textsuperscript{44}

Before statistical analysis, data was checked for normality and homogeneity of variances. One-way ANOVA and the Pearson correlation coefficients were calculated using SAS 9.1.3 Portable, while PCA analysis and the ecological index calculations were conducted using MVSP 3.1.\textsuperscript{45}

**Results**

**Soil analysis**

All soil chemical properties varied significantly among sites (Table 2). Accordingly to the PCA analysis, A4 and A6 were the most dissimilar locations (Fig. 1). A1 and A2 were more similar to each other than A3, A5 and A7. Total organic carbon and available phosphorus were the main factors contributing to the variance of most samples. PCA plot also showed a strong positive relationship between total nitrogen, soil pH and available phosphorus and 93.1% of the samples' variation was explained by the two axes.

**Spore abundance and root colonization**

Sites A4 and A5 did not significantly differ in spore abundance data, showing the highest values from all studied sites, averaging 16.6 spores g\textsuperscript{-1} soil and 15.3 spores g\textsuperscript{-1} soil, respectively (Fig. 2). In the remaining five sites, we found statistically significant differences (p < 0.05) when comparing A1, A2 and A6 with A3 and A7. A7 had the smallest value of spore abundance from all studied sites (6.8 spores g\textsuperscript{-1} soil). Statistically significant differences (p < 0.05) were also found in root colonization among all sites (averages ranging between 6.3 and 36.5%). A2 had the highest root colonization, while A1 the smallest of all studied sites (Fig. 2).

**AMF community diversity and structure**

Ecological indexes significantly differed among sites (p < 0.05). AMF total richness varied between 14 and 18 species, the diversity index ranged between 1.98 and 2.64, and the values for the dominance index averaged between 0.80 and 0.91 among sites. A4 site had the highest values for all ecological indexes calculated (species richness, 18 species; diversity index, 2.64; and dominance index, 0.91; Table 3).

Field-collected spores were from two orders, Diversisporales (four families and six genera), and Glomerales (two

| Study area | Location | Geographical coordinates | IVI (%)\textsuperscript{a} |
|------------|----------|--------------------------|---------------------------|
| A1         | Algodão de Jandaia, PB | 06°46'7.3" S 36°01'55.3" W | 59.02 ± 1.12 |
| A2         | Esperança, PB | 06°56'45.7" S 35°54'06.8" W | 58.12 ± 0.52 |
| A3         | Ibaretama, CE | 04°49'46.8" S 38°38'47.6" W | 60.01 ± 1.87 |
| A4         | Juazeirinho, PB | 07°06'33.3" S 36°34'54.2" W | 59.13 ± 1.13 |
| A5         | Monteiro, PB | 07°48'19.8" S 37°10'32.4" W | 58.01 ± 2.17 |
| A6         | Natuba, PB | 07°37'34.9" S 35°32'24.5" W | 59.18 ± 1.56 |
| A7         | Poçinhos, PB | 07°10'23.3" S 36°12'41.8" W | 60.12 ± 1.98 |

\textsuperscript{a} According to the Köppen classification.
\textsuperscript{b} WRB.\textsuperscript{45}
\textsuperscript{c} IVI (%) = RDi + RFi + RDoi; RDi, RFi and RDoi for relative density, relative frequency and relative dominance of M. tenuiflora in each study area accordingly to Magurran.\textsuperscript{25} Values are given as mean ± SE, n = 40.
families and four genera). Representatives belonging to four families of Diversisporales were identified: Acaulosporaceae, Entrophosporaceae, Gigasporaceae; while two families from Glomerales were recognized, Claroideoglomeraceae and Glomeraceae, representing ten genera – Acaulospora (2), Claroideoglomus (1), Denticutata (3), Entrophospora (1), Funneliformis (3), Gigaspora (3), Glomus (1), Racocetra (1), Rhizogolmus (2) and Scutellospora (1); in a total of 18 different AMF species. Accordingly to their occurrence frequency, AMF species were classified into two groups: rare AMF species (R, occurrence frequency lower than 10%), and common AMF species (C, occurrence frequency between 10 and 30%). The AMF species identified as rare species were: Acaulospora denticulata, Acaulospora tuberculata, Denticutata cerradensis, Denticutata erythropus, Denticutata heterograma, Entrophospora infrequens, Funneliformis caledonium, Funneliformis geosporum, Funneliformis mosseae, Gigaspora gigantea, Glomus multicule, Racocetra coralloidei, Rhizogolmus aggregatum, Scutellospora calospora, Claroideoglomus etunicatum, Gigaspora albida, Gigaspora decipiens, and Rhizogolmus clarea were classified as common species. No AMF species had an occurrence frequency higher than 30% (Table 4).

The PCA analyses showed that AMF species richness, Shanon index, Simpson index, soil pH, total organic carbon and available phosphorous were the main factors contributing to the variance of the samples (Fig. 3). The analysis also showed: (1) a strong negative relationship between total soil pH and available phosphorous with AMF species richness; and (2) a negative relationship between total organic carbon and

| Ecological index | A1 | A2 | A3 | A4 | A5 | A6 | A7 |
|------------------|----|----|----|----|----|----|----|
| Species richness | 18 a | 17 a | 17 a | 18 a | 15 b | 17 a | 14 b |
| Shanon index     | 2.20 ± 0.02 c | 2.17 ± 0.01 c | 2.42 ± 0.08 b | 2.64 ± 0.02 a | 1.98 ± 0.04 d | 2.58 ± 0.06 a | 2.16 ± 0.07 c |
| Simpson index    | 0.86 ± 0.01 b | 0.85 ± 0.01 b | 0.89 ± 0.01 a | 0.91 ± 0.01 a | 0.80 ± 0.01 c | 0.91 ± 0.01 a | 0.85 ± 0.01 b |

A1 – Algodão de Jandaíra, PB; A2 – Esperança, PB; A3 – Ibaretama, CE; A4 – Juazeirinho, PB; A5 – Monteiro, PB; A6 – Natuba, PB; A7 – Poçinhos, PB; different lower case letters represent statistically significant differences after LSD test (p < 0.05).
Our results provided evidence that AMF are common in M. tenuiflora roots and rhizosphere, supporting our hypothesis that M. tenuiflora rhizosphere has high AMF spore abundance and root colonization. However, AMF species were quantitatively variable among the studied sites. Mello et al.\textsuperscript{46} reported low values of spore abundance, <1 spore g\textsuperscript{-1} soil, and root colonization, <10\%, in the rhizosphere of native plants from Brazilian semi-arid region, as Aspidosperma pyrifolium, Bromelia sp., Myrrocardruon urundeuva, Tabebuia impetiginosa, and Zizyphus joazeiro. Contrarily, crop host plants, such as maize and sorghum, usually have high sporulation (>5 spores g\textsuperscript{-1} soil) and high root colonization values (approximately 40\%).\textsuperscript{47} Thus, our results suggest that M. tenuiflora have a good level of promiscuity as a host plant, presenting high values of sporulation and mycorrhizal colonization independently of the studied site. In addition to the variability found in spore abundance and root colonization for all studied sites, we also observed a significant variation in the content of vesicles and arbuscules, which probably indicates different physiological and/or phenological mechanisms of the symbiosis between the host plant and the fungi among sites (Unpublished data).

The differences in spore abundance and root colonization among sites are in agreement with previous work\textsuperscript{15,48} that reported variability in spore abundance during the dry season in Brazilian semiarid region for AMF species from Diversisporales order. Silva et al.\textsuperscript{15} concluded that the dry season is the

Shanon and Simpson indexes. The two axes explained 83.42\% of the samples’ variation.

### Discussion

Our results provided evidence that AMF are common in M. tenuiflora roots and rhizosphere, supporting our hypothesis that M. tenuiflora rhizosphere has high AMF spore abundance and root colonization. However, AMF species were quantitatively variable among the studied sites. Mello et al.\textsuperscript{46} reported low values of spore abundance, <1 spore g\textsuperscript{-1} soil, and root colonization, <10\%, in the rhizosphere of native plants from Brazilian semi-arid region, as Aspidosperma pyrifolium, Bromelia sp., Myrrocardruon urundeuva, Tabebuia impetiginosa, and Zizyphus joazeiro. Contrarily, crop host plants, such as maize and sorghum, usually have high sporulation (>5 spores g\textsuperscript{-1} soil) and high root colonization values (approximately 40\%).\textsuperscript{47} Thus, our results suggest that M. tenuiflora have a good level of promiscuity as a host plant, presenting high values of sporulation and mycorrhizal colonization independently of the studied site. In addition to the variability found in spore abundance and root colonization for all studied sites, we also observed a significant variation in the content of vesicles and arbuscules, which probably indicates different physiological and/or phenological mechanisms of the symbiosis between the host plant and the fungi among sites (Unpublished data).
period of greatest AMF spore production, because soil water availability plays an important role in the AMF-host plant relationship, namely in terms of the composition and functioning. Also, data available in the literature from elsewhere showed that spore abundance might vary from 0 to 13 spores g⁻¹ soil and root colonization ranges from 27.7 to 45.7% in other semi-arid and arid regions.²³,⁴⁹

Our results for total AMF species richness differ from the ones presented by Silva et al.,¹⁵ which found that AMF total species richness might be larger than 40 AMF species. This dissimilarity in results may be due to soil site-specific characteristics, as well as rhizosphere peculiarities that differ among sites that might determine the number of AMF species present, and consequently, the number of AMF species detected.⁶⁰ However, our results are similar to recently published data from semiarid and arid areas elsewhere, which showed total richness to vary between 3 and 32 AMF species.⁶⁰

There was a statistically significant difference in AMF diversity and dominance among sites. A4 was the site with the greatest diversity (2.64) and the highest dominance index (0.91). Other studies carried out in the Brazilian semiarid region found similar results.¹⁵,⁵¹ As pointed out above, soil properties may have more influence than the host plant on the AMF community,¹⁵,⁴⁷,⁵² because soil pH, water availability, phosphorous and nitrogen contents are determinant factors in the composition and functioning of the AMF-host plant relationship. Our study provides evidence that the soil properties, such as soil pH, total nitrogen and phosphorous available, are key aspects on the AMF community structure in the Brazilian semiarid region.

Species of Acaulospora, Claroideoglomus, Dentiscutata, Funneliformis, Gigaspora, Rhizoglomus and Scutellospora were among the identified taxa. These genera are common in semiarid sites,¹³,¹⁵,⁵³ and acid soils with low phosphorous availability.¹⁴ Previous studies have shown the presence of these AMF species associated with M. tenuiflora.³,¹²,¹³ Most studies have shown a certain constancy in the AMF genera associated with this plant species, like Acaulospora, Archaeospora, Gigaspora, Glomus, and Scutellospora,¹⁴,¹⁵ but there are no reports of dominant AMF genus in the M. tenuiflora rhizosphere.⁵⁰ Our results also support the lack of dominance of a given AMF genus or species associated with M. tenuiflora rhizosphere. Moreover, the high abundance of representatives from the Order Gigasporales found in our study, might give some support to the hypothesis proposed by Goto et al.⁶⁰ and Marinho et al.,¹⁵ which points out that Brazil is the diversification and dispersion center of species from Gigasporales.

Fig. 2 – AMF spore abundance, given as number of spores g⁻¹ soil, and percentage of root colonization (mean ± SE, n = 40) in the rhizosphere of M. tenuiflora. Different letters represent statistically significant differences (p < 0.05) among sites, after one-way ANOVA and LSD test for overall comparisons.

Fig. 3 – PCA score plot of soil properties and AMF community structure, here represented by the ecological indexes calculated (species richness, Shanon index and Simpson index). A1 – Algodão de Jandaíra, PB; A2 – Esperança, PB; A3 – Ilharetama, CE; A4 – Juazeirinho, PB; A5 – Monteiro, PB; A6 – Natuba, PB; A7 – Poçinhos, PB. Points represent samples from each plot by studied sites.
Soil properties differed among all studied sites. These results support our hypothesis that soil properties are important factors influencing the AMF community in the *M. tenuiflora* rhizosphere. Accordingly to Oehl et al. and Carneiro et al., soil pH, available phosphorus, and other soil properties can change AMF community composition, increasing the frequency of mutualists from genera *Acaulospora*, *Funneliformis*, *Gigaspora* and *Scutellospora*. There was a negative correlation between soil pH, total nitrogen and phosphorus availability and the different ecological indices calculated for each site (Fig. 3). Our results are in agreement with previous work carried out in 16 locations across Europe, which found that different soil types contained different AMF species. In Switzerland, a study analyzing 154 different agricultural soil samples reported that the abundance of some AMF taxa was also related with soil variables. More recently, a study performed along an environmental gradient in the Brazilian semiarid region found a strong correlation between soil attributes and AMF community composition, concluding that soil properties are key factors determining AMF community structure in a local scale, even though sites are geographically close to each other.

In conclusion, our results showed that (1) AMF species exhibit high sporulation during the dry season, and that *M. tenuiflora* is a common host plant, which points out its importance in the biome it inhabits and its potential use in reforestation programs in the Caatinga degraded areas. However, further research is needed to unravel the effect of AMF inoculation in the native plants, *M. tenuiflora* in this particular case, growing in different soil conditions and with different AMF communities before establish habitat restoration measures. Despite no dominant AMF species was found in *M. tenuiflora* rhizosphere, rare and common AMF species accordingly to Zhang’s classification were described. Based on these results, one can assume that this native plant species does not benefit any AMF particular genus and highlights its potential in AMF communities’ restoration in degraded soils. Further work is needed to understand whether this diverse AMF community has a positive differential effect in increasing native plant growth, resistance or tolerance to root pathogens in the Brazilian semiarid region.

**Conflicts of interest**

The authors declare no conflicts of interest.

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