Occurrence and distribution of Gigaspora under Cryptostegia madagascariensis Bojer Ex Decne in Brazilian tropical seasonal dry forest

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Abstract: The Brazilian tropical seasonal dry forest presents high abundance of arbuscular mycorrhizal fungal from Order Diversisporales, but the occurrence and distribution of Gigaspora species (Order Diversisporales) in invaded zones by exotic plant species in the Caatinga are not known yet. Here, we compare the occurrence and distribution of Gigaspora community in soils from invaded zone by the exotic plant species C. madagascariensis and soils from native zone by the native plant species M. tenuiflora from Brazilian tropical seasonal dry forest, Pombal, Paraíba, Brazil. We analysed and compared the number of spores and frequency of occurrence of Gigaspora species using 40 m transects and morphological analyses. In general, the most dominant Gigaspora species in the invaded zone was Gigaspora albida, whereas we did not find any dominance by Gigaspora species in the native zone. Differences in Gigaspora occurrence and distribution were associated with (1) the dominant plant species (C. madagascariensis vs. M. tenuiflora) that alter the occurrence and frequency of Gigaspora in field conditions from the Brazilian semi-arid region, (2) the transition zone, where G. albida and G. margarita did not occur, and (3) specific plant-AMF pairing, where we identified G. gigantea as the most resilient AMF species occurring in all the studied sections into the 40 m transects. These results contribute to a deeper view about the Gigaspora occurrence and distribution in invaded and native zones of the Brazilian semi-arid and open new perspective for ecological studies addressing specific AMF taxa and other exotic plant species in the Brazilian tropical seasonal dry forest.

Keywords: Brazilian semi-arid; Gigaspora albida; Gigaspora gigantea; Gigaspora margarita; arbuscular mycorrhizal fungi.

Ocorrência e distribuição do gênero Gigaspora em Cryptostegia madagascariensis Bojer Ex Decne na Caatinga

Resumo: A região semiárida brasileira apresenta elevada abundância de micorrizas da ordem Diversisporales, contudo a ocorrência e a distribuição de espécies de fungos micorrízicos do gênero Gigaspora (Ordem Diversisporales) em áreas em processo de invasão biológica dentro da Caatinga ainda é desconhecida. Avaliou-se a ocorrência e a distribuição de espécies do gênero Gigaspora em solos de ambientes invadidos pela espécie de planta exótica C. madagascariensis e solos de ambiente nativos pela espécie de planta nativa M. tenuiflora, da Caatinga brasileira, Pombal, Paraíba, Brasil. Foram analisados e comparados o número de esporos e a frequência de ocorrência de espécies do gênero Gigaspora usando transectos de 40 m e análise morfológica dos esporos. A comunidade de Gigaspora nas zonas invadidas e nativas foram dissimilares. Em geral, a espécie de Gigaspora observada como dominante na zona invadida foi Gigaspora albida, enquanto que não foi verificado a dominância das espécies de Gigaspora na zona nativa. Diferências na ocorrência e distribuição do gênero Gigaspora foram associadas com (1) o tipo de espécie de planta dominante (C. madagascariensis vs. M. tenuiflora) que alteram a ocorrência e frequência de espécies do gênero Gigaspora em condições de campo do semi-árido brasileiro, (2) a zona de transição, onde não foi observada a ocorrência de espécies G. albida e G. margarita, e (3) o paramento planta-fungo específico, onde constatou-se a espécie G. gigantea como a mais resiliente espécie de fungo micorríticos observada em todas as secções estudadas dentro do transeco de 40 m. Estes resultados contribuem com uma visão mais aprofundada sobre a distribuição e ocorrência de espécies do gênero Gigaspora em zonas invadidas e nativas do semi-árido brasileiro e abrem novas perspectivas para estudos ecológicos referentes a grupos específicos de FMA e outras espécies de plantas exóticas da Caatinga brasileira.

Palavras-chave: Fungos micorrízicos arbusculares; Gigaspora albida; Gigaspora gigantea; Gigaspora margarita; Semi-árido brasileiro.
1 Introduction

The Brazilian semi-arid region is characterized by high diversity and variability of arbuscular mycorrhizal fungi (AMF) (Souza and Freitas, 2017; Schüßler et al., 2001). According to Silva et al. (2014) during the dry season the frequency of occurrence of different AMF species in the Brazilian semi-arid increase through high fungal sporulation. Goto et al. (2012) and Marinho et al. (2014) describe the Brazilian semi-arid as a diversification and dispersion centre of species from Diversisporales. Nowadays, Diversisporales encompassed nine genus, within them genus Gigaspora (Souza, 2015) and there are many works showing the occurrence and distribution of Gigaspora around the world (Goto et al., 2012; Souza, 2015; Souza and Santos, 2018), however, the occurrence and distribution of this genus on invaded environments of Brazilian semi-arid remain unclear.

In this context, our work addressed the following question: Could Cryptostegia madagascariensis Bojer ex. Decne., an invasive plant species from tropical seasonal dry forest, alters the occurrence and distribution of Gigaspora species into invaded areas of Brazilian semi-arid? According to Souza et al. (2015) this invasive plant species can reduce AMF diversity and alter AMF community composition of sandy soils. Oehl et al. (2011) reported that some AMF species respond specifically to the intensity of land use and plant diversity, which suggests that genus Gigaspora occurrence also can be altered by C. madagascariensis presence.

Many studies have showed that AMF species from genus Gigaspora occur with high frequency in natural ecosystems from Brazilian semi-arid (i.e., Mimosa tenuiflora (Willd.) Poir, Licania rigida Benth., Copernicia prunifera (Miller) H. E. Moore and Ziziphus joazeiro Mart.), whereas in invaded ecosystems by exotic plant species this AMF genus occur with low frequency or it can be absent from the changed-AMF community by biological invasion (Goto et al., 2012; Marinho et al. 2014; Souza et al., 2015; Souza et al., 2016a; Souza et al., 2018; Souza e Freitas, 2017; Souza e Santos, 2018).

Our aim here was to investigate how C. madagascariensis alters the occurrence and distribution of Gigaspora species in a natural ecosystem on a Regosol occupied by M. tenuiflora (native plant species) and C. madagascariensis (invasive plant species).

2 Material and Methods

We selected study environments near Pombal, Paraíba, Brazil (6°47'34.01" S, 37°49'10.7" W, and average altitude 183 m). The climatic condition of the studied environments is Bsh (Köppen), hot semi-arid, temperature of 28°C and annual precipitation scanty and irregular of 963.07 mm (Souza et al., 2016a). The soil type was classified as an Eutric Cambisol with low contents of total organic carbon (8.61 g kg⁻¹) and available P (2.31 mg dm⁻³) (WRB, 2006).

The exotic Cryptostegia madagascariensis Bojer ex. Decne. and the native Mimosa tenuiflora (Willd.) Poir, which co-occur into the riparian forest in the Brazilian tropical seasonal dry forest from Paraíba, Brazil were selected. Both plant species were selected according to the following criteria: (1) the plant had a diameter near soil surface > 3 cm; (2) the plant had a height higher than 2 m; (3) in flowering stage; and (4) no individual from a different plant species were growing in a 3-m radius to the selected plant in all directions (Souza et al., 2018).

We collected samples containing soil and root fragments at 0.10 m depth, using 40 m transects (n = 10) in eight different position between an invaded and a native zone. So, the transect was divided into 5 meters sections and then four soil samples were collected within each 5 meters section: EXO0. - Under the drip line of C. madagascariensis (exotic plant species) and located 20 m from the transition zone; EXO15 – Within the invaded zone and located 15 m from the transition zone; EXO10 – Within the invaded zone and located 10 m from the transition zone; EXO5 – Within the invaded zone and located 5 m from the transition zone; NAT3 - Within the native zone and located 5 m from the transition zone; NAT10 - Within the native zone and located 10 m from the transition zone; NAT15 - Within the native zone and located 15 m from the transition zone; NAT10 – Under the drip line of M. tenuiflora (native plant species) and located 20 m from the transition zone (Fig. 1).

Spores of Gigaspora species extracted from native and invaded zone were classified as “Changed-Gigaspora community” if they occurred in the invaded zone only, and as “unaltered-Gigaspora community” if they...
occurred in the native zone only (Souza et al., 2018). Spores from field were extracted by the wet sieving technique (Gerdemann and Nicolson, 1963), followed by sucrose centrifugation (Jenkins, 1964). For this, we used 100 g of field soil. Initially, the extracted spores were examined in water under a dissecting microscope and they were separated based on morphology. After it, they were mounted in polyvinyl alcohol lactoglycerol (PVLG) with or without addition of Melzer’s reagent (Walker et al., 2007). The identification of Gigaspora species was based on the description provided by consulting the LBM website (http://glomeromycota.wixsite.com/lbmcorrizas/chaves-de-identificacao). In this work we adopted the classification proposed by Oehl et al. (2011). We assessed spore abundance (total number of spores of each Gigaspora species recorded), and the species occurrence frequency (FO) of each Gigaspora species. FO was calculated using the following equation: 
\[ FO = \frac{n}{N} \]
where n is the number of times a Gigaspora species was observed and N is the total of Gigaspora spores observed from each studied section into the transects.

The Kolmogorov-Smirnov test was applied to assess the normality of the data distribution. Student t test for independent samples was carried out to investigate differences between native and invaded zone in spore abundance of Gigaspora species. Two-way ANOVA was used to test for the effect of biological invasion by C. madagascariensis on occurrence and distribution of Gigaspora species. Data sets were arcsin log transformed. Notwithstanding, the results are presented in their original scale of measurement (mean ± standard deviation). Multiple comparisons of means were performed by the Bonferroni test \( (P < 0.05) \) after performing two-way ANOVA. The t test, two-way ANOVA, and Bonferroni’s multiple comparison tests were conducted using the stats package of the R statistical program.

### 3 Results

In total, we identified 3 different Gigaspora species corresponding to: Gigaspora albida N.C. Shenck & G.S. Sm., Gigaspora gigantea (T.H. Nicolson & Gerd.) Gerd. & Trappe, and Gigaspora margarita Becker & Hall. Significant differences between native (NAT) and invaded (EXO) zone were found for Gigaspora total abundance and Gigaspora species in all studied zones. Across the investigated sections into the transects, the invaded zone had a Gigaspora total abundance ranging from 1.1 ± 0.3 to 28.2 ± 0.4 spores/100 g soil, whereas in the native zone the Gigaspora total abundance ranged from 5.8 ± 0.4 to 45.0 ± 0.3 spores/100 g soil. The number of spores of G. albida \( (P < 0.05) \), G. gigantea \( (P < 0.05) \) and G. margarita \( (P < 0.05) \) were significantly higher in the native zone. However, no differences between invaded and native zone were obtained for G. albida into the EXO5 and NAT5 sections \( (P < 0.5698) \); and G. gigantea into the EXO10 and NAT10 \( (P < 0.7366) \) and EXO5 and NAT5 \( (P < 0.6598) \) sections. Not only G. albida abundance was particularly high in the native zone, but also G. gigantea and G. margarita abundance (Table 1).

The most abundant taxa in the invaded zone were G. gigantea (63.68 %), whereas G. margarita (38.13 %) were mostly found in the native zone. We also observed that G. albida and G. gigantea were both the most frequent and dominant Gigaspora species in the EXO5 and NAT5 sections, respectively. But, these conditions changed near the transition zone, where G. gigantea was the only one Gigaspora species.

Figure 1 Sampling methodology scheme of soil samples along a transect between native and invaded zone.
species observed in both EXO5 and NAT5 field soil samples. We did not find any *G. albida* species in the EXO10, and for the EXO5, we did not observe both *G. albida* and *G. margarita* species (Figure 2).

Table 1 Number of spores of *Gigaspora* species (mean ± SD, *N* = 320) from the four studied sections into the transects between invaded and native zone.

<table>
<thead>
<tr>
<th>Studied zones</th>
<th><em>Gigaspora</em> total abundance</th>
<th><em>G. albida</em></th>
<th><em>G. gigantea</em></th>
<th><em>G. margarita</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>EXO&lt;sub&gt;DL&lt;/sub&gt;</td>
<td>8.1 ± 0.1 d</td>
<td>4.1 ± 0.5 d</td>
<td>3.0 ± 0.1 d</td>
<td>1.0 ± 0.1 e</td>
</tr>
<tr>
<td>NAT&lt;sub&gt;DL&lt;/sub&gt;</td>
<td>45.6 ± 0.3* a</td>
<td>12.2 ± 0.3* b</td>
<td>17.2 ± 0.3* a</td>
<td>16.2 ± 0.1* a</td>
</tr>
<tr>
<td>EXO&lt;sub&gt;15&lt;/sub&gt;</td>
<td>17.5 ± 0.4* c</td>
<td>6.2 ± 0.7 c</td>
<td>8.1 ± 0.2 c</td>
<td>3.2 ± 0.2 d</td>
</tr>
<tr>
<td>NAT&lt;sub&gt;15&lt;/sub&gt;</td>
<td>40.0 ± 0.2 b</td>
<td>17.1 ± 0.5* a</td>
<td>11.0 ± 0.1* b</td>
<td>11.9 ± 0.6* b</td>
</tr>
<tr>
<td>EXO&lt;sub&gt;10&lt;/sub&gt;</td>
<td>4.2 ± 0.1 e</td>
<td>0.0 ± 0.0 f</td>
<td>3.0 ± 0.5 d</td>
<td>1.2 ± 0.3 e</td>
</tr>
<tr>
<td>NAT&lt;sub&gt;10&lt;/sub&gt;</td>
<td>17.5 ± 0.2* c</td>
<td>5.1 ± 0.3* c</td>
<td>4.0 ± 0.6* d</td>
<td>8.1 ± 0.1* c</td>
</tr>
<tr>
<td>EXO&lt;sub&gt;5&lt;/sub&gt;</td>
<td>1.0 ± 0.3 f</td>
<td>0.0 ± 0.0 f</td>
<td>1.0 ± 0.6 e</td>
<td>0.0 ± 0.0 f</td>
</tr>
<tr>
<td>NAT&lt;sub&gt;5&lt;/sub&gt;</td>
<td>5.1 ± 0.4* e</td>
<td>1.0 ± 0.1* e</td>
<td>2.1 ± 0.5* e</td>
<td>2.0 ± 0.1* e</td>
</tr>
</tbody>
</table>

*Independent sample t-test comparing invaded zone (EXO) x native zone (NAT) groups (*P* ≤ 0.05; *ns* not significant); Different letters indicate significant differences among the studied zones assessed by the Bonferroni test (*P* < 0.05) after performing two-way ANOVA.

Figure 2 Frequency of occurrence (FO) of *Gigaspora* species of each studied section into the 40m transect (*N* = 320) between invaded and native zone. FO = \( n_i/N \), where *n* is the number of times an AMF species was observed and *N* is the total of AMF spores observed from each studied condition.

4 Discussion

Our results provided evidence for changes in the occurrence and distribution of *Gigaspora* species caused by *C. madagascariensis* presence. In fact, biological invasion by *C. madagascariensis* may influence negatively AMF community by changing soil properties and native plants community composition as described by Souza et al. (2016a). These results support our main hypothesis that *C. madagascariensis*, an invasive plant species from Brazilian tropical seasonal dry forest may negatively alters the occurrence and distribution of *Gigaspora* by changing total abundance of gigasporoid spores and frequency of occurrence of *Gigaspora* species between the invaded and native zone. It is not usual to report *Gigaspora* community composition in invaded zones of the Brazilian semiarid (Souza et al., 2016a; Souza and Freitas 2017; Souza et al., 2018), but results...
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here showed that *Gigaspora* is a very common AMF species under natural ecosystem of Brazilian semiarid (Mello et al., 2012; Pereira et al., 2014; Silva et al., 2014; Souza et al., 2016b).

We also observed differences in the *Gigaspora* community composition between invaded and native zone. In fact, invasive plants species may influence arbuscular mycorrhizal fungi community composition in different ways (Zubek et al., 2016). In the transect sections, we observed that: i) *G. albida* was the *Gigaspora* species that presented most affinity with *C. madagascariensis*; ii) *G. gigantea* was the most resilient *Gigaspora* species, once we had find their spores in all studied sections; iii) Even the most frequent *Gigaspora* species being *G. gigantea* in the native zone, we did not observed any dominance by the three identified *Gigaspora* species in this field study; and iv) in transition zone between invaded and native plant communities, both *G. albida* and *G. margarita* were negatively affected by the reduction of native plants diversity. These results are in agreement with previous studies (Callaway et al., 2008; Mummey and Rillig, 2006; Vogelsang and Bever, 2009) and support the hypothesis proposed by Hausmann and Hawkes (2009) that plant neighbours are very important in structuring AMF communities and the presence of invasive plants changes AMF composition in roots of their native neighbours (Hawkes et al., 2006). Bini et al. (2018) reported that the number of *Gigaspora* spores and the frequency of occurrence of arbuscular mycorrhizal fungal species decreased 100.0 and 56.7 %, respectively because intercropping two exotic plant species, *Eucalyptus grandis* and *Acacia mangium* in a Rhodic Ferralsol, Brazil. Examining other studies around the world, we found negative effects of *Stevia rebaudiana* Bertoni on *Gigaspora* occurrence (Astuti et al., 2018) and a significant reduction (on average51.3 %) on *Gigaspora* frequency of occurrence as a result of initial exotic plant seedling mixture (Henning et al., 2018) in Indonesia and U.S.A., respectively.

The modification of occurrence and frequency of *Gigaspora* species suggests a functional adaptation of the arbuscular mycorrhizal fungal species to the soil conditions and to the physiological requirement of their host trees (e.g. *C. madagascariensis* in the invaded zone; and *M. tenuiflora* in the native zone) for the *Gigaspora* species (Courty et al., 2018). Souza et al. (2016a, b) demonstrated that the arbuscular mycorrhizal fungal communities were highly influenced by biological invasion and plant community diversity. According to Martínez-García et al. (2015) fungal alpha diversity decreased and beta diversity increased with undisturbed ecosystem age. In our study, we observed a reduction of the abundance of *Gigaspora* species from the native zone to the invaded zone. Interestingly, the same trend of reduction of the abundance of arbuscular mycorrhizal community has been reported in other studies (Zhang et al., 2010; Zubek et al., 2016; Erktan et al., 2018).

**5 Conclusion**

The main findings of this study may be summarized as follows: (1) invasive plant species as *C. madagascariensis* alter the occurrence and frequency of *Gigaspora* in field conditions from the Brazilian semiarid region, (2) *G. albida* and *G. margarita* did not occur in the transition zone between the invaded and native zone, and (3) among the three identified species from *Gigaspora*, *G. gigantea* was the most resilient AMF species occurring in all the studied sections into the 40 m transects. Our findings suggest that the biological invasion by *C. madagascariensis* can alter the composition of the *Gigaspora* community, an important taxon from the Order Diversisporales in the Brazilian semiarid region. Despite our results are an important contribution to our understanding on the importance of considering the impacts of biological invasion by exotic plant species on specific taxa of AMF species.

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