

Environmental degradation impact on native communities of arbuscular mycorrhizal fungi in an urban fragment of semideciduous plateau forest

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RESUMO

(Impacto da degradação ambiental sobre as comunidades nativas de fungos micorrízicos arbusculares em um fragmento urbano de floresta estacional semidecídua). No município de Maringá (PR) existem três parques abertos à visitação, todos com áreas degradadas. O impacto causado pelo corte de árvores, tráfego de automóveis e visitação sobre o estabelecimento de fungos micorrízicos arbusculares (FMA) foi avaliado em duas áreas do Horto Florestal Dr. Luis Teixeira Mendes, remanescente de floresta estacional semidecídua. Amostras de solo foram retiradas de três pontos em cada área. Os esporos foram isolados do solo via peneiramento úmido e centrifugação em sacarose, e montados em lâminas permanentes. Sob microscópio foram quantificados e identificados morfológicamente. Com os dados de abundância, calcularam-se os índices: diversidade, dominância, equitabilidade e similaridade. A área degradada apresentou maior número de esporos e comunidades com valores menores de riqueza, diversidade e equitabilidade. No entanto, convém esclarecer que a maior densidade de esporos foi ocasionada pela presença freqüente de esporocarpos de *G. sinuosum*. Na área preservada foram verificadas 10 a 12 espécies por ponto de coleta, enquanto na área degradada, esse número variou de 6 a 12. Na área degradada, o ponto II, localizado na região mais protegida do fragmento, apresentou comunidades bem diversificadas e equilibradas, à semelhança dos pontos da área preservada. Os resultados sugerem que a degradação ambiental teve reflexos negativos no estabelecimento e na diversidade dos FMA.

Palavras-chave: diversidade, Glomeromycetes, fragmentação florestal

ABSTRACT

(Environmental degradation impact on native communities of arbuscular mycorrhizal fungi in an urban fragment of semideciduous plateau forest). Three forest reserves, with highly degraded areas, are open to visitors in Maringá, Paraná, Brazil. Impact caused by tree cutting, heavy traffic and visitors on the establishment of arbuscular mycorrhizal fungi (AMF) was evaluated in two areas with different degradation stages of the Dr. Luis Teixeira Mendes Forest Garden, a remnant of semideciduous forest. Soil samples were removed from three locations within each area; spores were isolated from the soil by wet sieving and sucrose centrifugation and mounted on permanent slides. Spores were counted and identified taxonomically under a microscope. Diversity, dominance, equitability and similarity indexes were calculated from abundance data. The degraded area had the highest number of spores and featured communities with the lowest rates in richness, diversity and equitability. However, high spore density was caused by the frequent presence of *G. sinuosum* sporocarps. Ten to 12 species were verified in each site from the preserved area while this number varied from 6 to 12 in the degraded area. In the degraded area, Site II, lying in the most protected area of the forest fragment, diversified and equilibrated communities existed, similar to sites in the preserved area. Results suggest that environmental degradation had negative effects on the establishment and diversity of AMF.

Key words: diversity, Glomeromycetes, forest fragmentation

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Introduction

Arbuscular mycorrhizal fungi (AMF), linked to improvements in vegetation growth for more than one hundred years (Trappe 1987), are of paramount importance to recover disturbed natural areas. The number of plants dependent on arbuscular mycorrhizal (AM) association is generally high and nutrient deficiencies are an important restriction in their growth (Li *et al.* 1991).

Considered to be the most relevant components of soil microbiota (Oehl *et al.* 2003) and present in various regions of the world, AMF are involved in ecological succession and flora rebuilding, contributing to the diversification, productivity and stability of natural ecosystems (Allen *et al.* 1995; Klironomos *et al.* 2000).

There are three forest reserves in Maringá with remnant native vegetation representing sites of biological reserve. Conservation areas have been deficient and several sites are highly degraded.

Impact caused by pathways and tree removal within forest fragments, car traffic and visitors have caused a series of environmental changes (Kattan & Alvaréz-López 1996), which interfere in AMF communities and in the functioning of symbiosis. Microclimate variations (mainly temperature rise and decrease in humidity), loss of macro- and micronutrients, changes in floristic composition and genetic erosion are the consequences of forest clearings (Kattan & Alvaréz-López 1996; Schellas & Greenberg 1996). Such modifications may negatively affect establishment and diversity of AMF communities (Cuenca *et al.* 1998; Stahl *et al.* 1988; Zhang *et al.* 2004). Consequently, knowledge of several factors that affect AMF diversity is of great importance for the management, recovery and conservation of the environment.

Several studies have used population data, such as distribution and abundance of spores of species, and sociological data of AMF communities in cultivated and natural ecosystems, in Brazil and in other countries. Still, there are still few studies focusing on AMF diversity in urban forest fragments predominantly constituted by native vegetation, and in Brazil assessments have started in Paraná state, specifically in Maringá. In this municipality, two of seventeen parks have been evaluated (Dr. Luiz Teixeira Mendes Forest Garden and Cinquentenário Park), and in these, different communities of AMF were verified in terms of composition as well as diversity. In the first fragment, considered to be a forest reserve, two studies were made over a two-year period in which 114 soil samples were evaluated. During the first year, the diversity of AMF was examined under two environmental conditions, preserved and degraded (this paper), using samples with different volumes of soil. Since the recovery of species was not better in the biggest samples and data dispersal was higher, we considered only data from the smallest ones. In the second year, Carrenho & Santos (2006) investigated the edge effect on the composition of species in those areas and verified lower number of species

(1 to 6 in the preserved area and 2 to 4 in the degraded area) and spores (averages of 36.46 and 29.2 in the preserved and degraded areas, respectively). It is interesting to stress that the first study was done in the summer and the second in the winter, period which no additional species was identified. In the second fragment (Cinquentenário Park), smaller and in a less advanced stage of succession and anthropization, 85 samples were collected from different sites in an unique period from summer, resulting in two studies whose main objectives were to verify the efficiency of different trap plants (Lippert 2009) or homeopathic solutions on peanut (Santos 2009) under pot culture in detecting the diversity of species coming from field.

Little is known about the effects of urbanization on AMF established in the referred fragments, but results from other studies have evidenced negative responses when plants imported from other areas are introduced (Helgason *et al.* 2002) or when the area is exposed to common pollutants, such as nitrogenous compounds, toxic metals and ozone (Cairney & Meharg 1999; Egerton-Warburton & Allen 2000). Thus, current research evaluates if the loss of environmental quality, caused by deforestation, paths and visitors influences AMF communities.

Material and methods

Site characterization

The Forest Garden is delimited by the coordinates Latitude 22°30'-24°30'S and Longitude 51°30'-54°W, at a mean altitude of 556 meters, featuring tropical deciduous plateau vegetation (Embrapa/Iapar 1984). The soil in Maringá is a dystrophic Dusky-Red Latosol, originated from basalt decomposition (www.codem.org.br). According to Köppen classification, a humid tropical mesothermal climate, or Cw'h, predominates. Data from the Main Climatology Station of Maringá at the State University of Maringá show that in 1999 mean, maximum and minimum temperatures were 28.1°C and 17.4°C, respectively; relative humidity was 68%; total rainfall was 1,412.8 mm; total evaporation was 1,920.4 mm; and total sunshine reached 2,701.6 h (www.codem.org.br).

Two distinguishable areas may be detected in the Forest Garden: a protected area, prohibited to visitors, and a more exposed area with several sites lacking vegetation, in which visitors and cars are allowed. Soil chemical properties of the two areas show statistically significant differences, seemingly related to vegetation (Tab. 1).

Sampling

Soil was collected in PVC tubes, 15 cm long and 5 cm diameter, with a volume of 294.37 mL. Samples were removed at three randomized sites (I, II, III) from the two areas (preserved and degraded) of the Dr. Luis Teixeira Mendes

Table 1. Chemical and physical-chemical soil characteristics in different sites of Dr. Luis Teixeira Mendes Forest Garden in Maringá, PR.

Characteristics	P	Preserved Area				Degraded Area			
		S I ⁽¹⁾	S II	S III	SD ⁽²⁾	S I	S II	S III	SD
pH (CaCl ₂)	0.004	5.56	5.62	5.80	0.12	6.45	6.89	7.05	0.31
H + Al (mmol _c .dm ⁻³)	0.001	2.32	1.89	1.78	0.28	2.87	2.81	3.07	0.14
Ca (mmol _c .dm ⁻³)	0.030	20.35	21.19	24.34	2.10	11.45	13.83	18.12	3.38
Mg (mmol _c .dm ⁻³)	0.017	4.08	3.16	4.03	0.52	2.18	2.55	2.65	0.25
K (mmol _c .dm ⁻³)	0.020	2.36	1.77	2.57	0.42	1.13	1.07	0.21	0.51
P (mg.dm ⁻³)	0.005	24.75	36.52	38.39	7.39	8.36	11.55	7.92	1.98
C (%)	0.186	13.01	12.27	17.41	2.78	8.37	14.22	4.16	5.05

⁽¹⁾ S I, S II and S III correspond to the sampling sites. ⁽²⁾ SD = standard deviation.

Forest Garden on December 13, 1999. Three replications from each site, totaling 18 samples, were taken in order to extract the spores, and another six samples were collected to be used as source of propagules for culture in pots.

Trap culture

For culturing the native AM fungal communities, traps were established by placing 200 g of soil samples with root pieces as layers sandwiched between two layers of sterile soil (methyl bromide). Plastic pots used were 10 cm wide and 15 cm tall having the capacity to accommodate 1 kg of soil. Pots were first filled with sterile soil up to 8 cm, the soil inoculum was added and topped with another layer of sterile soil. Four replications from each site, totaling 24 samples, were used to multiply the AMF. *Sorghum bicolor* (L.) Mönch was used as bait plant, and at the end of the four-month growing cycle, 100 g soil was sampled from each pot for AMF spore extraction, counting and identification. Nevertheless, this trial failed to establish a successful production of spores, although the roots exhibited some sites colonized by AMF (unpublished data)³. It is interesting to emphasize that those roots were also colonized by dark septate fungi, which were more frequently observed than AMF (unshown data).

Soil processing and preservation of spores

Soil from tubes was dried at room temperature and AMF spores were isolated by wet sieving technique (Gerdemann & Nicolson 1963) and sucrose centrifugation (Jenkins 1964).

Spores were mounted on semi-permanent slides in two separate groups: one group with PVLG resin and the other with PVLG resin + Melzer, and counted under a microscope (Morton *et al.* 1993). The sporocarps were carefully broken and the spores were counted. All of the spores are kept in the personal collection from Rosilaine Carrenho, and those in good state of conservation are mounted on slides which are being incorporated into Herbário da Universidade Estadual

de Maringá (HUEM). Photographs can be obtained from the responsible author.

Identification of AFM species

Species taxa were identified using Schenck & Pérez (1988) Carrenho & Trufem (2001), INVAM - International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (<http://invam.caf.wvu.edu>) and other species descriptions.

Sociological parameters

Behavior of AMF communities was evaluated by spore density and ecological indexes (richness, diversity, dominance, equitability, similarity), following Magurran (1988). Total density of spores consists of spores found in the tubes (samples); species density is the number of spores of each species. Frequency of occurrence refers to the number of samples (replications) in which a certain species occurred. Richness was represented by number of species. Dominance was shown by Simpson's index; diversity was determined by Shannon's index; equitability was calculated by Pielou's index. Similarity of communities in the different environments was determined by Sorensen's qualitative coefficient.

Statistic analysis

Data on spore density were analyzed by non-parametric ANOVA and Mann-Whitney test.

Results and discussion

When soil volume analyzed in each collecting site (883.11 mL) is taken into account, total number of spores was low (Table 2). In studies related to fragmented forest environment, means ranged between 288 and 2,797 in 100 g of dry soil (Santos 2001; Alves 2004) and in urban forest fragments, means ranged from 83.67 to 165 (Lippert 2009;

³ The data were not shown or evaluated by statistical tests because many plants died, mainly those cropped on soil from degraded areas, thereby weakening the statistical analysis.

Table 2. Influence of environmental degradation on spore density of AMF established in two areas of Dr. Luis Teixeira Mendes Forest Garden.

Areas	Average number of spores ⁽¹⁾	SD	Normality ⁽²⁾	Homocedasticity ⁽³⁾
Preserved	136.44	79.55	Yes	No
Degraded	228.20	279.78	Yes	No

p = 0.359

⁽¹⁾ n=9; ⁽²⁾ Kolmogorov & Smirnov test; ⁽³⁾ Bartlett's test.

Santos 2009). In those studies, the highest numbers of spores were verified in fragments of larger dimensions and under lesser anthropic intervention, different from this study, whose higher number of spores occurred in the degraded area (Tab. 2). This fact was, however, due to the constant presence of sporocarps of *Glomus sinuosum*, whose spore numbers were considered to calculate population abundance and related ecological indexes (Tab. 3). When the above species is not taken into account, AMF communities in sites I and III have very low number of spores (18 and 16, respectively). These sites, close to paths with traffic of cars and people, show poor vegetation as well as low soil fertility (Tab. 1) different from site II, that lies in the middle of the fragment and exhibits better environmental conditions (higher humidity; richer and denser vegetation; higher rates of phosphorus and carbon). Stahl *et al.* (1988) noted fewer propagules and lower species richness of AMF in environmentally disturbed areas that is partially concordant with the present data. Considering only spore numbers, which are one of the types of mycorrhizal propagules, and excluding *Glomus sinuosum* from samples, it is possible to observe the referred relationship. Since soil organic matter is derived mainly from plant residues, it contains all of the essential plant nutrients. Accumulated organic matter, therefore, is a storehouse of plant nutrients. Loss of plant cover affects both quality and quantity of soil organic matter, which modify many chemical and physical properties, including soil pH. Organic matter decomposition generally increases soil acidity by addition of hydrogen and this will depend on input rates. The less input on soil, the less acidification, as verified in the present study and pH has been considered a modulating factor on AMF growth. Santos (2001) and Barbosa (2004) found that spore numbers and soil pH were negatively related and corroborate data in current study (excluding *G. sinuosum* from the samples). It is possible that species forming free spores or in small groups and species forming dense sporocarps are differently affect by soil properties, that can explain the opposite responses verified for *G. sinuosum*.

All sites in the preserved area are well structured with many herbaceous plants in the lower layer and well-closed top covering. In this area, soil properties were generally evaluated as uniform with sharp differences only in phosphorus and carbon rates. Site I, more exposed to light and wind, had a lower P rate while the innermost site (Site III), more shaded and with extensive vegetation, had the highest C and P rates (Table 1). However these contrasts in environ-

mental variables and edaphic properties were not enough to change the structure of AMF communities, which showed higher richness, diversity and equability (Tab. 4). In this area, interspecific competition seemed to be symmetric and differences in spore abundance may be due to reproductive effort of each species.

Intensity, frequency and duration of natural or anthropic disturbances are directly related to restoration and regeneration capacity of the ecosystem. Low environmental perturbations may stimulate proliferation and diversification of biotic communities and cause subtle changes in their composition. Serious disturbances produce heterogeneous environments with communities exhibiting mosaicism, which may induce unrepairable metabolic errors and development instability in these organisms, causing loss of genetic variability (White & Jentsche 2001). However, it is important to keep in mind that in clonal organisms, such AMF, the genetic heterogeneity originating from somatic mutations, mitotic recombinations, mitotic gene conversions, genome duplications, or transfer of nuclei between hyphae can represent an important source of variation within a population. Experimental studies on this topic are lacking and genetic processes in AMF are critically discussed by Pawlowska (2005).

Burrows & Pflieger (2002) reported that an increase in plant diversity in a given environment may cause a increase in the number of spores (30% to 150%) and in AMF spore volume (40% to 70%), or rather, a greater quantity of large spores. Table 2 shows that species with the above qualities (*A. laevis*, *A. tuberculata*, *G. macrocarpum*, *G. versiforme* and *Scutellospora rubra*) sporulated preferentially in the preserved area with richer and more diversified vegetation, supporting the above authors' report. Zhang *et al.* (2004) recorded that unfavorable soil conditions for plant growth also decrease the density of propagules of the organisms. Soil samples of the degraded area had quite lower rates of Ca, Mg, K, P and C than those of the preserved area and this condition seemed to limit the sporulation of almost all species (Tab. 1). On the other hand, *G. sinuosum* occurred with high population abundances and high frequency on those conditions, which increased the dominance on AMF communities from those sites (Tab. 4)

Glomus macrocarpum and *G. versiforme* were also abundant at Site II of the degraded area, which is the most protected site, with higher P and C rates in the soil. *Glomus macrocarpum* is widely distributed in Brazil and its sporulation has been positively related to soils with high

Table 3. Total (TD) and specific density (SD) of spores and frequency of occurrence of arbuscular mycorrhizal fungi in different areas of Dr. Luis Teixeira Mendes Forest Garden according to level of anthropic perturbation.

Species	Preserved area				Degraded area			
	S I	S II	S III	AD ^(1,2)	S I	S II	S III	AD
<i>Acaulospora</i>								
<i>A. foveata</i> Trappe & Janos	-	27(1) ⁽³⁾	2(1)	29	-	16(3)	-	16
<i>A. laevis</i> Gerd. & Trappe	5(1)	25(1)	131(2)	161	-	48(3)	2(1)	50
<i>A. mellea</i> Spain & N.C. Schenck	16(1)	58(3)	3(1)	77	4(2)	50(3)	4(2)	58
<i>A. morrowiae</i> Spain & N.C. Schenck	-	-	1(1)	1	-	6(1)	1(1)	7
<i>A. scrobiculata</i> Trappe	-	1(1)	1(1)	2	-	-	-	0
<i>A. tuberculata</i> Janos & Trappe	1(1)	-	39(3)	40	-	-	-	0
<i>Entrophospora</i>								
<i>E. infrequens</i> (Hall) Ames & Schneider	1(1)	2(1)	-	3	-	-	-	0
<i>Glomus</i>								
<i>G. claroideum</i> N.C. Schenck & Sm.	-	-	-	0	7(1)	2(2)	1(1)	10
<i>G. clavispurum</i> (Trappe) Almeida & N.C. Schenck	100(1)	-	-	100	-	-	-	0
<i>G. etunicatum</i> Becker & Gerd.	27(2)	44(3)	11(1)	82	2(1)	10(3)	-	12
<i>G. geosporum</i> (N.T. Nicolson & Gerd.) C. Walker	-	-	-	0	-	1(1)	1(1)	2
<i>G. macrocarpum</i> Tul. & C. Tul.	127(3)	126 (3)	65(2)	318	4(1)	88(3)	-	92
<i>G. microaggregatum</i> Koske, Gemma & Olexia	-	5(1)	-	5	-	20(1)	-	20
<i>G. sinuosum</i> (Gerd. & Bakshi) Almeida & N.C. Schenck	120(1)	-	-	120	1000(2)	86(2)	550(2)	1636
<i>G. versiforme</i> (P. Karst.) Berch	100(3)	95(3)	82(2)	277	1(1)	137(3)	1(1)	139
<i>Glomus</i> sp. ⁽⁴⁾	-	5(2)	3(1)	8	-	5(1)	6(2)	11
<i>Glomus</i> aff. <i>clarum</i> T.H. Nicolson & N.C. Schenck	-	-	-	0	-	1(1)	-	1
<i>Scutellospora</i>								
<i>S. rubra</i> Stürmer & J.B. Morton	-	5(2)	-	5	-	-	-	0
TD	497	393	338	1228	1018	470	566	2054
NTAX ⁽⁵⁾	9	11	10	15	6	13	8	13

⁽¹⁾ n=3. ⁽²⁾ AD means the accumulated number of spores in each one of the sampling sites. ⁽³⁾ number inside parenthesis correspond to the frequency of occurrence. ⁽⁴⁾ *G. = macrocarpum*: spores globose, subglobose, elliptical or oblong, 150.0-180.0 µm diam., 170.0-200.0 µm long x 140.0-160.0 µm wide, dark-orange-brown to reddish-brown, with an structural wall formed by two adherent layers: the first is laminated, 5.0-7.5 µm esp., brown, and the second is unitary, yellow, 0.8-1.5 µm diam esp.; inside there is another layer, hyaline, semi-flexible, 0.7-1.0 µm esp., that separate almost always from those others. ⁽⁵⁾ NTAX refers to number of specific taxa.

Table 4. Ecological indexes for communities of AMF established in two areas of Dr. Luis Teixeira Mendes Forest Garden, Maringá – PR, under different environmental conditions.⁽¹⁾

Compared Aspects	Species Richness	Diversity (Shannon)	Equability (Pielou)	Dominance (Simpson)	Similarity (Sorensen)
Areas					
Preserved (P)	15	2.05	0.72	0.16	P x D = 74.1%
Degraded (D)	13	0.88	0.31	0.64	
Preserved Area					
S I	10	1.67	0.62	0.21	S I x S II = 54.5%
S II	12	1.80	0.66	0.20	S I x S III = 60.0%
S III	10	1.53	0.57	0.26	S II x S III = 72.7%
Degraded Area					
S I	6	0.11	0.04	0.97	S I x S II = 66.6%
S II	12	1.93	0.77	0.18	S I x S III = 61.5%
S III	7	0.17	0.07	0.94	S II x S III = 73.7%

⁽¹⁾. All of the AMF communities showed log distribution.

phosphorus levels (Carrenho 1998). According to Bononi & Trufem (1983) the generalized occurrence of a specific AMF taxon may indicate greater ecological disturbance in the environment. Likewise, restricted occurrence may indicate lesser disturbances and a trend towards AMF association with specific phytobionts.

Although *G. versiforme* is a restricted species in Brazil, a high representation of the species in protected natural forest areas and in deforested areas may indicate, according to Zhang *et al.* (2004), a wide adaptive and tolerance capacity to environmental disturbances.

Acaulosporaceae and Glomeraceae predominate in AMF communities in terms of spore abundance and in species richness. Six species of *Acaulospora*, one species of *Entrophospora*, ten species of *Glomus* and one species of *Scutellospora* (Tab. 2) were reported. Sporulation of *Acaulospora* was more extensive in the preserved area and seemed to be related to high acid pH in the soil. Above data have been confirmed by Johnson *et al.* (1991) and Carrenho *et al.* (2002). Since a similar behavior has been verified for *Glomus*, C rate of soil is probably the modulating factor of sporulation of these organisms, as has been previously reported by Johnson & Wedin (1997) and Carpenter *et al.* (2001). Within the most frequent and abundant species, *Acaulospora laevis*, *Glomus etunicatum*, *G. macrocarpum* and *G. versiforme* showed great differences in total number of spores and were dominant in the preserved area. Forest fragment size (more extensive in this area) may have contributed to such behavior, as Mangan *et al.* (2004) have reported in their studies in Panama.

Fifteen AMF species have been reported in the preserved area with slight variations (9, 11 and 10 species for sites I, II and III respectively) among the sampling sites. Further, the number of species restricted to a single site was also low (*G. clavisporum* at site I and *G. microaggregatum* and *Scutellospora rubra* at site II). This fact induced the formation of similar communities, as Sorensen's similarity coefficients have shown (Tab. 4). A similar behavior has been reported for total density of spores (Tab. 3). Since communities are assessed according to the proportionality of species occurrence and abundance, site II had the highest richness, diversity and equitability and site III had the highest dominance. However, differences were slight and revealed good AMF performance in this area (Tab. 4). It was also observed that the similarity between the sites was lower when Site I was confronted (Tab. 4) and this can be due to the edge effect on plant communities and their associated AMF.

Thirteen species, distributed in a variety of manners among the sampling sites (6, 13 and 18 respectively at sites I, II and III), were found in the degraded area. Site II (less disturbed and with higher P and C rates in soil) revealed communities with the highest richness, diversity and equitability. This fact contrasted greatly with communities at sites I and III (Tab. 4). In spite of such unequal distributions, similarity indexes between sites were high, or rather, between 61.5% and 73.7%, being the lowest ones related with Site I, located

on the edge of the fragment that indicates the edge effect altered the distribution and densities of AMF, a fact also verified in the preserved area. It must also be emphasized that dominance in sites I and III is accountable to *G. sinuosum* (Tab. 3), which occurs in compact sporocarps, and assumes a typical "phalanx" growth strategy, whose tightly packed arrangement of spores and profuse population aggregation restrict local occupation by other species, that evidences a clear example of asymmetric competition. This can be due to direct competition for space and resources or by indirect influence, through the production of high concentrations of extracellular enzymes (related to mineralization of organic P compounds, that may enhance mycorrhizal utilization of an important nutrient pool in soil) or pigments such as melanin (given the supposed involvement of melanin in protecting fungal structures, improving hyphal longevity), which may interfere negatively with the spread of other species.

Current data show that the production of spores of most species and the diversity of AMF communities were jeopardized by stresses produced by disturbances in the original environment. *Acaulospora laevis*, *Glomus etunicatum*, *G. macrocarpum* and *G. versiforme* were highly affected by deforestation, forest paths and visitors, while *G. sinuosum* was resistant to the conditions of the degraded area.

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