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Occurrence of arbuscular mycorrhizal fungi after organic fertilization in maize, cowpea and cotton intercropping systems

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ABSTRACT. The effect of organic manure on the community of mycorrhizal fungi (AMF) was evaluated in maize, cowpea and cotton intercropping systems in a semi-arid region of Brazil over two consecutive years. The experiment was conducted using a randomized block design with four replicates and six soil treatments: (M) goat manure incorporated in the soil before planting; (G) gliricidia prunings incorporated before planting; (M+G) manure and gliricidia incorporated before planting; (GS) gliricidia applied on the soil surface 45 days after planting; (M+GS) manure incorporated before planting and gliricidia applied to the surface 45 days after planting; and (C) control treatment without incorporation. It was not possible to identify the best treatment for both years in terms of spore density, mycorrhizal colonization and glomalin content. However, there was a significant effect from the fertilization treatments when compared to the control in most treatments. In both years, M+GS favored glomalin and AMF sporulation in the cotton plots. In the second year, which had low rainfall, there was an increase in production of spores, glomalin, colonization and AMF species richness irrespective of soil incorporation and culture. Plots that were fertilized with manure presented greater AMF species richness regardless of the year and crop type.

Keywords: mycorrhizal association, *Gliricidia sepium*, goat manure, green fertilizer.

Ocorrência de fungos micorrízicos arbusculares após adubação orgânica em sistemas consorciados de milho, feijão e algodão

RESUMO. O efeito da adubação orgânica sobre a comunidade de fungos micorrízicos arbusculares (FMA) em sistemas consorciados com milho, feijão e algodão foi avaliado no semi-árido paraibano, ao longo de dois anos. O experimento foi conduzido em delineamento de blocos casualizados com quatro repetições e seis tratamentos: (M) esterco incorporado no solo antes do plantio; (G) ramas de gliricídia incorporadas antes do plantio; (M+G) esterco e gliricídia incorporados no solo antes do plantio; (GS) gliricídia aplicada em cobertura 45 dias após o plantio; (M+GS) esterco incorporado antes do plantio e gliricídia aplicada em cobertura 45 dias após o plantio; (C) tratamento controle. Não foi possível identificar um melhor tratamento, em ambos os anos, em termos de densidade de esporos, colonização micorrízica e teor de glomalina. Entretanto, houve efeito significativo das incorporações na maioria dos tratamentos quando comparados ao controle. Em ambos os anos, M+GS favoreceu a produção de glomalina e a esporulação de FMA no sistema com algodoeiro. No segundo ano, de poucas chuvas, houve aumentos na produção de esporos, glomalina, colonização e riqueza de espécies de FMA, independentemente do efeito das incorporações e da cultura. Os tratamentos com esterco apresentaram maior riqueza de espécies, independentemente do ano e da cultura.

Palavras-chave: associação micorrízica, *Gliricidia sepium*, esterco caprino, adubos verdes.

Introduction

In the semi-arid region of northeastern Brazil, high rainfall variability, recurrent droughts and low soil fertility limit the agriculture and cattle productivity of the land (SILVA; MENEZES, 2007). The use of chemical fertilizers to improve soil fertility is not viable for most producers in this region due to irregular rainfall and low agriculture profitability. Therefore, organic fertilization is the most viable alternative to replenish the soil nutrients (TIESSEN et al., 1994).

Livestock manure is the main organic fertilizer used in the region; however, the amount produced is insufficient to fertilize all of the agricultural land

(GARRIDO et al., 2009). Furthermore, the available manure is usually low quality and contains low nitrogen and high lignin content, which can lead to N immobilization and hinder crop growth (MENEZES; SALCEDO, 2007; SILVA; MENEZES, 2007).

In addition to livestock manure, the use of green manure, which consists of biomass from plant species, is a low-cost practice that may increase crop yield in low-input agricultural systems such as those in the semi-arid region of NE Brazil (TIESSEN et al., 1994). When used in combination with livestock manure, green manure can supply the necessary N to enable faster decomposition of the low quality livestock manure and supply other nutrients such as P (SILVA; MENEZES, 2007).

An example of green manure with a potential use in semi-arid conditions is gliricidia, which provides high quality forage to feed livestock (BARRETO; FERNANDES, 2001) and is able to fix significant amounts of atmospheric N in association with diazotrophic bacteria (BALA et al., 2003). In addition, gliricidia produces biomass under conditions of low water availability (MARIN et al., 2007). Pruned gliricidia leaves and thin twigs (< 1 cm in diameter) have a low secondary metabolite content and high N mineralization rate (MAFONGOYA et al., 2000).

Arbuscular mycorrhizal fungi (AMF) are important organisms in plant nutrition because they contribute to increased root absorption of low mobility nutrients in the soil, such as P, Zn and Cu (MIRANDA et al., 2008). The beneficial effects of organic fertilizers were also observed for infectivity (PALENZUELA et al., 2002), mycorrhizal colonization (GRYNDLER et al., 2005) and production of propagules in the field (GAUR; ADHOLEYA, 2005).

The organic fertilizers benefit AMF by improving the soil properties and production of certain substances during their decomposition (GRYNDLER et al., 2005). Although the use of organic sources favors the intra-root phase, factors such as dose, type of residue, plant species and AMF should be considered for the success of symbiosis in organic cultivation (SILVA et al., 2007).

Negative responses of AMF to the incorporation of organic residues have been observed and attributed to the high nutrient content of these materials, presence of phytotoxic substances (MARTIN et al., 2002), the specific composition of the residue (BORIE et al., 2002) and/or presence of pathogens (ELORRIETA et al., 2003).

The effects of organic fertilization on AMF are variable, and more studies are needed to clarify their relationship. Therefore, the objective of the present work was to evaluate the effect of organic fertilization with gliricidia and/or goat manure on AMF in an intercropped system of maize, cowpea and cotton in the semi-arid region of Brazil.

Material and methods

Description of the experimental area

The experiment was performed in 2006 and 2007 at the Agroecological Station of Vila Maria Rita, in Taperoá municipality, Paraíba State, Brazil, which is located at 7º 12”23” South and 36º 49”25” West and has anaverage altitude of 520 m. The soil from the experimental area is classified as a Fluvent. The average annual precipitation is 558 mm, and the average annual temperature is 26ºC. The total monthly rainfall was recorded at the study site during the two years of the experiment (Figure 1).

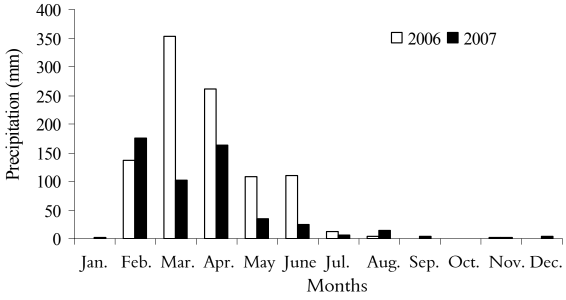

Figure 1. Monthly precipitation recorded in Taperoá, Paraíba State, in 2006 and 2007 (AESA, 2008).

Experiment

The experiment was established in random blocks with four replicates. The intercropping systems included maize, *Zea mays* L. (Sergipano variety); cowpea, *Vigna unguiculata* (L.) Walp (Moitinha variety); herbaceous cotton, *Gossipium hirsutum* L. (H8 variety, Embrapa CNPA) and the following organic fertilization systems: M - goat manure incorporated in the soil before planting; G - gliricidia prunings incorporated before planting; M+G - gliricidia and manure incorporated before planting; GS - gliricidia applied on the soil surface 45 days after planting; M+GS - manure incorporated before planting and gliricidia applied 45 days after planting; and C - control treatment without incorporation. The experimental plots were 5 x 7 m with a harvest area of 4 x 5 m. The intercropping system consisted of alternating rows with holes planted with cotton, cowpea and maize. Four seeds of each crop species were placed in each hole, and the seedlings were thinned to maintain only two plants per hole approximately two weeks after emergence.

Goat manure and gliricidia were applied in doses equivalent to 20 ton. ha^{-1} of dry matter for manure and 20 ton. ha^{-1} of fresh matter for gliricidia in treatments with a single fertilizer source. For treatments with both fertilizers, 10 ton. ha^{-1} of dry matter of manure and 10 ton. ha^{-1} of fresh matter of gliricidia were applied. The average dry matter content of the gliricidia fresh prunings was 250 g kg^{-1}. The fertilizers were incorporated to a depth of 15 cm using a manual hoe when applied to the soil before planting or were spread evenly on the soil throughout the entire experimental plot when surface applied.

Fertilizer incorporation and crop planting were performed immediately after the first rains in 2006 and 2007, which was between January and March. Weeding was manually performed using a hoe three times throughout the crop cycle, and insect pests were controlled with neem oil. The nutrient content of the gliricidia and manure used, on a dry matter basis, was 34 and 12 g N kg^{-1}; 6.1 and 6.5 g P kg^{-1}; and 21 and 27 g K kg^{-1}, respectively, as determined by Embrapa (1999).

Soil samples were collected from the 0-15 cm layer from each experimental plot for chemical and physical analysis (EMBRAPA, 1999) in 2006 and 2007 (Table 1). Rhizospheric soil and root samples were collected in 2006 and 2007. The samples were air-dried, separated, homogenized and sieved with 2-mm mesh. Fine roots (< 2 mm) were separated from the soil, washed in water and stored in plastic containers with 50% alcohol for conservation until analysis.

AMF spore density and species identification

AMF spores were extracted from 50-g subsamples using the humid sieving technique (GERDEMANN; NICOLSON, 1963), which employs superposed sieves of 50-μm, 100-μm and 250-μm sieves followed by centrifugation in water (3000 g) and 45% sucrose (2000 g) for 3 and 1 min., respectively. After, the spores were counted in channeled plates using a stereomicroscope (40x).

For the identification of the AMF species, trap cultures were prepared using soil samples diluted in autoclaved sand (1:1), which were, transferred to 500 mL plastic pots planted with Italian millet (*Panicum miliaceum* L.). After three millet multiplication cycles, the spores were extracted from the soil, separated according to their morphological characteristics and mounted on glass slides with PVLG (polyvinyl-lactoglycerol alcohol) and Melzer + PVLG (1:1; v v^{-1}). Species richness was considered as the number of species that occurred in the plot. The frequency of occurrence was estimated according to Equation 1: Fi = Ji/k*100, where: Ji was the number of samples in which the species was observed, and k was the number of total soil samples.

Quantification of glomalin-related soil proteins

The content of glomalin-related soil proteins was quantified by the Wright and Upadhyaya (1998) method, in which 0,25 g of soil were autoclaved with 2 mL of sodium citrate (20 mM; pH 7.0) for 30 min. at 121°C, and afterwards centrifuged at 10000 g for 5 min. An aliquot of 50 μL of the supernatant together with 2,5 mL of the shiny comassie brilliant blue dye G-250 was used for the quantification of glomalin content. Bovine serum albumin was used as standard.

Mycorrhizal colonization

The percentage of mycorrhizal colonization was determined using the split-plate intersect method (GIOVANNETTI; MOSSE, 1980). The preparation of the samples began with the clarification of the roots with KOH (10%) for 24 hours, at room temperature.

Table 1. Physical and chemical soil characteristics during two years of evaluation in intercropping systems with maize, cowpea and cotton under different organic fertilization systems in Taperoá, a semi-arid region in Paraíba State, Brazil.

Organic fertilization treatments[1]	pH	P	Na	K	Ca	Mg	C.O.	Granulometry (g kg^{-1})		
	H_2O	mg kg^{-1}		$cmol_c$ kg^{-1}			g kg^{-1}	sand	clay	silt
				2006						
M	7.6	145.6	0.02	0.77	8.7	1.5	14.7	475	286	240
G	7.7	158.2	0.06	1.12	10.0	2.1	13.9	447	326	253
M+G	7.8	174.7	0.07	0.49	8.4	1.5	13.9	577	231	193
GS	7.7	159.2	0.08	0.46	8.5	1.7	11.6	607	206	188
M+GS	7.5	167.5	0.05	0.66	10.3	1.8	10.2	525	258	218
C	7.1	161.8	0.09	0.79	6.9	1.8	14.5	532	266	203
				2007						
M	7.5	173.7	0.04	1.22	4.5	2.7	16.8	475	286	240
G	7.5	187.3	0.07	1.64	3.7	1.8	12.2	447	326	253
M+G	7.6	180.3	0.06	1.06	3.6	1.9	13.8	577	231	193
GS	7.9	164.5	0.04	0.86	3.2	1.8	12.7	607	206	188
M+GS	7.7	170.2	0.03	1.37	4.2	2.2	14.9	525	258	218
C	7.9	171.9	0.05	1.41	5.3	2.0	14.7	532	266	203

Organic fertilization treatments[1]: M, goat manure incorporated to the soil before planting; G, gliricidia prunings incorporated before planting; M + G, gliricidia and manure incorporated before planting; GS, gliricidia applied on the soil surface 45 days after planting; M + GS, manure incorporated before planting and gliricidia applied 45 days after planting; and C, control treatment without incorporation.

After this, the roots were treated with H_2O_2 for 45 minutes, with HCl (1%) for 3 minutes and colored with Trypan blue (0.05%). One-hundred colored root segments were separated for visualization of fungal structures (arbuscules, vesicles and hyphae) using a stereomicroscope (40x).

Statistical analysis

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

Results and discussion

In 2006, treatment with G, M+G and GS increased AMF spore production (81, 73 and 75 spores in 50 g soil, respectively) in the rhizosphere of maize (Table 2). In the following year, the M+G treatment continued favoring sporulation (278 spores in 50 g soil); however, the spore numbers did not significantly differ from the M system (258 spores in 50 g soil). No significant differences were observed among the fertilization treatments regarding mycorrhizal colonization; however, in 2006, they were superior to the control treatment. The contents of glomalin-related soil proteins (GRSP) in all treatments, except for the M+G treatment, were superior to the control treatment in 2006 but not in 2007.

M and GS favored spore production in the rhizosphere of cowpea in 2006 (53 and 61 spores in 50 g soil, respectively). In the following year, greater values were observed in the G system (292 spores in 50 g soil). There was no significant effect on the fertilization treatments in terms of mycorrhizal colonization regardless of the year. In both years, the GRSP contents increased in all fertilization systems, except G, when compared to the control treatment.

Spore density, mycorrhizal colonization and GRSP content varied among the fertilization treatments; therefore, it was not possible to identify the consistently best system for maize and cowpea for both years. However, in most situations, there was a significant effect of the fertilization systems when compared to the control.

There are many explanations for the beneficial effects of organic fertilization on AMF. According to Gryndler et al. (2005), humic substances, such as fulvic acids that result from the decomposition of organic fertilizers, adsorb free cations from the soil solution and may favor the physiological functions of the fungal mycelia (absorption and transport). The improvement in soil structure also contributes to AMF mycelia development because it reduces the mechanical resistance to hyphal growth. Furthermore, bacterial communities that promote spore germination and increase the rate of AMF colonization (JOHANSSON et al., 2004) are favored by the addition of organic fertilizers (CRECCHIO et al., 2001). According to Muthukumar and Udaiyan (2002), conditions that favor plant growth, especially growth of the root systems, also promote the establishment of the mycorrhizal association because they increase the chance for contact between the surface of the roots and AMF propagules in the soil.

Table 2. Spore density (SD), root colonization (RC) and glomalin-related soil protein (GRSP) in an intercropping system with maize, cowpea and cotton with different organic fertilization treatments during the growing seasons of 2006 and 2007 in Taperoá, a semi-arid region in Paraíba State, Brazil.

Fertilization treatments	SD (50 g^{-1} soil)			RC (%)			GRSP (mg g^{-1} soil)		
Crops	Maize	Cowpea	Cotton	Maize	Cowpea	Cotton	Maize	Cowpea	Cotton
2006									
M	58bB	53aB	46cB	45.3aB	32.0aB	52.3bB	0.81aB	0.53aB	0.44cA
G	81aB	23bB	22dB	50.0aB	33.2aB	50.9bB	0.71aA	0.22bB	0.45cA
M+G	73aB	35bB	67bB	42.9aB	29.8aB	52.6bA	0.46bB	0.53aB	0.60bA
GS	75aB	61aB	53cB	42.9aB	23.9aB	55.9aA	0.69aA	0.49aB	0.46cA
M+GS	39bB	23bB	100aB	48.8aB	26.1aB	59.7aA	0.59aB	0.43aB	0.74aA
C	43bB	44bB	80bB	25.1bB	35.3aB	47.6bA	0.25bB	0.23bB	0.41cA
CV (%)	14.74	14.49	15.67	19.06	14.29	15.00	18.30	17.49	24.91
2007									
M	258aA	181cA	248bA	60.4aA	61.4aA	58.6aA	1.16aA	1.14aA	0.06cB
G	174bA	292aA	156cA	61.8aA	62.9aA	59.0aA	0.76bA	0.82bA	0.10bB
M+G	278aA	224bA	158cA	61.2aA	61.5aA	50.1bA	1.28aA	1.35aA	0.18bB
GS	198bA	181cA	226bA	58.6aA	61.4aA	56.6aA	1.22aA	1.29bA	0.13bB
M+GS	225bA	229bA	376aA	59.8aA	60.0aA	59.9aA	1.49aA	1.36aA	0.24aB
C	202bA	242bA	235bA	60.2aA	62.3aA	52.2bA	1.44aA	0.80bA	0.12bB
CV (%)	14.74	14.49	15.67	19.06	14.29	15.00	18.30	17.49	24.91

Organic fertilization treatments[1]: M, goat manure incorporated to the soil before planting; G, gliricidia prunings incorporated before planting; M+G, gliricidia and manure incorporated before planting; GS, gliricidia applied on the soil surface 45 days after planting; M+GS, manure incorporated before planting and gliricidia applied 45 days after planting; and C, control treatment without incorporation. Results followed by similar letters were not statistically different using the test of Scott and Knott at 5% probability. Capital letters show a comparison of each fertilization system between the two years, while the small letters compare the fertilization treatments within each year.

The M+GS system favored spore and glomalin production in the rhizosphere of cotton in both years. The cotton plants had the greatest mycorrhizal colonization; however, in 2006, it did not significantly differ from the GS system (60 and 56%, respectively). In 2007, except for the M+G fertilization system, mycorrhizal colonization of cotton did not significantly differ among the fertilization systems; however, they were superior to the control.

Garrido et al. (2009) reported that the M+GS treatment had the highest cotton biomass production, which was linked to slow crop growth in phase with the gradual nutrient release by the manure. The large biomass production likely resulted in an increase of photosynthesis and photosynthate allocation to the roots, which stimulated sporulation, mycorrhizal colonization and glomalin production by the AMF associated with these plants.

It has been shown that moderate quantities of organic fertilizers have small less adverse effects on AMF than the equivalent quantities of mineral fertilizers, which is likely due to a temporal difference in P availability that results from its gradual release that is concomitant with demands by the plants (BODDINGTON; DODD, 2000).

Significant increases in spore density (215-1270%) and mycorrhizal colonization (95-257%) occurred in the 2007 crop cycle compared to the 2006 cycle regardless of the fertilization system. These increases may represent a resistance mechanism of AMF to adverse conditions because the rainfall was much lower in 2007 (Figure 1). Root colonization and sporulation are crucial strategies for survival of AMF under adverse conditions (HART; READER, 2002).

An increase in the GRSP content was observed in the rhizosphere of maize and cowpea in all fertilization systems in 2007 when compared to 2006, which varied from 107 to 576%. This increase was not observed in the rhizosphere of cotton. It is possible that the AMF increased the number of propagules, which is used as a survival strategy, and favored the increase of glomalin production. According to Driver et al. (2005), glomalin deposition in the soil is a result of the decomposition of spores and hyphae (> 80%), and to a lesser degree, is due to the passive release or secretion of the hyphae.

A total of 21 AMF species that belong to the genera *Glomus* (12), *Acaulospora* (6), *Ambispora* (1), *Entrophosphora* (1) and *Kuklospora* (1) were registered during both crop years (Table 3). With the highest number of known species, the genus *Glomus* had the greatest presence, which may indicate a possible relationship with the soil pH in the region (above 7.5) because species belonging to this genus generally predominate in a pH from 6.0 to 8.0 (MOREIRA; SIQUEIRA, 2006). According to Carrenho et al. (2001), species of *Glomus* have the capacity to adapt to different soil organic matter contents, such as liming and texture. In other studies with organic fertilization (FOCCHI et al., 2004; OEHL et al., 2004; PURIN et al., 2006), the predominance of *Glomus* species have also been reported.

Species of *Acaulospora* tend to be found in soils with a pH lower than 6.5 (GAI et al., 2006). However, in our work, species of this genus were observed even when the pH was greater than 7.5. Similarly, Silva et al. (2005) reported the occurrence of *Acaulospora* species in regions with a pH above 7.0, which indicates that this species is likely tolerant to a broad pH spectrum.

Genera such as *Glomus* and *Acaulospora*, which produce small spores, are more able to survive by adapting their sporulation patterns under unfavorable conditions, such as in semi-arid environments (GAI et al., 2006; LI et al., 2007; SHI et al., 2007; TAO; ZHIWEI, 2005). This dominance may also indicate a selective adaptation to water stress in hot and arid regions.

In general, there were more species (64%) in 2007 regardless of the fertilization system, and the presence of *A. elegans*, *A. spinosa*, *E. infrequens*, *G. aggregatum*, *G. constrictum*, *G.* sp resembling, *G. eburneum*, *G. glomerulatum*, *G. sinuosum*, *G. tortuosum* and *Kuklospora colombiana* were all detected. It is possible that the increase in sporulation in this year enabled the recovery of a greater number of AMF species. The M+GS system had an increased species richness (12 species) compared to the other fertilization systems regardless of the crop and year. Oehl et al. (2004) observed greater species diversity of AMF in organic systems than in other cultivation systems; among these species were *A. longula, G. etunicatum*, *G. constrictum* and *G. mosseae*, which were also observed in the present study. Purin et al. (2006) reported the presence of 20 AMF species in conventional orchards and 30 AMF species in organic apple orchards in the southern region of Brazil and found eight species (*A. morrowiae*, *A. spinosa*, *A. scrobiculata*, *E. infrequens*, *G. claroideum*, *G. etunicatum*, *G. mosseae* and *G. sinuosum*) that were also detected in the present study. In organic systems in western Pennsylvania, USA, Franke-Snyder et al. (2001) detected the presence of *G. mosseae*, *G. etunicatum* and *G. claroideum*.

Table 3. AMF species in intercropping systems with maize, cowpea and cotton under different organic fertilization systems for two years of evaluation in Taperoá, Paraíba State.

AMF species	Maize												Cowpea												Cotton												*RF (%)	
	Organic fertilization treatments[1]																																					
	M		G		M+G		GC		M+GC		C		M		G		M+G		GC		M+GC		C		M		G		M+G		GC		M+GC		C			
Year	2007	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Acaulospora elegans														X																							-	5,5
Acaulosporalongula																									X							X		X			5,5	11,1
Acaulospora morrowiae						X															X																5,5	5,5
Acaulosporascrobiculata																					X	X															5,5	5,5
Acaulospora foveoreticulata		X								X			X	X	X	X						X			X	X				X				X			22,2	44,4
Acaulospora spinosa		X																X																			-	11,1
Ambispora sp. *gerdemannii-like*					X		X																		X												16,6	-
Entrophospora infrequens						X																															-	5,5
Glomus aggregatum																						X															-	5,5
Glomus sp. *'ambisporum-like'*	X						X										X				X								X		X						33,3	-
Glomus claroideum	X		X		X		X		X		X		X		X		X		X		X		X		X		X		X		X		X		X		100	-
Glomus constrictum		X								X		X		X						X				X								X					-	38,9
Glomus sp. *'eburneum-like'*		X																																			-	5,5
Glomus etunicatum	X	X	X	X	X		X	X	X	X	X		X		X	X		X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	83,3	83,3
Glomus glomerulatum																				X																	-	5,5
Glomus intraradices	X		X					X	X		X			X			X		X	X	X		X		X	X	X	X	X	X	X	X	X		X		77,8	38,9
Glomus macrocarpum	X	X		X	X			X	X			X	X			X			X	X							X				X						33,3	27,8
Glomus mosseae	X		X	X	X	X			X				X	X	X	X	X	X	X	X	X		X		X	X	X	X				X	X	X			66,6	61,1
Glomus sinuosum																						X															-	5,5
Glomus tortuosum																				X				X													-	11,1
Kuklospora colombiana																																				X	-	5,5
Total of species	6	6	4	3	5	3	4	3	5	3	3	2	5	4	4	4	4	3	4	7	7	5	4	3	7	4	5	3	3	3	5	5	4	4	3	2	-	-

Organic fertilization treatments[1]: M, goat manure incorporated to the soil before planting; G, gliricidia prunings incorporated before planting; M+G, gliricidia and manure incorporated before planting; GS, gliricidia applied on the soil surface 45 days after planting; M+GS, manure incorporated before planting and gliricidia applied 45 days after planting; and C, control treatment without incorporation.* RF = Relative frequency.

Usually, the actual AMF species richness may be greater than what is reported because the size and morphological resemblance among *Glomus* spores may hinder species identification (MAIA et al., 2006). The eventual spore production by some species and the presence of non-viable spores may also hinder species identification (SOUZA et al., 2010). Furthermore, spore multiplication in pot cultures likely did not enable the recovery and identification of all species present in the plots.

Entrophosphora infrequens was exclusively registered in the M+G system in maize rhizospheric soil while *A. scrobiculata*, *G. sinuosum* and *G. aggregatum* were identified only in the M+GS system associated with cowpea. In addition, *A. elegans* and *G. eburneum*-like were only detected in the M system in maize and cowpea soil, respectively. *Glomus glomerulatum* was only observed in the GS system with cowpea. *Kuklospora colombiana* was identified only incotton plots without organic fertilizer application. Oehl et al. (2010) stated that abiotic factors were more important than biotic factors to the establishment of AMF populations. Furthermore, local conditions determine the selection of AMF species, which are more demanding for environmental adaptation and produce favorable responses to plant development.

Thirteen, fifteen and eleven AMF species were observed in the maize, cowpea and cotton rhizosphere when considering all organic fertilization systems and both years. *Glomus claroideum* and *G. etunicatum* had the greatest presence in 2006 and 2007, respectively. However, it is important to highlight that species with a low frequency of occurrence may be more efficient than species that were more frequent despite the fact that the latter may be more adapted to the soil and climate conditions (PURIN et al., 2006).

Conclusion

The application of organic fertilizers favored AMF activity and diversity in the rhizosphere of maize and cowpea when compared to the control treatment.

The system M+GS favored spore and glomalin production in the rhizosphere of cotton.

Plots that were fertilized with systems M or M+GS had greater species richness than the other fertilization systems, regardless of the crop and year.

In the second crop year there was a significant increase in sporulation, mycorrhizal colonization, glomalin production (except for cotton) and AMF species richness, regardless of the fertilization system and the crop.

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