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Artigo Científico

Occurrence and distribution of *Gigaspora* under *Cryptostegia madagascariensis* Bojer Ex Decne in Brazilian tropical seasonal dry forest[1]

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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 know 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, Paraiba, 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 semiarid 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 semiarid 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 semiarid; *Gigaspora albida; Gigaspora gigantea; Gigaspora margarita*; arbuscular mycorrhizal fungi.

Ocorrência e distribuição do gênero *Gigaspora* em C*ryptostegia 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 frequencia de ocorrência de espécies do gênero *Gigaspora* usando transectos de 40 m e análises morfológicas do 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 dominância das espécies de *Gigaspora* na zona nativa. Diferenças na ocorrência e distribuição do gênero *Gigaspora* foram associadas com (1) o tipo de espécie de planta dominante *(C. madagascariensis vs. M. tenuiflora)* que alteraram a ocorrência e frequência de espécies do gênero *Gigaspora* em condições de campo do semiárido brasileiro, (2) a zona de transição, onde não foi observada ocorrência das espécies *G. albida* e *G. margarita*, e (3) pareamento planta-fungo específico, onde constatou-se a espécie *G. gigantea* como a mais resiliente espécie de fungo micorrízicos observada em todas as seções estudadas dentro do transecto 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 semiárido 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*; Semiárido brasileiro.

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1 Introduction

The Brazilian semiarid region is characterized by high diversity and variability of arbuscular mycorrhizal fungi (AMF) (Souza and Freitas, 2017; Schuβler et al., 2001). According to Silva et al. (2014) during the dry season the frequency of occurrence of different AMF species in the Brazilian semiarid increase through high fungal sporulation. Goto et al. (2012) and Marinho et al. (2014) describe the Brazilian semiarid 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 semiarid 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 semiarid? 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 semiarid (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, Paraiba, Brazil (6º47’34.01” S, 37º49’10.7” W, and average altitude 183 m). The climatic condition of the studied environments is Bsh (Köppen), hot semi-arid, temperature of 28ºC 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 Paraiba, 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: EXO_{DL} - Under the drip line of *C. madagascariensis* (exotic plant species) and located 20 m from the transition zone; EXO_{15} – Within the invaded zone and located 15 m from the transition zone; EXO_{10} – Within the invaded zone and located 10 m from the transition zone; EXO_5 – Within the invaded zone and located 5 m from the transition zone; NAT_5 - Within the native zone and located 5 m from the transition zone; NAT_{10} - Within the native zone and located 10 m from the transition zone; NAT_{15} - Within the native zone and located 15 m from the transition zone; and NAT_{DL} - 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 lacto-glycerol (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/lbmicorrizas/chaves-de-identificao). 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_i) of each *Gigaspora* species. FO_i was calculated using the following equation: $FO_i = n_i/N$, where n_i 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.

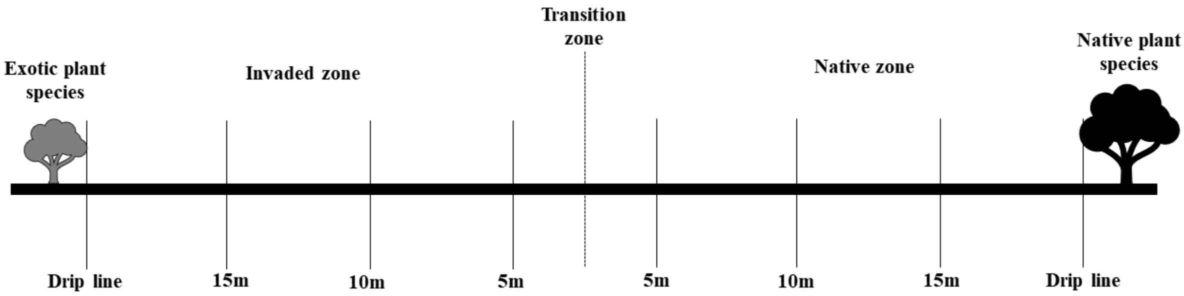

Figure 1 Sampling methodology scheme of soil samples along a transect between native and invaded zone.

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 $\log_n$ 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 testes 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/100g 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 EXO_5 and NAT_5 sections ($P < 0.5698$); and *G. gigantea* into the EXO_{10} and NAT_{10} ($P < 0.7366$) and EXO_5 and NAT_5 ($P < 0.6598$) 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 EXO_{DL} and NAT_{DL} sections, respectively. But, these conditions changed near the transition zone, where *G. gigantea* was the only one *Gigaspora* species observed in both EXO_5 and NAT_5 field soil samples. We did not find any *G. albida* species in the EXO_{10}, and for the EXO_5, 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 section into the transects between invaded and native zone

Studied zones	*Gigaspora* total abundance	*G. albida.*	*G. gigantea*	*G. margarita*
EXO_{DL}	8.1 ± 0.1 d	4.1 ± 0.5 d	3.0 ± 0.1 d	1.0 ± 0.1 e
NAT_{DL}	45.6 ± 0.3*[a] a	12.2 ± 0.3* b	17.2 ± 0.3* a	16.2 ± 0.1* a
EXO_{15}	17.5 ± 0.4* c	6.2 ± 0.7 c	8.1 ± 0.2 c	3.2 ± 0.2 d
NAT_{15}	40.0 ± 0.2 b	17.1 ± 0.5* a	11.0 ± 0.1* b	11.9 ± 0.6* b
EXO_{10}	4.2 ± 0.1 e	0.0 ± 0.0 f	3.0 ± 0.5 d	1.2 ± 0.3 e
NAT_{10}	17.5 ± 0.2* c	5.1 ± 0.3* c	4.0 ± 0.6^{ns} d	8.1 ± 0.1* c
EXO_5	1.0 ± 0.3 f	0.0 ± 0.0 f	1.0 ± 0.6 e	0.0 ± 0.0 f
NAT_5	5.1 ± 0.4* e	1.0 ± 0.1^{ns} e	2.1 ± 0.5^{ns} e	2.0 ± 0.1* e

[a]Independent sample *t* test comparing invaded zone (EXO) x native zone (NAT) groups (*$P \leq 0.05$; ^{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.

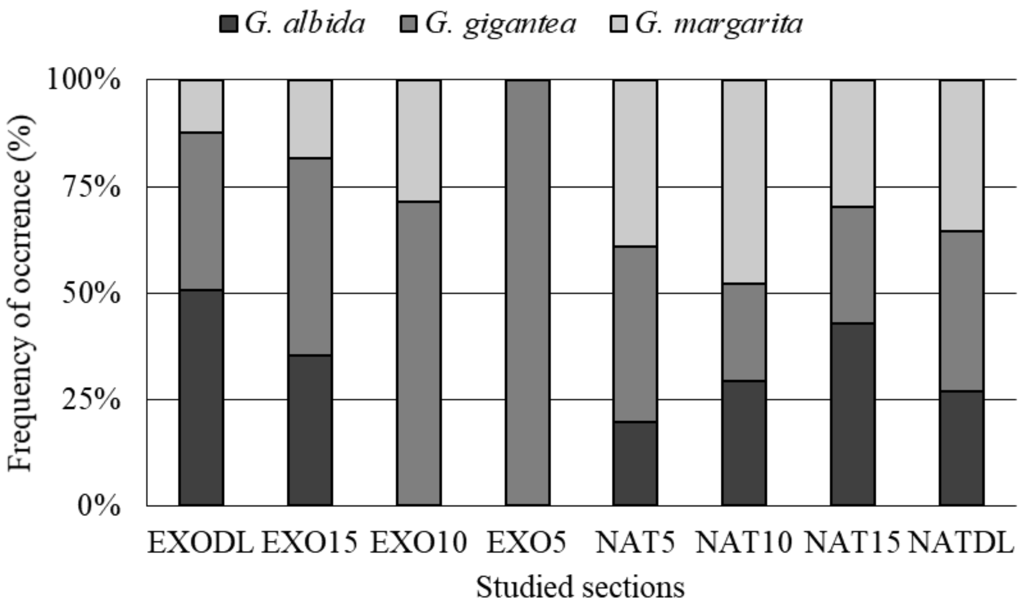

Figure 2 Frequency of occurrence (FO_i) of *Gigaspora* species of each studied section into the 40m transect (*N* = 320) between invaded and native zone. $FO_i = n_i/N$, where n_i is the number of times an AMF species was observe and N is the total of AMF spores observed from each studied condition.

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 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 seeding 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 Martinez-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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