



Arbuscular mycorrhizal fungi within agroforestry and traditional land use systems in semi-arid Northeast Brazil

Carla da Silva Sousa^{1*}, Rômulo Simões Cezar Menezes², Everardo Valadares de Sá Barreto Sampaio², Francisco de Sousa Lima¹, Fritz Oehl³ and Leonor Costa Maia⁴

¹Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia, Rua Rui Barbosa, s/n, Campus Universitário, 44380-000, Cruz das Almas, Bahia, Brazil. ²Departamento de Energia Nuclear, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil. ³Agroscope Reckenholz-Tänikon Research Station, Reckenholzstrasse, Zürich, Switzerland. ⁴Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil. *Author for correspondence. E-mail: cssagro@yahoo.com.br

ABSTRACT. The diversity of arbuscular mycorrhizal fungi (AMF) can be a critical factor in enhancing both the productivity and the diversity of plants in ecosystems, and the plants in the ecosystem also strongly influence the occurrence of these fungi. The relationships between different land use systems and AMF communities in the semi-arid region of the State of Paraíba, NE Brazil were evaluated. The experiment followed a split-plot randomized block design, with four replicates. The main plots were defined by the presence or absence of trees (glicírcia and maniçoba), while the split plots were defined by three land use systems: 1) traditional cropping of maize + beans, 2) buffel grass pasture, and 3) prickly pear forage crop. The presence of trees increased sporulation, mycorrhizal colonization and the production of infective propagules of AMF in all three land use systems. Greater production of glomalin-related soil protein (GRSP) occurred in the prickly pear plots regardless of the presence or absence of trees. Species belonging to the *Glomus* genus predominated regardless of the presence of trees, land use system or soil sampling period.

Keywords: mycorrhizae, agroecosystems, glomalin.

Fungos micorrízicos arbusculares em sistemas agroflorestais e sistemas tradicionais de uso da terra no semi-árido do nordeste do Brasil

RESUMO. A diversidade de fungos micorrízicos arbusculares (FMA) pode ser um fator crucial para aumentar a produtividade e diversidade de plantas nos ecossistemas, e em contrapartida, as plantas também influenciam fortemente a ocorrência desses fungos. Foi avaliada a relação entre diferentes sistemas de uso da terra e as comunidades de FMA na região semi-árida do Estado da Paraíba, Nordeste do Brasil. O experimento foi conduzido em esquema de parcelas subdivididas em blocos casualizados, com quatro repetições. A presença ou ausência de árvores (glicírcia e maniçoba) foram parcelas principais e três sistemas de uso da terra foram as subparcelas: 1) cultivo tradicional de milho + feijão; 2) pastagem de capim buffel e 3) cultivo de palma forrageira. A presença das árvores aumentou a colonização micorrízica, esporulação e produção de propágulos de FMA em todos os três sistemas de uso da terra. Houve maior produção de proteínas do solo relacionadas à glomalina pelos FMA em parcelas com o cultivo de palma forrageira, independentemente da presença das árvores. Espécies de FMA pertencentes ao gênero *Glomus* predominaram independentemente da presença das árvores, do sistema de uso da terra e do período de amostragem do solo.

Palavras-chave: micorrizas, agroecossistemas, glomalina.

Introduction

In the semi-arid region of Brazil there are many tree species adapted to the environmental conditions that could provide diverse products if cultivated in agroecosystems. However, human population growth, the land tenure system and the succession of commercial cultivars have exerted strong pressures on these natural resources, particularly on the native dry forest (SABOURIN et al., 2000). One of the consequences of this human occupation

process in the semi-arid region of Paraíba is the near elimination of the native vegetation and a decrease in the presence of tree species in existing agroecosystems (LIMA; SIDERSKY, 2002). Many studies indicate that the elimination of tree cover usually results in accentuated decreases in soil organic matter and nutrient contents and, therefore, increased erosion rates (SAMPAIO; MENEZES, 2003).

To minimize the fragility of the production systems as well as the loss of trees in the semi-arid

region of Paraíba State, many producers are turning to agroforestry systems. In these systems, crops may be established between rows of one or more arboreal species, which are periodically pruned (SAMPAIO; MENEZES, 2003).

It has been shown that the presence of arbuscular mycorrhizal fungi (AMF) may enhance the re-establishment of vegetation cover in some ecosystems. These fungi play a significant role in increasing the establishment and productivity of tree species, especially in semi-arid regions where plant productivity is limited by low soil fertility and water availability (RAO; TARAFDAR, 1998).

According to Heijden et al. (2003), the diversity of fungal species exerts an important effect on the co-existence of plants as well as on the distribution of nitrogen (N) and phosphorus (P). Furthermore, the diversification of plant hosts can be responsible for an increase in the diversity of AMF (MOREIRA; SIQUEIRA, 2006). The objective of the present study was to evaluate the relationships between land use systems and communities of arbuscular mycorrhizal fungi in the semi-arid region of Paraíba State.

Material and methods

Description of the experimental area

The study was conducted at the Agroecological Station of Vila Maria Rita in the municipality of Taperoá (7°12'23" S., 36°49'25" W. Gr., and 520 m of altitude), in the State of Paraíba, Brazil. The soil in the experimental area is classified as Neossolo Flúvico according to the Brazilian soil classification system (Fluvent, in the USA soil classification system). The average annual rainfall is 558 mm, and the average temperature is 26°C (AESAs, 2008). Monthly precipitation was recorded in 2007, the year the experiment was conducted (Figure 1).

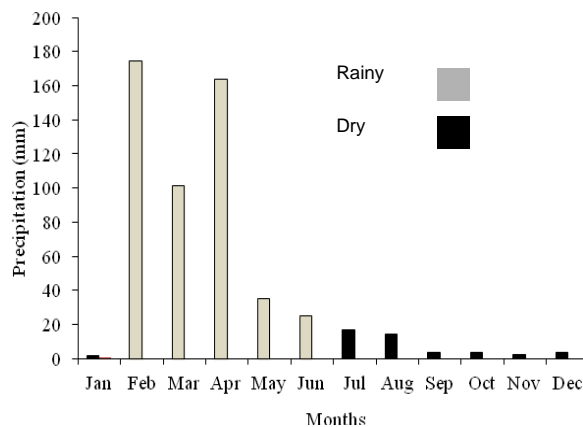


Figure 1. Monthly precipitation recorded in 2007, in Taperoá, Paraíba State (AESAs, 2008).

Installation of the experiment

The experiment was initiated in February 2006 and followed a split-plot randomized block design, with four replicates. The main plots consisted of two treatments: presence or absence of trees. The split plots consisted of three land use systems: 1) traditional agricultural cultivation, intercropping maize (*Zea mays* L.) + beans (*Phaseolus vulgaris* L.), 2) pasture planted with perennial herbaceous buffel grass (*Cenchrus ciliaris* L.) and 3) prickly pear, also known as opuntia (*Opuntia ficus-indica* (L.) Mill.). Each main plot had dimensions of 10 × 30 m and each split plot 10 × 6 m. Tree main plots (agroforestry plots) were planted with alternating rows, 6 m apart, of maniçoba (*Manihot glaziovii* Muell. Arg.), a native caatinga Euphorbiaceae species, and gliricidia (*Gliricidia sepium* (Jacq.) Steud), an exotic Leguminosae species introduced several years ago to the semi-arid area of Northeastern Brazil. The trees were at spaced 1 m intervals along the rows. Maize + beans, maize and opuntia were planted at 1 m intervals between rows with 50 cm between plants, and buffel grass was planted at 50 cm intervals between rows with 10 cm between plants.

Soil and root sampling

Soil and root samples were collected during both the rainy (May) and the dry (November) periods of 2007. For this procedure, ten topsoil (0-15 cm depth) samples were collected in each experimental plot, air dried, sieved (2 mm mesh) and mixed to create a composite sample. Thin roots (< 2 mm) of maize, beans, opuntia and buffel grass were collected in each experimental plot, washed in water and stored in plastic receptacles containing 50% alcohol, for preservation until the analysis.

Chemical and physical characterization of the soil

Chemical and physical analyses of the soil were performed (Table 1) according to the following procedures: pH in H₂O (soil : water, 2.5 v v⁻¹); Ca⁺² and Mg⁺² (extracted with KCl and determined by atomic absorption); P, K⁺ and Na⁺ (extracted with Mehlich I, K⁺ and Na⁺ determined by flame photometry and P by colorimetry); organic C (determined by humid oxidation in potassium dichromate); and granulometry by the densimetric method. A detailed description of the analytical methods can be found in Embrapa (1999).

Table 1. Physical and chemical soil characteristics in areas under different land use systems in Taperoá, Paraíba State.

Land use Systems ¹	pH	P	K	Na	Ca	Mg	C.O.	Granulometry (g kg ⁻¹)		
	H ₂ O	mg kg ⁻¹		cmol. kg ⁻¹			g kg ⁻¹	sand	clay	silt
Rainy Season										
MT	7.69	77.45	0.55	0.16	9.02	1.61	7.82	698	172	130
OT	7.46	72.30	0.43	0.18	6.53	0.90	10.34	658	182	160
BT	7.70	65.23	0.50	0.31	5.84	0.99	9.51	685	172	143
M	7.15	51.63	0.51	0.12	6.42	1.03	8.05	665	185	150
O	7.09	60.81	0.41	0.17	7.15	1.16	8.13	633	197	170
B	7.30	78.91	0.63	0.16	5.71	0.84	9.47	723	147	130
Dry Season										
MT	7.71	79.55	0.33	0.27	7.51	1.41	11.62	698	172	130
OT	7.59	79.74	0.35	0.24	7.22	1.14	12.08	658	182	160
BT	7.85	72.60	0.39	0.24	5.95	1.10	9.63	685	172	143
M	7.28	55.71	0.36	0.22	5.70	1.07	9.75	665	185	150
O	7.41	51.37	0.35	0.16	6.65	1.20	10.72	633	197	170
B	6.91	58.41	0.42	0.24	4.52	0.73	10.03	723	147	130

¹Land use systems of: (O) = opuntia (*Opuntia ficus-indica*) without trees; (OT) = opuntia with maniçoba (*Manihot glaziovii*) and gliricidia (*Gliricidia sepium*); (M) = Maize (*Zea mays*) and beans (*Phaseolus vulgaris*) without trees; (MT) = Maize and beans with maniçoba and gliricidia; (B) = Buffel grass (*Cenchrus ciliaris*) without trees; (BT) = Buffel grass with maniçoba and gliricidia. Means followed by same letters do not differ by the Scott & Knott test at 5% probability. Small letters compare systems with each other and capital letters compare the presence or absence of trees in the land use systems.

Identification of AMF species, spore number and variability and ecological indices

AMF spores were extracted from 50 g of the composite soil samples by the humid sieving technique (GERDEMANN; NICOLSON, 1963) using superposed sieves of 50, 100 and 250 μm , followed by centrifugation in water (3000 rpm) and a sucrose solution 45% (2000 rpm) for 3 and 1 minutes, respectively (JENKINS, 1964). Five milliliters of idonitrotetrazolium chloride (INT) at 0.1% was added to the spores, which were incubated for 5 days at room temperature to evaluate their viability, according to the methodology proposed by Walley and Germida (1995). Spores were considered viable when they turned red from reacting with INT and non-viable when their original color was maintained with INT. Afterwards, the spores were counted in channeled plates using a stereomicroscope (40 times magnification).

To identify the AMF species, trap cultures were created using a portion of the soil samples from each plot, diluted in autoclaved sand (1:1) and transferred to 500 mL plastic pots with 500 mL capacity, using Italian millet (*Panicum miliaceum* L.) as the host plant. After three multiplication cycles, the spores were extracted from the soil, separated according to their morphological characteristics (color, size and form) and mounted on slides with PVLG (polyvinyl-lactoglycerol alcohol) and with Melzer + PVLG (1:1; v v⁻¹) (MORTON et al., 1993). AMF species richness was defined as the number of species occurring in an area. The frequency of occurrence of species (Fi) was estimated according to the following equation: $F_i = J_i/k \cdot 100$, where J_i = number of samples in which the species occurred and k = total number soil samples.

Soil infectivity

Soil infectivity was evaluated according to the most probable number technique (MPN) for AMF infective propagules, described by Feldmann and Idczak (1992). A bioassay was conducted with soil from each plot and harvest period (rainy and dry). Sieved sand (0.5 cm mesh) was washed, autoclaved for 1 h at 120°C for three alternating days and oven dried at 105°C. It was then used to dilute the soil-inoculum samples to the following proportions: 1:1, 1:10, 1:100 and 1:1000 soil: sand (v v⁻¹). The mixtures were transferred to 100 g plastic tubes, with five replicates per plot and dilution. Two maize seeds were sown in each tube, and after germination (± 5 days), only one seedling was left. Plants were harvested after 30 days, and all the root systems were prepared for observation of AMF structures (KOSKE; GEMMA, 1989).

Quantification of glomalin-related soil protein (GRSP)

The proteins related to glomalin in the soil (GRSP) were quantified by the Wright and Upadhyaya (1998) method, in which 0.25 g of soil was autoclaved with 2 mL of sodium citrate (20 mM; pH 7.0) for 30 minutes at 121°C, followed by centrifugation at 10000 g for 5 minutes. An aliquot of 50 μL of the supernatant and 2.5 mL of the shiny Coomassie Brilliant Blue dye G-250 were used for quantification of the glomalin content. Bovine serum albumin was used as the standard.

Mycorrhizal colonization

The percentage of mycorrhizal colonization was determined using the split-plate intersect method (GIOVANNETTI; MOSSE, 1980) after processing of the roots. Processing consisted of clarification with KOH (10%) for 24h at room temperature, followed by alkaline H₂O₂ treatment for 45 minutes,

then HCl (1%) treatment for three minutes and staining with Trypan blue (0.05%) (KOSKE; GEMMA, 1989). One hundred stained root segments were separated for the visualization of fungal structures (arbuscules, vesicles and hyphae) using a stereomicroscope (40x).

Statistical analysis

Results were submitted to analysis of variance, and averages were compared with the Scott and Knott test at 5% probability, using the SISVAR software package. Data on spore numbers were transformed to $(x + 0.5)^{1/2}$ and the percentage of mycorrhizal colonization to $\arcsin \sqrt{x/100}$.

Results and discussion

Regardless of the presence or absence of gliricidia and maniçoba trees, a low number of viable spores was detected in general, compared with the number of non-viable spores. The fraction of viable spores in the total number of spores varied between 4.2 and 11.5% during the rainy period and between 3.9 and 9.8% during the dry period. According to McGee (1989), the number of viable spores in arid and semi-arid regions is usually relatively low. Lima et al. (2007) also observed that the number of viable spores was low, oscillating between 1.5 and 3.7% of the total number of spores, regardless of the land use system. The authors attributed these results to the environmental conditions of the semi-arid region in which the work was conducted.

During the rainy period, in the presence of gliricidia and maniçoba trees, a greater number of spores was observed (67 spores g^{-1} soil) for the intercropped system with maize + beans (Table 2). The intercropped systems of maize + beans and the opuntia system did not differ statistically with regard to spore number (58 and 52 spores $50 g^{-1}$ of soil, respectively) in the presence of gliricidia and maniçoba trees during the dry period. In the absence of gliricidia and maniçoba trees, there was no significant difference between land use systems regarding spore number for either sampling period. During the rainy period, the presence of gliricidia and maniçoba trees promoted increases of 92 and 41% in the total number of spores for the intercropped maize + beans and the buffel grass systems, respectively. We also observed increases of 56 and 31% in the total number of spores for the maize + beans and the opuntia systems with the presence of gliricidia and maniçoba trees during the dry period.

Studies conducted under field conditions (BENEDETTI et al., 2005; CARRENHO et al.,

2002; COLLOZZI-FILHO; CARDOSO, 2000) demonstrated that the cultivation of leguminous plants, such as gliricidia, may enhance sporulation and diversity of AMF species, possibly due to the composition of its root exudates. Flavonoids produced by leguminous plants, such as daidzein, genistein and coumestrol, stimulate spore germination and mycorrhizal colonization (ANTUNES et al., 2006; LAROSE et al., 2002). The concentration of flavonoids encountered in leguminous plants is influenced both by genotype and by environmental conditions (HOECK et al., 2000). Therefore, the results of the present study may reflect the effect of study site conditions on the gliricidia trees.

Table 2. Spore number (SD), root colonization (RC), most probable number (MPN) of infective propagules and glomalin-related soil protein (GRSP) in areas under different land use systems, during the rainy and dry seasons, in Taperoá, Paraíba State.

Land use systems ¹	SD (50 g^{-1} of soil)			RC (%)	MPN cm^{-3} of soil	GRSP (mg g^{-1} soil)
	Viable	Non viable	Total			
Rainy season						
MT	39aA	297aA	336aA	54.3cA	180cA	0.84bA
OT	14bA	159cA	173cA	78.6aA	350bA	1.00aA
BT	10bA	233bA	243bA	42.2bA	540aA	0.69cB
M	12aB	163aB	175aB	49.4bA	140aA	0.91bA
O	11aA	150aA	161aA	72.6aA	39cB	1.05aA
B	15aA	158aB	173aB	33.1cA	180aB	0.86bA
CV(%)	25.37	16.16	16.25	14.78	10.15	11.85
Dry season						
MT	20aA	269aA	290aA	61.0bA	45cA	1.13aA
OT	18aA	243aA	261aA	72.1aA	180bA	1.25aA
BT	8bB	197bA	205bA	37.1cA	250aA	0.86bB
M	13aB	173aB	186aB	33.2bB	52aA	1.06aA
O	13aA	185aB	198aB	44.5aB	62aB	1.27aA
B	19aA	177aA	196aA	35.8bA	45aB	1.18aA
CV(%)	30.54	19.06	18.97	15.33	16.25	15.95

¹Land use systems of: (O) = opuntia (*Opuntia ficus-indica*) without trees; (OT) = opuntia with maniçoba (*Manihot glaziovii*) and gliricidia (*Gliricidia sepium*); (M) = Maize (*Zea mays*) and beans (*Phaseolus vulgaris*) without trees; (MT) = Maize and beans with maniçoba and gliricidia; (B) = Buffel grass (*Cenchrus ciliaris*) without trees; (BT) = Buffel grass with maniçoba and gliricidia. Means followed by same letters do not differ by the Scott & Knott test at 5% probability. Small letters compare systems with each other and capital letters compare the presence or absence of trees in the land use systems.

According to Eom et al. (2000), a greater density of AMF spores associated with leguminous plants can also be attributed to reciprocal benefits between the rhizobium and the AMF. The mycorrhizal association and the symbiosis with the rhizobia contribute to increasing the photosynthetic rate and, consequently, the drain of photosynthetic compounds to the microsymbionts (MERGULHÃO et al., 2001). Furthermore, the nutritional benefits from the AMF allow plants to be better supplied with P and with essential nutrients for nodule formation and biological fixation of N_2 . In addition to P, the absorption of Cu, Zn and Mo also favors nodulation and N_2 fixation (PACOVSKY

et al., 1986). In addition, the rhizobium, as well as other microorganisms, are producers of hydrolytic enzymes known as “helpers”, which facilitate the penetration of the fungus into the roots (MOREIRA; SIQUEIRA, 2006).

In the opuntia systems (both under monoculture and under agroforestry), plants demonstrated the highest percentages of mycorrhizal colonization for both sampling periods. Cactus plants are found in arid and semi-arid regions and are therefore subjected to various types of stress, such as high temperatures and low availability of water and nutrients caused by irregular precipitation. They are found in soils where P exists in insoluble forms and is largely unavailable to plants (AZCÓN; BAREA, 1997). These are favorable conditions for the establishment of mycorrhizal symbiosis (SMITH; READ, 1997). For this reason, according to Bashan et al. (2000), cactus plants develop their roots slowly until they are colonized by AMF.

In the present study, there was no significant effect from the presence of gliricidia and maniçoba trees on mycorrhizal colonization during the rainy period. However, in the dry period, an increase to 84 and 62% was observed in mycorrhizal colonization in the maize + beans and in the opuntia systems, respectively, in the presence of gliricidia and maniçoba trees. Due to their deep root systems, the gliricidia trees possibly had greater tolerance for water stress, which promoted greater stability and resistance in the production systems, as they may have been able to take up water and nutrients from deeper layers of the soil (MARIN et al., 2007).

During the rainy period, a greater number of infective AMF propagules were recorded in the buffel grass system, regardless of the presence or absence of trees. This system also demonstrated a larger number of infective AMF propagules during the dry period in the presence of trees. However, in the absence of trees, higher values were measured in the opuntia system. Ganesan and Veeralkshmi (2006) considered buffel grass a good host plant for propagating *Glomus fasciculatum* because it favored the production of infective propagules by this species, possibly due to its rapid growth and abundant root system. In addition, the cultivation of highly mycorrhizal leguminous plants may increase the potential amount of AMF inoculum in the soil (COLLOZZI-FILHO; CARDOSO, 2000).

Higher GRSP levels were measured in the soil of the opuntia system regardless of the presence or absence of gliricidia and maniçoba trees during the rainy period. During the dry period, in the presence

of trees, the maize + beans and the opuntia systems did not differ and showed the highest GRSP levels. However, it was noticed during this period that in the absence of gliricidia and maniçoba trees, the soils in the land use systems did not differ significantly with regard to their GRSP levels.

In general, higher GRSP levels were measured for the opuntia system. In this system, the plants also showed a greater percentage of mycorrhizal colonization, suggesting that greater amounts of photosynthetic compounds were being allocated to the AMF by the plants, and this may have stimulated the production of this protein. We also observed higher levels of soil organic C in the opuntia system (Table 1). Several other studies have also reported positive correlations between the fractions of GRSP and soil organic C content, both for natural and for cultivated soils (BIRD et al., 2002; NICHOLS; WRIGHT, 2005; RILLIG et al., 2003; WRIGHT; UPADHYAYA, 1996).

In the present study, 17 AMF species were identified (Table 3). *Glomus* was represented by eight species, followed by *Acaulospora* (five), *Scutellospora* (one), *Racocetra* (two) and *Entrophosphora* (one). Some studies (GAI et al., 2006; LI et al., 2007; SHI et al., 2007; TAO; ZHIWEI, 2005) also demonstrated that the *Acaulospora* and *Glomus* species are dominant in semi-arid areas.

Although these genera both contain the largest numbers of cataloged species, it is also possible that they are more flexible with regard to their response to environmental variables, adjusting their sporulation standards to the environmental conditions and tolerating or avoiding unfavorable conditions in the semi-arid region (PICONE, 2000). The AMF species have different tolerances and behave distinctively according to environmental conditions (KLIRONOMOS et al., 1993).

In the land use systems without gliricidia and maniçoba trees, a total of 15 AMF species were detected. From these, *G. tortuosum*, *S. cerradensis*, *R. fulgida*, *R. novaum*, *A. excavata* and *A. morrowiaewere* were found exclusively in treeless systems, whereas *Acaulospora longula* and *G. clavispurum* were detected only in the land use systems with trees present, where a total of 11 AMF species were observed. Changes in plant composition may cause AMF species to lose their hosts, creating difficulties for sporulation (MUNYANZIZA et al., 1997). The species *R. novaum* and *A. foveoreticulata* were not included on the species list at the Blaszkowski site.

Table 3. AMF species in areas under different land use systems during the rainy and dry seasons in Taperoá, Paraíba State.

AMF species ² Season ³	Land use systems ¹														*RF(%)	
	O		OT		M		MT		B		BT					
	R	D	R	D	R	D	R	D	R	D	R	D	R	D		
<i>Acaulospora foveoreticulata</i>	X	X			X		X	X				X			50	50
<i>Acaulospora excavata</i>												X			-	16.7
<i>Acaulospora longula</i>			X				X								33.3	-
<i>Acaulospora scrobiculata</i>			X		X							X			33.3	16.7
<i>Acaulospora morrowiae</i>							X								-	16.7
<i>Entrophospora infrequens</i>			X				X		X	X	X				66.7	-
<i>Glomus ambisporum</i>	X				X				X	X					50	16.7
<i>Glomus clavisorum</i>												X			16.7	-
<i>Glomus claroideum</i>	X		X		X		X								-	66.7
<i>Glomus etunicatum</i>	X	X	X	X			X	X	X	X	X	X	X	X	83.3	83.3
<i>Glomus intraradices</i>	X	X	X	X	X		X		X	X	X	X	X	X	50	66.7
<i>Glomus macrocarpum</i>	X	X	X	X	X		X	X	X	X	X	X	X	X	100	66.7
<i>Glomus mosseae</i>	X		X		X	X	X	X	X	X		X			66.7	50
<i>Glomus tortuosum</i>									X						16.7	-
<i>Racocetra novaum</i>		X													-	16.7
<i>Racocetra fulgida</i>	X														16.7	-
<i>Scutellospora cerradensis</i>									X						16.7	-
Total of species	5	6	6	4	5	4	6	6	7	4	7	4	4	-	-	-

¹Land use systems of: (O) = opuntia (*Opuntia ficus-indica*) without trees; (OT) = opuntia with maniçoba (*Manihot glaziovii*) and gliricidia (*Gliricidia sepium*); (M) = Maize (*Zea mays*) and beans (*Phaseolus vulgaris*) without trees; (MT) = Maize and beans with maniçoba and gliricidia; (B) = Buffel grass (*Cenchrus ciliaris*) without trees; (BT) = Buffel grass with maniçoba and gliricidia. ²Spore identification based on spores extracted from soil in trap cultures. ³R = Rainy; D = Dry. *RF = Relative frequency.

Twelve AMF species were identified in the opuntia as well as in the buffel grass systems, whereas 11 species were identified in the maize + beans system. *Glomus clavisorum*, *G. tortuosum*, *S. cerradensis* and *A. excavata* were observed only in the buffel grass system and *R. fulgida* and *R. novaum* only in the opuntia system. *Acaulospora morrowiae* was identified only in the maize + beans system.

Glomus macrocarpum and *G. etunicatum* showed a greater index of occurrence during the rainy and dry periods, respectively, which could be attributed to their greater resistance to soil stresses that persist longer in the soil. Furthermore, according to Caproni et al. (2003), *G. macrocarpum* demonstrates high infective potential in the soil and can therefore constantly reinfect roots. Over time, if other species do not establish themselves, this species can become predominant (CARRENHO et al., 2001).

According to Carrenho et al. (2001), rare fungal species, or species of low frequency, can be present in the environment in other forms (auxiliary cells, hyphae, colonized roots) or may simply appear as remnants of a pre-existing community of plants with short life cycles or plants that may have been produced in close proximity to the area and then dispersed, without having had much success in occupying the new environment.

Seasonal variations in the frequency of AMF species do not necessarily reflect their elimination from the environment (PURIN et al., 2006). Annual hosts, such as leguminous plants and grasses that can be present in one season and absent in

another hinder, or at least do not facilitate, the production of spores by some species and, consequently, their detection in the soil.

Conclusion

In general, greater AMF species richness was observed in the traditional land use systems, i.e., those without trees present. However, the establishment of agroforestry systems, by intercropping gliricidia and maniçoba trees with grain or forage crops, increased sporulation, mycorrhizal colonization and the number of AMF infective propagules in the area of the present study. The production of glomalin by AMF was greater in areas cultivated with opuntia, regardless of the presence or absence of trees or of the sampling season.

Acknowledgements

The authors thank the financial support provided by CNPq - Edital MCT/CNPq 15/2007 (Processo 478138/2007-5), Edital MCT/CNPq 01/2005 (Projeto Imsear- Processo no.: 420294/2005-8) e Edital MCT/CNPq/CT-Agro 43/2008 (Processo 574893/2008-3). The authors also thank the support of the Inter American Institute for Global Change Research (IAI) - Project Amfoods (CRN2-014) and from Facepe - Edital PPP 2006 (Processo APQ-0633-5.01/06).

References

- AESA-Agência Executiva de Gestão das Águas do Estado da Paraíba. 2008. Available from: <www.http://site2.aesa.pb.gov.br/aesa/monitoramentoPluviometria.do?metodo=listarMesesChuvadasMensais>. Access on: Nov. 25, 2008.
- ANTUNES, P. M.; RAJCAN, I.; GOSS, M. J. Specific flavonoids as interconnecting signals in the tripartite symbiosis formed by arbuscular mycorrhizal fungi, *Bradyrhizobium japonicum* (Kirchner) Jordan and soybean (*Glycine max* (L.) Merr.). **Soil Biology and Biochemistry**, v. 38, n. 3, p. 533-543, 2006.
- AZCÓN, R.; BAREA, J. M. Mycorrhizal dependency of a representative plant species in Mediterranean shrublands (*Lavandula spica* L.) as a key factor to its use for revegetation strategies in desertification-threatened areas. **Applied Soil Ecology**, v. 7, n. 1, p. 83-92, 1997.
- BASHAN, Y.; DAVIS, E. A.; CARRILLO-GARCIA, A.; LIDERMAN, R. G. Assessment of mycorrhizal inoculum potential in relation to the establishment of cactus seedlings under mesquite nurse-trees in the Sonoran Desert. **Applied Soil Ecology**, v. 14, n. 2, p. 165-175, 2000.
- BENEDETTI, T.; ANTONIOLLI, Z. I.; GIRACCA, E. M. N.; STEFFEN, R. B. Diversidade de fungos micorrízicos na cultura do milho após uso de espécies de

- plantas de cobertura de solo. **Revista de Ciências Agroveterinárias**, v. 4, n. 1, p. 44-51, 2005.
- BIRD, S. B.; HERRICK, J. E.; WANDER, M. M.; WRIGHT, S. F. Spatial heterogeneity of aggregate stability and soil carbon in semi-arid rangeland. **Environmental Pollution**, v. 116, n. 3, p. 445-455, 2002.
- CAPRONI, A. L.; FRANCO, A. A.; BERBARA, R. L. L.; TRUFEM, S. B.; GRANHA, J. R. D. O.; MONTEIRO, A. B. Ocorrência de fungos micorrízicos arbusculares em áreas revegetadas após mineração de bauxita em Porto Trombetas, Pará. **Pesquisa Agropecuária Brasileira**, v. 38, n. 12, p. 1409-1418, 2003.
- CARRENHO, R.; TRUFEM, S. F. B.; BONONI, V. L. R. Fungos micorrízicos arbusculares em rizosferas de três espécies de fitobiontes instaladas em área de mata ciliar revegetada. **Acta Botanica Brasílica**, v. 15, n. 1, p. 115-124, 2001.
- CARRENHO, R.; TRUFEM, S. F. B.; BONONI, V. L. R. Effects of using host plants on the detected biodiversity of arbuscular mycorrhizal fungi from an agroecosystem. **Revista Brasileira de Botânica**, v. 25, n. 1, p. 93-101, 2002.
- COLLOZZI-FILHO, A.; CARDOSO, J. B. N. Detecção de fungos micorrízicos arbusculares em raízes de cafeeiro e de crotalária cultivada na entrelinha. **Pesquisa Agropecuária Brasileira**, v. 35, n. 10, p. 2033-2042, 2000.
- EMBRAPA-Empresa Brasileira de Pesquisa Agropecuária. **Manual de análises químicas de solos, plantas e fertilizantes/Embrapa Solos, Embrapa Informática agropecuária**: organizador Fábio César da Silva. Brasília: Embrapa Comunicação para Transferência de Tecnologia de Tecnologia, 1999.
- EOM, A. H.; HARTNETT, D. C.; WILSON, G. W. T. Host plant species on arbuscular mycorrhizal fungal communities in tallgrass prairie. **Oecologia**, v. 122, n. 3, p. 435-444, 2000.
- FELDMANN, F.; IDCZAK, E. Inoculum production of vesicular-arbuscular mycorrhizal fungi for use in tropical nurseries. In: NORRIS, J. R.; READ, D. J.; VARMA, A. K. (Ed.). **Techniques for mycorrhizal research methods in microbiology**. London: Academic Press, 1992, p. 799-817.
- GAI, J. P.; FENG, G.; CAI, X. B.; CHRISTIE, P.; LI, X. L. A preliminary survey of the arbuscular mycorrhizal status of grassland plants in southern Tibet. **Mycorrhiza**, v. 16, n. 3, p. 191-196, 2006.
- GANESAN, V.; VEERALAKSHMI, M. Assessment of suitable host for the mass multiplication of arbuscular mycorrhiza *Glomus fasciculatum*. In: JAYABALAN, N. (Ed.). **Plant Biotechnology**. New Delhi: Kul Bhushan Nangia APH Publishing Corporation, 2006. p. 3003-3016.
- GERDEMANN, J. W.; NICOLSON, T. H. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. **Transactions of the British Mycological Society**, v. 46, n. 2, p. 235-244, 1963.
- GIOVANNETTI, M.; MOSSE, B. An evaluation of techniques to measure vesicular-arbuscular mycorrhizal infection in roots. **New Phytologist**, v. 84, n. 3, p. 484-500, 1980.
- HEIJDEN, M. G. A. V. D.; WIEMKEN, A.; SANDERS, I. R. Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. **New Phytologist**, v. 157, n. 3, p. 569-578, 2003.
- HOECK, J. A.; FEHR, W. R.; MURPHY, P. A.; WELKE, G. A. Influence of genotype and environment on isoflavone contents of soybean. **Crop Science**, v. 40, n. 1, p. 48-51, 2000.
- JENKINS, W. R. A. A rapid centrifugal-flotation technique for separating nematodes from soil. **Plant Disease Report**, v. 48, p. 692, 1964.
- KLIRONOMOS, J. N.; MOUTOGOLIS, P.; KENDRICK, B.; WIDDEN, P. A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils. **Canadian Journal of Botany**, v. 71, n. 11, p. 1472-1480, 1993.
- KOSKE, R. E.; GEMMA, J. N. A Modified procedure for staining roots to detect mycorrhizas. **Mycological Research**, v. 92, n. 4, p. 486-488, 1989.
- LAROSE, G.; CHÊNEVERT, R.; MOUTOGLIS, P.; GAGNE, S.; PICHE, Y.; VIERHEILIG, H. Flavonoid levels in roots of *Medicago sativa* are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus. **Journal of Plant Physiology**, v. 159, n. 12, p. 1329-1339, 2002.
- LI, L. F.; LI, T.; ZHAO, Z. W. Differences of arbuscular mycorrhizal fungal diversity and community between a cultivated land, and old field, and a never-cultivated field in a hot and arid ecosystem of southwest China. **Mycorrhiza**, v. 17, n. 8, p. 655-665, 2007.
- LIMA, M.; SIDERSKY, P. O papel das plantas nativas nos sistemas agrícolas familiares no Agreste da Paraíba. In: SILVEIRA, L. M.; PETERSEN, P.; SABOURIN, E. (Org.). **Agricultura familiar e agroecologia no semi-árido**: Avanços a partir do Agreste da Paraíba. Rio de Janeiro: AS-PTA, 2002. p. 201-218.
- LIMA, R. L. F. A.; SALCEDO, I. H.; FRAGA, V. S. Propágulos de fungos micorrízicos arbusculares em solos deficientes e, fósforo sob diferentes usos, da região semi-árida no nordeste do Brasil. **Revista Brasileira de Ciência do Solo**, v. 31, n. 2, p. 257-268, 2007.
- MARIN, A. M. P.; MENEZES, R. S. C.; SALCEDO, I. H. Produtividade de milho solteiro ou em aléias de gliricídia adubadas com duas fontes orgânicas. **Pesquisa Agropecuária Brasileira**, v. 42, n. 5, p. 669-677, 2007.
- McGEE, P. A. Variation in propagule numbers of vesicular-arbuscular mycorrhizal fungi in a semi-arid soil. **New Phytopathology**, v. 92, n. 1, p. 28-33, 1989.
- MERGULHÃO, A. C. E. S.; SILVA, M. L. R.; BURITY, H. A.; STAMFORD, N. P. Influência da dupla inoculação rizóbio e fungos micorrízicos arbusculares em plantas de sabiá sob solos de diferentes texturas. **Revista Ecosystemas**, v. 26, n. 1, p. 42-47, 2001.
- MOREIRA, F. M. S.; SIQUEIRA, J. O. **Microbiologia e bioquímica de solo**. Lavras: UFLA, 2006.
- MORTON, J. B.; BENTIVENGA, S. P.; WHEELER, W. W. Germplasm in the international collection of arbuscular and vesicular-arbuscular mycorrhizal fungi (INVAM) and

- procedures for culture development, documentation, and storage. **Mycotaxon**, v. 48, n.1, p. 491-528, 1993.
- MUNYANZIZA, R.; KEHRI, H.; BAGYARAJ, D. J. Agricultural intensification, soil biodiversity and agro-ecosystem function in the tropics: the role of mycorrhiza in crops and tree. **Applied Soil Ecology**, v. 6, n. 1, p. 77-85, 1997.
- NICHOLS, K. A.; WRIGHT, S. F. Comparison of glomalin and humic acid in eight native US soils. **Soil Science**, v. 170, n. 12, p. 985-997, 2005.
- PACOVSKY, R. S.; PAUL, E. A.; BETHLENTALVAY, G. J. Response of mycorrhizal and P-fertilized soybeans to nodulation by *Bradyrhizobium* or ammonium nitrate. **Crop Science**, v. 26, n. 1, p. 145-150, 1986.
- PICONE, C. Diversity and abundance of arbuscular-mycorrhizal fungus spores in tropical forest and pasture. **Biotropica**, v. 32, n. 4, p. 734-750, 2000.
- PURIN, S.; FILHO KLAUBERG, O.; STÜRMER, S. L. Mycorrhizae activity and diversity in conventional and organic apple orchards from Brazil. **Soil Biology and Biochemistry**, v. 38, n. 7, p. 1831-1839, 2006.
- RAO, A. V.; TARAFDAR, J. C. Significance of micro-organisms in afforestation programmes in arid zone. **Annals of Arid Zone**, v. 37, n. 4, p. 337-346, 1998.
- RILLIG, M.; RAMSEY, P. W.; MORRIS, S.; PAUL, E. A. Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to soil-use change. **Plant and Soil**, v. 253, n. 2, p. 293-299, 2003.
- SABOURIN, E.; SILVEIRA, L. M.; TONNEAU, J. P.; SIDERSKY, P. **Fertilidade e agricultura familiar**: um estudo sobre o manejo da biomassa. Esperança: AS-PTA/Cirad, 2000.
- SAMPAIO, E. V. S. B.; MENEZES, R. S. C. Sustainable soil use in tropical South America - with emphasis on Brazil. In: TIESSSEN, H. (Org.). **Capacity of soils for sustaining production**. A global overview, in Encyclopedia of Life Support Systems. Oxford: Unesco-Eolss, 2003. p. 1-11.
- SHI, Z. Y.; ZHANG, L. Y.; LI, X. L.; FENG, G.; TIAN, C. Y.; CHRISTIE, P. Diversity of arbuscular mycorrhizal fungi associated with desert ephemerals in plant communities of Junggar Basin, northwest China. **Applied Soil Ecology**, v. 35, n. 1, p. 10-20, 2007.
- SMITH, S. E.; READ, D. J. **Mycorrhizal symbiosis**. London: Academic Press, 1997.
- TAO, L.; ZHIWEI, Z. Arbuscular mycorrhizas in hot and arid ecosystem in southwest China. **Applied Soil Ecology**, v. 29, n. 2, p. 135-141, 2005.
- WALLEY, F. L.; GERMIDA, J. J. Estimating the viability of vesicular-arbuscular mycorrhizae fungal spores using tetrazolium salts as vital stains. **Mycologia**, v. 87, n. 2, p. 273-279, 1995.
- WRIGHT, S. F.; UPADHYAYA, A. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. **Soil Science**, v. 161, n. 9, p. 575-586, 1996.
- WRIGHT, S. F.; UPADHYAYA, A. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. **Plant and Soil**, v. 198, n. 1, p. 97-107, 1998.

Received on March 1, 2012.

Accepted on June 21, 2012.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited