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Arbuscular mycorrhizal fungi in a semi-arid, limestone mining-impacted area of Brazil

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ABSTRACT

The main goal of this study was to determine the diversity and activity of arbuscular mycorrhizal fungi (AMF) in an area degraded by limestone mining within the semi-arid region of Brazil known as the *caatinga* (shrublands). Near a limestone quarry, we selected two areas of *caatinga* (preserved and degraded) for study. The number of glomerospores did not differ significantly between the two areas. There was a trend toward the most probable number of infective propagules being higher in the degraded area. Twenty AMF taxa were identified in the two sampled areas, species richness, diversity and evenness being higher in the preserved area. Two species of *Racocetra* represent new records for the semi-arid region of Brazil. Glomerospore production and AMF species richness were unaffected by mining activity in the study area.

Key words: caatinga, glomerospores, infective propagules, mining activities, mycorrhiza

Introduction

Many interactions take place among the physical, chemical and biological components of soil, whose functionality depends on the environment in which it is located (Nannipieri *et al*. 2003). Particularly in the rhizosphere, various microbiological communities are essential for the occurrence of fundamental processes, such as biochemical cycling of the nutrients that influence the formation and maintenance of the soil and consequently the sustainability of land ecosystems (Barea *et al.* 2005).

The majority of plants form mycorrhizae, symbiotic associations with fungi that live in the root system. Those associations exercise an essential function for the structure and stability of plant communities. Arbuscular mycorrhizal fungi (AMF) are associated with representatives of more than 90% of the vascular plant families and, as obligate symbionts, are nutritionally dependent on the host plant (Smith & Read 2008). In exchange for plant-derived carbon, AMF facilitate plant absorption and uptake of nutrients from the soil, such as phosphorus (Jakobsen *et al.* 2001), and can increase plants' tolerance to toxic elements (Cumming & Ning 2003; Gattai *et al*. 2011), plant pathogens (Declerck *et al*. 2002), saline stress (Yano-Melo *et al*. 2003) and erosion (O'Dea 2007). The AMF can also help stabilize and aggregate soil, increasing the levels of soluble carbon and enzymes (e.g., urease, acid phosphatase and β-glucosidase), making them an important component to improve the growth of young plants under severe climate conditions (Caravaca *et al*. 2002).

Mining activity occurs in 26% of the municipalities within the semi-arid region of Brazil (DNPM 2009) and constitutes one of the human activities that most alters the land surface in the region. This activity can cause serious problems related to soil structure, water availability, biological activity, as well as to the supply of sulfur, phosphorus and nitrogen to plants, resulting in reduced productivity (Ferreira *et al*. 2007).

In arid and semi-arid regions, the generally low soil fertility makes plants highly dependent on mycorrhization (Tarafdar & Praveen-Kumar 1996), and it is therefore important to quantify the diversity of AMF in these regions, mainly in areas subject to degradation, such as those affected by mining activities. Due to the relevant role these fungi play in ecosystems, in this study we investigated the diversity, number of glomerospores and potential infectivity of AMF in an area of the Brazilian *caatinga* (shrublands) degraded by limestone mining, comparing the results with those obtained for a nearby preserved area.

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Material and methods

Study areas

The investigated areas are located on a property owned by the company Cimpor Cimentos Brazil, in the municipality of Campo Formoso (10°30'27"S; 40°19'17"W), located within the São Francisco River Valley, which is in the northern part of the state of Bahia, Brazil. The climate is semi-arid, with irregular rainfall, ranging from 300 to 700 mm/year, most of the rainfall occurring between November and March. The average annual temperature is 22-23°C, and the average relative air humidity is 20%, making the region highly arid. The terrain is mostly flat, covered by spiny deciduous shrubs and bushes (typical of the *caatinga*), forming a generally open landscape, interspersed with a few emergent trees and virtually no herbaceous layer in the dry season (PRAD Cimpor 2004).

Data collection

Soil samples were collected in October 2008 (dry season), during which the average monthly temperature was 23.8°C and evapotranspiration was 36.4 mm. We selected two areas of *caatinga* vegetation for study: a preserved area; and an area degraded by limestone mining. In each area, an imaginary zig-zag line was traced and 20 soil samples (each comprising three subsamples of ca. 0.5 kg) were collected and placed in plastic bags for AMF studies. A single composite soil sample (ca. 1.0 kg) for each area was taken to the Soil Laboratory of the Pernambuco Institute of Agronomy for physical and chemical analyses (Tab. 1).

Evaluation of mycorrhizae

Glomerospores were extracted from 100 g of 20 soil samples by wet sieving (Gerdemann & Nicolson 1963), followed by centrifuging in water and sucrose at 50.0% (modified from Jenkins 1964). The glomerospores were then quantified in Petri dishes with a stereomicroscope (40×).

The most probable number (MPN) of infective propagules in the soil was evaluated according to Feldmann & Idzack (1994). For each area, we prepared a compound sample, which was diluted in washed, autoclaved sand at four dilution levels (no dilution; 1/10; 1/100; and 1/1000), with five replicates. Two corn seeds (*Zea mays* L.) were planted in each pot (150 ml). At 10 days after sowing, the seedlings were culled, leaving only one plant per pot. The plants were harvested at 30 days after culling. The roots were washed in running water, stained according to Phillips & Haymann (1970) and evaluated for the presence of mycorrhizal colonization.

We used the soil samples in order to quantify the glomerospores and to identify the AMF species directly or after growth in trap cultures using sorghum (*Sorghum bicolor* (L.) Moench) and peanut (*Arachis hypogaea* L.) as host plants. The trap cultures were kept in a greenhouse for three months and watered every other day. Glomerospores were extracted as described above, mounted on slides with polyvinyl-lactoglycerol (PVLG) or with Melzer's reagent + PVLG (1:1 v/v) and observed under microscopy. For species identification, we consulted Schenck & Pérez (1990) and the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu), as well as new descriptions. The classification follows Oehl *et al.* (2011).

Data analysis

The numbers of glomerospores were analyzed by one-way ANOVA using the two areas (preserved and degraded) as variables. Means were compared by the Tukey's test ($p < 0.05$).

The Shannon diversity index (H') was calculated according to the following equation:

$$H' = -\Sigma\ (Pi\ ln\ [Pi])$$

where *Pi* is the relative abundance (the number of individuals of the species *i* in relation to the total number of individuals of all species), and *ln* is the natural log. Pielou's evenness index (J') was obtained by the following equation:

$$J' = H'/\log\ (S)$$

where *S* is the total number of species obtained with the PAleontological STatistics (PAST) program (Hammer *et al.* 2001). The species accumulation curve and the first-order Jackknife (Jackknife 1) index were estimated using the Primer 6.0 program (Clarke & Gorley 2006). The similarity between the two areas was estimated by the Sørensen index (Brower & Zar 1984). In accordance with Zhang *et al.* (2004), we defined the following categories of frequency of occurrence: dominant (> 50.0%); quite common (31.0-50.0%); common (10.0-30.0%); and rare (<10.0%).

Results and discussion

The number of glomerospores did not differ significantly between the preserved and degraded areas (164.0 ± 44.0/100 g^{-1} soil vs. 186.0 ± 47.0/100 g^{-1} soil). In both areas, the number of glomerospores was relatively low, as is common in arid and semi-arid regions (Requena *et al.* 1996). The high pH and limited availability of nutrients in the soil (Tab. 1), especially P, may have contributed to the small number of glomerospores (Entry *et al.* 2002). In an earlier study, Silva *et al.* (2001) obtained a similar result for an area of *caatinga* degraded by copper mining, reporting < 160.0 glomerospores/100 g^{-1} soil, suggesting that this response is related to host plant or soil toxicity in the area. However, in areas replanted after bauxite mining, Caproni *et al.* (2005) found an average of 2335.0 glomerospores/100 ml^{-1} soil, which can be considered a high density. More recently, La-

Table 1. Physico-chemical characteristics of the soil samples collected in preserved and degraded areas of *caatinga* (shrublands) in the municipality of Campo Formoso, in the state of Bahia, Brazil.

Area	pH	P	Ca	Mg	Na	K	Al	Sand	Silt	Clay
	H_2O	mg/dm^{-3}	mg/dm^{-3}	mg/dm^{-3}	mg/dm^{-3}	mg/dm^{-3}	mg/dm^{-3}	%	%	%
Preserved	7.3	42	11.00	1.55	0.07	1.2	0.0	35	33	32
Degraded	7.8	18	11.25	1.95	0.13	0.8	0.0	32	43	25

bidi *et al*. (2011) reported that $CaCO_3$ has harmful effects on the colonization and number of mycorrhizal structures (arbuscules, vesicles, external mycelia and glomerospores) of *Glomus irregulare* Blaszk., Wubet, Renker & Buscot, with a negative impact on the fungus life cycle during the pre-symbiotic (germination) and symbiotic phases in the root systems of transformed chicory (*Cichorium intybus* L.). In the present study, the limestone quarry did not appear to have a negative influence on the formation of glomerospores, given that similar number was found in both areas.

We observed a trend toward the number of infective propagules being higher in the degraded area than in the preserved area (35.0/cm^{-3} soil vs. 13.0/cm^{-3} soil). The distribution and abundance of AMF propagules can also be related to the complexity of the mycorrhizal community (Ramos-Zapata *et al*. 2011). The number of infective propagules, together with the low number of glomerospores, can indicate that the infectivity of the AMF in the sampled areas is more dependent on colonized roots than on glomerospores, and the stressful conditions in the area surrounding the mine possibly induces lower infectivity, corroborating the results of other studies conducted in the *caatinga* (Lima *et al*. 2007; Mergulhão *et al*. 2007). A similar result was found in a mined area covered with molasses grass, guandu beans (pigeon peas) and native plants, where the authors observed 39.3 propagules/g^{-1} soil, while in a closed forest area containing tree species the number was only 11.2 propagules/g^{-1} soil (Melloni *et al*. 2003). In another area of *caatinga* in the state of Bahia, Silva *et al*. (2001) found a much lower number of propagules (0.15/cm^{-3} soil). Although the methods used by Melloni *et al*. (2003) and Silva *et al*. (2001) to evaluate the MPN of propagules were different from that used in the present study, the fact that the MPN of infective propagules identified here was nearly 90 times higher than that reported by Silva *et al*. (2001) suggests a significant difference between the two studies, underscoring the need for standardization of methods in mycorrhizal studies (Maia *et al*. 2010).

In the present study, we found no difference between the preserved and degraded areas in terms of AMF diversity, evenness or richness, as measured by the Shannon index (H' = 2.30 and H' = 2.12, respectively), Pielou's index (J' =0.81 and J' = 0.78, respectively) and the total number of species (17 and 15, respectively). Regarding AMF diversity, 12 species occurred in both areas, representing a 75.0% similarity, which can be related to the nonspecific nature of the AMF, independent of the local conditions (Dandan & Zhiwei 2007). In a study conducted in India, Radhika & Rodrigues (2010) compared AMF diversity between two areas with different levels of available P. The authors found that other physico-chemical conditions of the soil, such as texture and pH, also influenced AMF diversity and dominance. Although AMF are commonly found in arid regions, their diversity declines as aridity increases (Stutz & Morton 1996). In another study conducted in the *caatinga* of northeastern Brazil, Maia *et al*. (2010) identified over 70 species of AMF. In the Xingó region, located in the state of Alagoas, Souza *et al*. (2003) identified 24 AMF taxa, some of which were also found in this study: *Acaulospora longula*, *Claroideoglomus etunicatum*, *Funneliformis mosseae* and *Paraglomus occultum*. In the municipality of Jaguarari, in the state of Bahia, Silva *et al*. (2005) identified 15 AMF species, six of which were also registered in our study: *Entrophospora infrequens*, *C. etunicatum*, *Glomus macrocarpum*, *G. microcarpum*, *F. mosseae* and *P. occultum*.

In the present study, we identified a total of 20 AMF taxa (Tab. 2). We observed more AMF species in the preserved area than in the degraded area, with an overall dominance of *Glomus macrocarpum*, followed by *Claroideoglomus etunicatum*, which was dominant in the degraded area and quite common in the preserved area. The other species were classified as common or rare in both areas. The sampling effort was sufficiently efficient to recover 83.0% of the total species, as determined by the Jackknife 1 estimator (Figure 1). Species observed in the trap cultures did not differ from those found in the field.

The predominance of representatives of Glomeraceae (*Glomus* and *Funneliformis*) and Entrophosporaceae (*Claroideoglomus* and *Entrophospora*) confirms the tolerance of these individuals to soil disturbances and anthropogenic environments and acid to neutral soils (Boddington & Dodd 2000; Sieverding & Oehl 2006). Species of the genus *Acaulospora* have often been associated with acidic soils (Morton 1986; Abbott & Robson 1991). However, in the present study, various species of that genus were found to occur on alkaline soil, also a frequent finding in arid areas of tropical zones (Tao & Zhiwei 2005), although less so in subtropical zones and rarely in colder climates (Yang *et al*. 2011). Representatives of the *Glomus* genus are also commonly found in arid regions (Pande & Tarafdar 2004; Silva *et al*. 2005; Tian *et al*. 2009; Mello *et al*. 2012), where their occurrence is related to variations in the edaphic conditions,

Table 2. Arbuscular mycorrhizal fungi in preserved and degraded areas of *caatinga* (shrublands) in the municipality of Campo Formoso, in the state of Bahia, Brazil.

AMF species	Preserved area		Degraded area	
	NS	F (%)	NS	F (%)
Acaulospora lacunosa J.B. Morton	0	0	1	5
Acaulospora longula Spain & N.C. Schenck	1	5	1	5
Acaulospora mellea Spain & N.C. Schenck	1	5	4	20
Acaulospora morrowiae Spain & N.C. Schenck	1	5	0	0
Acaulospora scrobiculata Trappe	0	0	1	5
Ambispora sp.	2	10	1	5
Claroideoglomus etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schuessler	10	50	13	60
Entrophospora sp.	10	50	2	10
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneid.	2	10	6	30
Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schuessler	6	30	4	20
Glomus sp. 1	2	10	0	0
Glomus sp. 2	1	5	1	5
Glomus sp. 3	1	5	2	10
Glomus brohultii Sieverd.	0	0	1	5
Glomus macrocarpum Tul. & C. Tul.	16	75	21	80
Glomus microcarpum Tul. & C. Tul.	1	5	0	0
Pacispora boliviana Sieverd. & Oehl	1	5	4	20
Paraglomus occultum (C. Walker) J.B. Morton & D. Redecker	5	25	1	5
Racocetra fulgida (Koske & C. Walker) Oehl, F.A. Souza & Sieverd.	1	5	0	0
Racocetra verrucosa (Koske & C. Walker) Oehl, F.A. Souza & Sieverd.	1	5	0	0
Total number of species	17		15	
Total number of spores	62		63	

AMF – arbuscular mycorrhizal fungi; NS – number of spores; F – frequency of occurrence of species.

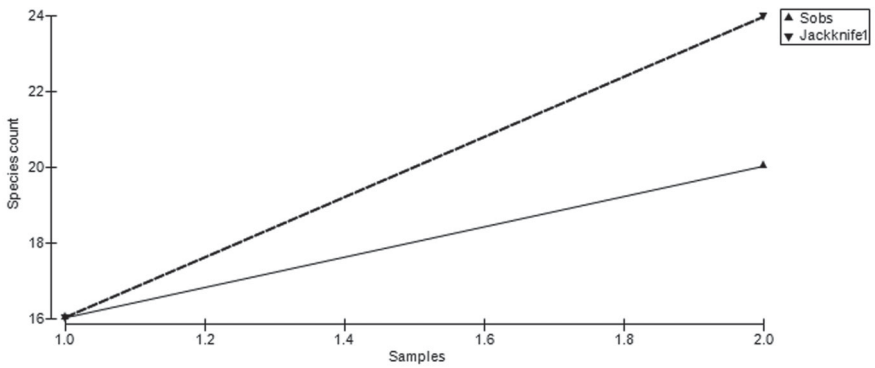

Figure 1. Species accumulation curve, based on the numbers of species observed (Sobs), and the species richness index, determined by the Jackknife first-order (Jackknife 1) estimator, in preserved and degraded areas of *caatinga* (shrublands) vegetation in the municipality of Campo Formoso, in the state of Bahia, Brazil.

as well as to their high adaptability to varying conditions of soil and temperature, enabling them to survive in acidic and alkaline soils alike. In addition, the great quantity and small size of their glomerospores facilitate distribution in arid environments (Stutz *et al.* 2000).

Representatives of *Ambispora* and *Entrophospora* occurred in both of the areas evaluated in the present study, as did *Acaulospora longula*, *Acaulospora mellea*, *Claroideoglomus etunicatum*, *Entrophospora infrequens*, *Funneliformis mosseae*, *Glomus macrocarpum*, *Pacispora*

boliviana and *Paraglomus occultum*. However, some species (*Acaulospora morrowiae*, *Glomus microcarpum*, *Racocetra fulgida* and *R. verrucosa*) were exclusive to the preserved area, whereas others (*Acaulospora lacunosa*, *Acaulospora scrobiculata* and *Glomus brohultii*) were exclusive to the degraded area.

The application of limestone to the soil did not alter the community of native AMF in degraded areas of the Brazilian savanna (Martins *et al*. 1999). However, soil disturbances have a large impact on the AMF community (Schnoor *et al*. 2011), possibly reducing the richness of sensitive species and increasing that of tolerant species. In a area of transition between open-pit copper mining and a preserved area of *caatinga*, Silva *et al*. (2005) registered *Acaulospora scrobiculata*, *Claroideoglomus etunicatum*, *Glomus macrocarpum*, *Funneliformis mosseae* and *Paraglomus occultum*, species also observed in this study (degraded area). Melloni *et al*. (2003) identified six AMF species in reclaimed areas after bauxite mining, among them *A. scrobiculata* and *P. occultum*, species that can be classified as generalists (Oehl *et al*. 2010; Tchabi *et al*. 2009), at least in tropical zones.

Information on the diversity of AMF can be used to investigate the function of these fungi in maintaining plant biodiversity and the function of the ecosystem during conservation and restoration of various natural ecosystems, especially in semi-arid zones (Dandan & Zhiwei 2007). The knowledge of the diversity and activity of AMF obtained here adds to that provided by previous studies conducted in arid areas and areas degraded by mining. New species of AMF have recently been described in the *caatinga* biome (Goto *et al*. 2010) and could be exclusive to Brazil. In the present study, the two species of *Racocetra* are among the first reported for the semi-arid region of Brazil. Our results indicate that the production of glomerospores is not altered by limestone mining activity. Our findings also confirm the predominance of *Glomus* in areas with a semi-arid climate, as well as showing that species that are more tolerant can be dominant in stressful environments, in contrast to rare species in unaltered environments. Knowledge of AMF diversity in Brazil can inform decisions related to public policies for environmental preservation and programs to produce seedlings of native plants for reclamation of degraded areas in the semi-arid region of the country.

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