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Arbuscular mycorrhizal communities in different tropical pastures of the brazilian northeast

Comunidades micorrízicas arbusculares em diferentes pastagens tropicais do nordeste brasileiro

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Abstract

The composition of the arbuscular mycorrhizal fungi (AMF) community was evaluated after the native vegetation was replaced by pasture. The systems: *Andropogon gayanus*, *Brachiaria brizantha*, *Panicum Maximum* and *Cynodon dactylon* and an agrosilvipastoril system (AFS) were studied; and a fragment of native forest. Soil samples were collected at depth of 20 cm. Eight families, 11 genera and 19 species of AMF were characterized. Glomeromycota species predominated in most pasture systems for both seasons, indicating the adaptation of these fungi.

Keywords: grassland, ecological indices, seasonality

Resumo

A composição da comunidade de fungos micorrízicos arbusculares (FMA) foi avaliada após a substituição da vegetação nativa por pastagem. Foram estudados os sistemas Andropogon gayanus, Brachiaria brizantha, Panicum Maximum e Cynodon dactylon e um sistema agrosilvipastoril (AFS); e um fragmento de mata nativa. Amostras de solo foram coletadas na profundidade de 20 cm. Oito famílias, 11 gêneros e 19 espécies de FMA foram caracterizados. Espécies de Glomeromycota predominaram na maioria dos sistemas de pastagens em ambas as estações, indicando a adaptação destes fungos.

Palavras-chave: pastagem, índices ecológicos, sazonalidade

Introduction

The soil microorganisms play a fundamental role in the maintenance of ecosystems. Among these microorganisms are arbuscular mycorrhizal fungi (AMF) that play a key role in the function of terrestrial ecosystems (OEHL et al., 2010; van DER HEIJDEN et al., 2011), are also essential in improving the soil quality (CARDOSO et al., 2013) and to establish physiological and ecological performance of plants (ZHANG et al., 2017).

For the past five decades, the intensive use of soil for pasture establishment has increased because of the need to promote higher animal production, particularly in soils with low fertility, such as tropical soil in the northeast of Brazil. Thus, the intensive management of soil can promote loss of soil biodiversity as well as select adapted species (SILVA et al., 2015), mainly by increasing the use of chemical fertilizers to maintain the nutritional quality of plants, bringing a strong dependency on these fertilizers.

Currently, more of 285 species of AMF have been identified (JANSA et al., 2014; http://glomeromycota.wix.com), with 119 species recorded only in Brazilian ecosystems (SOUZA et al., 2008; SOUZA et al., 2010). Studies on AMF biogeography, ecology, taxonomy, and molecular biology have provided information about the adaptation of these fungi to different environmental and soil conditions (ALGUACIL et al., 2015). Some studies have compared AMF diversity in disturbed and undisturbed areas (MERGULHÃO et al., 2010; PEREIRA et al., 2014). Furthermore, information about native AMF communities can be useful for evaluating the soil quality, particularly for crops and pastures. Studies on the dynamics of AMF in tropical pasture systems will indicate by means of the AMF species or its attributes more sustainable pasture systems, particularly where the native vegetation has been replaced with crops or pastures (CARUSO et al., 2012; REDECKER et al., 2013).

According SILVA et al. (2014) there are evidences of co-variation of plant and AMF communities composition on environmental gradients. Moreover, this phenomenon is not completely understood. Based on that, we formulate the hypothesis of this study is that the dynamics of the AMF community are influenced by different pasture systems; thus, they can be an indicator of sustainable systems. In this context, the objectives of this study were to determine the diversity and community structure of AMF and to assess aspects such as frequency, the Shannon index, dominance, equitability and abundance of AMF species, as well as the glomerospores density, root colonization and the most probable number of infective propagules.

Materials and Methods

A long-term experiment was conducted using pastures belonging to the Animal Science Department, Agriculture Science Center, Federal University of Piauí, Brazil (latitude 05°05′21″S and longitude 42°48′07″W; 74 m above sea level). The climate is dry and tropical, with a mean precipitation of 1,300 mm yr^{-1}. The soil type is Orthic Acrisol.

The experimental area was divided into approximately 0.5-ha sized plots each based on a different pasture system: 1) *Andropogon gayanus* Kunth [AND; plot without both liming and chemical fertilization; production of 2.1 tons ha^{-1} of dry weight; 2.21% of soil N; and a C:N ratio of

2:1]. 2) *Brachiaria brizantha* Stapf [BRA; plot annually fertilized with 120, 180, and 100 kg ha^{-1} urea, super triple phosphate, and potassium chloride, respectively; production of 4.35 tons ha^{-1} of dry weight; 0.91% of soil N; and a soil C:N ratio of 3:7]. 3) *Panicum maximum* Jacq. [PAN; plot annually fertilized with 70, 80, and 50 kg ha^{-1} urea, super triple phosphate, and potassium chloride, respectively; production of 3.0 tons ha^{-1}dry weight; 1.22% of soil N; and a soil C:N ratio of 3:1]. 4) *Cynodon dactylon* (L.) Pers. [CYN; plot annually fertilized with 75, 30, and 30 kg ha^{-1} urea, super triple phosphate, and potassium chloride, respectively; production of 1.3 tons $ha^{-1}$of dry weight; 1.37% of soil N; and a soil C:N ratio of 36:9]. 5) Agroforestry system [AFS; plot composed of grass (*A. gayanus* Kunth) and trees (*Mimosa* sp. and *Thiloa glaucocarpa* Benth); and production of 7.4 tons $ha^{-1}$of plant litter of dry weight]. 6) Native vegetation [NV; plot composed of native plant species, including *Cenostigma macrophyllum* Tul., *Tabebuia serratifolia* Vahl, *Hymenaea courbaril* L., *Orbignya phalerata* Mart., *Combretum leprosum* Mart., *Guarea kunthiana* A. Juss., and *Lecythis pisonis* Cambess; and production of 9.5 tons $ha^{-1}$of dry weight of plant litter].

Soil sampling was conducted in March (wet season) and September (dry season) 2017. Soil samples were obtained from 10 independent points along a set of parallel lines in each plot at a depth of 0–20 cm (DICK et al., 1996). Soil samples were air dried and passed through a 0.21-mm sieve to determine soil organic carbon using a wet combustion method with a heated mixture of potassium dichromate and sulfuric acid (YEOMANS; BREMMER, 1998).

Soil chemical analyses (Table 1) were performed at the Soil Quality Laboratory at the Federal University of Piauí. Soil pH was determined in a 1:2.5 soil:water extract. Exchangeable Al, Ca, and Mg were determined by extraction with 1 M KCl. Available P was extracted using the Mehlich-1 method and determined by colorimetry (TEDESCO et al., 1995).

Glomerospores were extracted from soil samples according to the methods of GERDEMANN and NICOLSON (1963). They were identified using the Manual of Schenck and Pérez 1988; websites http://invam.wvu.edu/ and www.zor.zut.edu.pl/Glomeromycota/. The classification system was proposed by OEHL et al. (2011), GOTO et al. (2012), BŁASZKOWSKI (2012), BŁASZKOWSKI and CHWAT (2013), and SIEVERDING et al. (2014). The glomerospores were counted on a plate in a stereomicroscope (40×), mounted on slides with PVLG (polyvinyl-alcohol in lactoglycerol) and PVLG+Melzer's reagent (1:1 v/v) and observed under a microscope for taxonomic study and species identification.

Table 1. Chemical properties of soil under different pastures: AND - *Andropogon gayanus* Kunth *;* BRA - *Brachiaria brizantha* Stapf*;* PAN - *Panicum maximum* Jacq.*;* CYN - *Cynodon dactilon* (L.) Pers.*;* AFS – Agroforestry system; NV - native vegetation.

	Wet						Dry					
	AND	BRA	PAN	CYN	AFS	NV	AND	BRA	PAN	CYN	AFS	NV
Ca ($cmol_c\ kg^{-1}$)	1.55	3.29	2.38	1.98	2.91	1.45	2.05	2.64	1.52	2.03	2.96	1.25
Mg ($cmol_c\ kg^{-1}$)	0.63	1.01	1.31	1.34	1.02	0.59	0.68	0.90	0.95	0.65	0.55	0.52
H+Al ($cmol_c\ kg^{-1}$)	2.88	1.37	0.29	0.23	1.65	3.15	2.84	1.78	0.50	0.41	1.92	2.56
Al ($cmol_c\ kg^{-1}$)	0.05	0.01	0.06	0.06	0.12	0.11	0.25	0.12	0.01	0.01	0.01	0.17
pH	5.68	7.34	7.80	7.82	6.16	5.92	5.76	7.34	8.04	7.80	5.47	5.40
P ($mg\ kg^{-1}$)	14.4	26.8	17.7	8.7	4.8	13.2	12.7	29.7	7.5	8.8	3.5	15.0

The most probable number of AMF was determined according to the method of FELDMANN and IDCZAK (1994), using dilutions of 1:10, 1:100, and 1:1000. *Panicum miliaceum* L was cultivated for one month in 100-ml plastic pots. The plants were harvested and their roots were stained and evaluated according to the methods of GIOVANNETTI and MOSSE (1980).

The extraction and quantification of proteins related to soil glomalin (GRSP) and its fractions, extractable (EE) GRSP and total (TG) GRSP, were performed according to the procedure of WRIGHT and UPADHYAYA (1998). The mean concentrations C content of glycoprotein extracted from soil (glomalin) were calculate based on NICHOLS and WRIGHT (2005).

The Shannon index ($H' = \Sigma p_i \ln p_i$, where p_i = glomerospore number of each species/total glomerospores), Simpson dominance (to the equation $C = \Sigma(n_i(n_i - 1)/N(N - 1)$ where n_i = the abundance of species i and N = total abundance), Pielou equitability (where: $R = H'/\log S$, where H' = value obtained using Shannon and S = total number of AMF species present in the sample), specific richness (the number of species observed), and frequency of occurrence (to the formula: $F_i = J_i/k$, where F_i = occurrence frequency of species i, J_i = number of samples in which species i occurred and k = total number of soil samples) were calculated according to the procedure of BROWER and ZAR (1984). The species were classified according to the procedure of STÜRMER and SIQUEIRA (2011), as dominant (FO > 50%), very common (31% < FO < 50%), common (10% < FO < 30%), rare (FO < 10%), general (present in all six areas), intermediate (present in two to five areas), and exclusive (present in all areas).

The Kolmogorov–Smirnov and Shapiro–Wilk test were performed to assess the normality and homogeneity of variance in Glomerospore count data. Then, data were transformed (log x + 1) according to STÜRMER et al. (2013) and analysis of variance was performed for AMF. The differences between means were compared using the Tukey test ($p < 0.05$). The ecological diversity indexes and their statistical comparisons were calculated using the program PAST 1.99. The statistical analyses were conducted using the software IBM SPSS 21. Statistical differences were evaluated using the multi-response permutation procedure (MRPP) on the basis of the Sorensen distance. Similar groups of system were clustered on the basis of NMS ordination ($p < 0.05$).

Results

Most of the identified species belonged to the genera *Acaulospora*, *Glomus*, and *Fuscutata* with six, three, and three species, respectively; there were two species belonging to each of the genera *Racocetra* and *Scutellospora* (Table 2). No species were classified as general because none appeared simultaneously in the six evaluated areas. Fifteen species were classified as intermediate and ten as exclusive.

Table 2. Frequence of occurrence and Species richness of AMF under different pastures: AND - *Andropogon gayanus* Kunth*;* BRA - *Brachiaria brizantha* Stapf*;* PAN - *Panicum maximum* Jacq.*;* CYN - *Cynodon dactilon* (L.) Pers.*;* AFS – Agroforestry system; NV - native vegetation. *D=dominant (FO > 50%); VC= very common (31% < FO < 50%); C=common (10% < FO < 30%); R= rare (FO < 10%)

AMF	**Wet**						**Dry**					
family/species	AND	BRA	PAN	CYN	AFS	NV	AND	BRA	PAN	CYN	AFS	NV
Acaulosporaceae												
A. excavata Ingleby & Walker 1994		C		C							VC	
A. foveata Trappe & Janos 1982	C	C				VC						
A. herrerae Furrazola, Goto, Silva, Sieverd & Oehl 2013	R		R		C	R					R	
A. morrowiae Spain & Schenck 1984			R		D	VC					C	D
A. rehmii Sieverd & Toro 1987			R			C						
A. spinosa C. Walker & Trappe 1981	C	C				C				C		
Ambisporaceae												
A. appendicula (Spain, Sieverd & Schenck) C. Walker 2008				C	R							
Dentiscutataceae												
D. cerradensis Sieverd, Souza & Oehl 2009									C			
Fuscutata												
F. aurea Oehl, Mello & Silva 2012				C								
F. rubra (Stürmer & Morton)	*C*			*C*			*C*		*R*			
F. heterogama Oehl, Souza, Maia & Sieverd. 2009					C							
Entrophosporaceae												
E. baltica Błaszk, Madej & Tadych 1998				R								
Gigasporaceae												
G. decipiens Hall &								C			R	

Abbott 1984												
Glomeraceae												
G. halonatum Rose & Trappe 1980			C				C		R	R		
G. microaggregatum Koske, Gemma & Olexia 1986											C	
G. rubiforme (Gerd & Trappe) Almeida & Schenck 1990											C	
S. titan Goto & Silva 2013										C		
Racocetraceae												
R. tropicana Oehl, Goto & Silva 2011	R	C	R					VC	C			
Scutellosporaceae												
S. pernambucana Oehl, Silva, Freitas & Maia 2009			C								C	
Species richness	*5*	*4*	6	5	4	5	2	2	4	3	7	1

We observed seasonality in the AMF community composition whereby some species were identified only during the wet season (*Acaulospora foveata*, *A. rehmii*, *Ambispora appendiculata*, *Fuscutata aurea*, *Entrophospora baltica*), and other species were identified only in the dry season (*Dentiscutata cerradensis*, *Gigaspora decipiens*, *G. microaggregatum*, *G. rubiforme*, *Septoglomus titan*). In addition, *Acaulospora herrerae* and *A. morrowiae* were found only in the dry season in AFS and NV systems. This occurrence of these AMF species in AFS and NV may be explained because the highest plant diversity in these systems (LOU et al., 2011).

The Shannon index was sensitive to studied systems and separated areas according to season (**Table 3**). The index was high in NV (wet) and AFS (dry) systems and the lowest in AND (wet) and BRA (dry) systems. BRA, PAN, and CYN systems had fewer glomerospores in the dry season.

Table 3. Shannon Index (H'), Simpson dominance (D'), and Pielou equitability (J') of arbuscular mycorrhizal fungi under different pastures: AND - *Andropogon gayanus* Kunth *;* BRA - *Brachiaria brizantha* Stapf*;* PAN - *Panicum maximum* Jacq.*;* CYN - *Cynodon dactilon* (L.) Pers.*;* AFS – Agroforestry system; NV - native vegetation.

Sites	**Wet**			**Dry**		
	Shannon Index (H')	**Simpson Dominance (D')**	**Pielou Equitability (J')**	**Shannon Index (H')**	**Simpson Dominance (D')**	**Pielou Equitability (J')**
AND	1.03^{c}	0.21^{a}	0.81^{a}	0.61^{c}	0.43^{a}	0.81^{a}
BRA	1.62^{a}	0.14^{b}	0.90^{a}	0.23^{d}	0.81^{a}	0.34^{b}
PAN	1.65^{a}	0.35^{a}	0.63^{b}	1.12^{b}	0.64^{a}	0.49^{b}
CYN	1.69^{a}	0.26^{a}	0.82^{a}	0.98^{b}	0.35^{a}	0.84^{a}
AFS	1.41^{b}	0.24^{a}	0.84^{a}	1.85^{a}	0.12^{b}	0.91^{a}
NV	1.73^{a}	0.11^{b}	0.87^{a}	0.53^{c}	0.65^{a}	0.78^{a}

The differences between means were compared using the Tukey test ($p < 0.05$)

The largest quantity of TG-GRSP occurred in NV (dry and wet) and AFS (wet) systems, whereas the smallest quantity of EE-GRSP occurred in AND and PAN (dry) systems. The highest percentage of root colonization occurred in the BRA system in both seasons. In dry and wet seasons, there were no statistical differences between pasture systems for NMP (**Table 4**).

Table 4. Glomerospores (GV, nº $100mL^{-1}$ soil), root colonization (RC, %), extractable (EE-GRSP, $mg.g^{-1}$ soil) and total glomalin (TG-GRSP, $mg.g^{-1}$ soil), glomalin-C (GLOM-C, $mg.g^{-1}$ soil), and most problable number (MPN, $propagules.cm^{-3}$) of AMF under different pastures: AND - *Andropogon gayanus* Kunth*;* BRA - *Brachiaria brizantha* Stapf*;* PAN - *Panicum maximum* Jacq.*;* CYN - *Cynodon dactilon* (L.) Pers.*;* AFS – Agroforestry system; NV - native vegetation.

	Wet						**Dry**					
	AND	**BRA**	**PAN**	**CYN**	**AFS**	**NV**	**AND**	**BRA**	**PAN**	**CYN**	**AFS**	**NV**
VG	55^{e}	144^{b}	248^{a}	121^{b}	95^{c}	56^{d}	175^{a}	39^{d}	68^{c}	109^{b}	87^{c}	61^{c}
RC	29^{e}	72^{a}	56^{c}	69^{a}	61^{b}	40^{d}	39^{c}	64^{a}	62^{a}	56^{b}	52^{b}	24^{d}
EE-GRSP	0.7^{b}	1.0^{a}	0.9^{a}	0.9^{a}	1.6^{a}	1.1^{a}	0.8^{b}	1.0^{a}	0.8^{b}	1.1^{a}	1.0^{a}	1.2^{a}
TG-GRSP	3.6^{c}	4.0^{b}	4.0^{b}	4.1^{b}	4.5^{a}	4.6^{a}	3.0c	4.1^{b}	4.2^{b}	4.1^{b}	4.2^{b}	5.3^{a}
GLOM-C	18^{c}	21^{b}	20^{b}	22^{a}	15^{d}	23^{a}	14^{c}	18^{b}	22^{a}	19^{b}	15^{c}	21a
MPN	52^{a}	44^{a}	48^{a}	56^{a}	49^{a}	58^{a}	24^{a}	41^{a}	22^{a}	21^{a}	36^{a}	32^{a}

The differences between means were compared using the Tukey test ($p < 0.05$)

Discussion

AFS and CYN systems had more exclusive species, followed by PAN and BRA systems. The variability in the frequency and occurrence of AMF species provides some indication that they

change in response to soil management change. *Gigaspora decipiens* was found only in BRA and AFS systems during the dry season. Usually, the order Gigasporaceae is more dominant in sandy soil with a pH > 6.0 and low organic matter (STÜRMER et al., 2013). This was partially confirmed in our study because the BRA system had a pH > 6.0. Also, the presence of *Gigaspora* sp. indicates reduced negative effects on AMF since Gigasporaceae are a good indicator of soil disrturbance (OEHL et al., 2010; GUADARRAMA et al., 2014).

Maintenance of the same plant species continually promotes the establishment of preferential associations between some species of AMF and plants; thus, reducing the diversity of AMF (MIRANDA et al., 2005). The conversion of forest to cultivated areas influences not only the soil chemical properties but also the composition of the AMF community (WAKELIN et al., 2008; PEREIRA et al., 2014).

According to JEFWA et al. (2012), species belonging to the genera *Glomus* and *Acaulospora* are common in natural or managed environment, and it is attributed to their specific characteristic related with propagation and adaptability. Both species have been identified in our pasture systems at two seasons. Acaulosporaceae develops easily in soils with low pH and were overrepresented in the tropics (STÜRMER et al., 2006; STÜRMER et al., 2018) and, therefore, we found species from this family in the AND and NV as these areas had the lowest soil pH.

Some AMF characteristics, such as size, metabolic activity, and maturity level, might affect glomerospores in the soil (LI et al., 2007). Reduced carbohydrate translocation toward the root may explain the decreased activity of AMF during the dry season and the consequent reduction in the glomerospore density (NISHA et al., 2010).

On the other hand, the increase of environmental stress may lead to an increment of sporulation. This is a natural mechanism of the species to assure its perpetuation. The raise of environmental stress occurs mainly during the dry season, when the vegetation growth is limited.

In pasture systems, the soil structuration and cover is lower than forest system and these expose the soil to high temperatures and nutrient loss, which can contribute to a decrease in glomalin (LYNCH; HO, 2005; OEHL et al*.,* 2010). The intense soil handling influences the GRPS concentration (SOUSA et al., 2014). The mechanical operations for cutting trees, planting grass and applying fertilizers are frequent in the pasture systems under monoculture. These practices reduce the aggregation of soil and increase the loss of nutrients and probably contributed to the decrease in TG-GRPS (OEHL et al., 2010).

The greater values of Shannon indices were observed in the TAM area for the ecological parameters studied here. Moreover, we measured a high Simpson dominance (D') in that area. Furthermore, we got lower values for the Pielou equitability (J) in the wet period. These results are partially in contrast to those in SILVA et al. (2015); in this reference, there are higher values for the Pielou equitability (J), mainly in herbaceous plants. In this context, JEFWA et al. (2012) emphasized that the Shannon index identify the rare species in the area. Consequently, if the disturbances strenghten the rare species would be the first to be impact.

High AMF dependence of *B. brizantha* and presence of fine roots favor an increase in root colonization (HOWELER et al., 1982). Less root colonization occurred in the NV system over two seasons compared with that in BRA, PAN, and CYN systems. However, in the NV system, roots were generally thicker and root colonization was higher (p<0.05) than those in AND, although only in the wet season. A reduced intensity of AMF infection in tropical plants is attributed to the high

density of root cortical cell walls, which is caused by suberization and lignification (ZANGARO et al*.,* 2012). In undisturbed soil, root infection arises from the contact of roots with the hyphae of extraradical mycelium, which is less dependent on the germination of glomerospores.

Although *Andropogon gayanus* Kunth presents fine roots, we did not find higher AMF colonization. Problably, in wet season, the reduction of soil temperature reduced the length of roots and promoted a negative interaction between AMF and roots (ZANGARO et al., 2012).

In the dry season, BRA and AFS systems presented the highest values for NMP. The presence of Gigasporaceae occurred only in these two areas, which may have contributed to the high number of infective propagules. The species belonging to the family are able to colonize roots (BRUNDRETT et al., 1996; KLIRONOMOS; HART, 2002). According to BARTZ et al., (2008), the values may be underestimated even with the use of trap cultures for estimating MPN (Most Probable Number). These authors emphasize that many species remain in the vegetative form for a long time, suggesting the MPN technique for longer periods.

Conclusions

Conversion of native vegetation to pasture system causes changes in the structure and composition of AMF community. On the other hands, the agroforestry system showed the presence of *Gipaspora* and *Scutellospora* which indicates a reduction of the negative effects of native vegetation replacement.

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