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Indigenous arbuscular mycorrhizae in areas with different successional stages at a tropical dry forest biome in Brazil

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Arbuscular mycorrhizal fungi mycelium creates multiple fungal links between roots of plants and could be an important component of plant succession in ecosystems. Our objective was to compare the spore number (SN), genus contribution and indigenous arbuscular mycorrhizal colonization (AMC) capacity of soils from a tropical dry forest (caatinga) ecosystem in Brazil at different levels of successional stages after culturing for 3 months in a greenhouse *Brachiaria decumbens* (= *Urochloa decumbens* Staph) as trap plants. Soil samples were collected from Parque Estadual Mata Seca (Manga, Minas Gerais State, Brazil) in four different areas: (a) Pasture (5 years without human activity, covered mainly by *Panicum maximum* Jacq.); and three different forest successional stages: (b) Initial (8 years in process of spontaneous recovery), (c) Intermediate (17 years without human activity) and (d) Late (no recorded human activity). At 90 DAP plants growing at intermediate disturbance soil had the lowest dry matter production, probably because of the lowest available soil P and Ca contents of this soil. The highest SN was found in soils from pasture while the lowest was in soils from the intermediate area. *Glomus* was, in general, the most abundant genus followed by *Acaulospora*, *Gigaspora* and *Scutellospora* (always present in intermediates rates). The contribution of *Archaeospora* was the lowest; however, in some cases it was absent. AMC was higher in plant roots growing in soils from pasture but the lowest AMC was in plant roots from the intermediate area. This could be associated with the soil acidity, Al and H contents of this area which could be toxic to AMF. Mycorrhiza formation and SN appeared to be more related to the physicochemical characteristics of the soil and the host plant development to the soil successional stage. Further studies are needed in order to identify appropriate management strategies for restoration of altered lands in order to contribute to the biodiversity conservation and microbial activity of ecosystems.

Key words: Arbuscular mycorrhizae, plant succession, soil factors.

INTRODUCTION

There is an emerging consensus that changes occurring aboveground (in the atmosphere or by consequences of

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Abbreviations: AMC, Arbuscular mycorrhizal colonization; **AMF,** arbuscular mycorrhizal fungi; **Ar,** arbuscule content; **DAP,** days after planting; **SDM,** shoot dry matter; **SN,** spore number.

land use) undoubtedly will affect plant, animal and belowground microbial biodiversity and that those compartments are intimately linked (van der Putten et al., 2009). Thus, recent studies are focused to understanding these relationships. Arbuscular mycorrhizal fungi (AMF) are belowground plant symbionts of roots that add another dimension to the plant-soil-microbe system because it can directly and indirectly influence other soil microbes and an adequate soil nutrient cycling. Mycorrhizal fungi are able to develop a symbiotic association with more than 90% of vascular or

non-vascular terrestrial plants (van der Heijden et al., 1998; Pongrac et al., 2007; Feddermann et al., 2010). AMF are a diverse taxon, both systematically and functionally and mechanisms governing the formation and maintenance of the symbiosis have not been yet fully clarified. They belong to a monophyletic phylum, the Glomeromycota (Schüßler et al., 2001; Smith and Read, 2008), its taxonomic classification is constantly changing and a new list of families and genera has recently been published (Schüßler and Walker, 2010).

Mycorrhizal symbiosis is usually not host-specific, and displays abundant ecological differentiation and speciali-zation to both their biotic and abiotic environments, including types that are highly specialized to unusual ecological niches (Fitter et al., 2004). AMF form a uniformly distributed mycelium in soil and hyphal proliferation occurs in response to several types of organic material deposition (Rillig and Mummey, 2006), connecting host plants and improving physical and chemical soil quality. The contribution of AMF on plant nutrition is widely documented, specially where available soil P limit plant growth of native plants as well agricultural crops (Menéndez et al., 2001; Covacevich et al., 2006, 2007; Siddiqui et al., 2008; Smith and Read, 2008; Guissou, 2009; Ndiaye et al., 2009). AMF provide other benefits to their host plants, stabilizing soil structure and increasing the tolerance to water stress, soil compaction, salinity and drought, root pathogens and heavy metals or others toxic substances present in the soil (Davies et al., 1993; Augé, 2001; Feng et al., 2002; Trotta et al., 2006; Hildebrandt et al., 2007; Siddiqui and Pichtel, 2008; Raviv, 2010; Miransari, 2011). The high amount of hyphae produced by AMF is correlated with significant increases in the aggregate stability of soils (Jastrow et al., 1998; Tisdall et al., 1997; Rillig, 2004; Treseder and Turner, 2007), modifying the soil's ability to mobilize nutrients, maintaining water content, facilitating roots penetration in soil and diminishing soil erosion potential.

The mycelium of AMF also interconnects the root systems of neighboring plants both of the same as different species (Allen et al., 2003). In this sense, mycorrhizal networks can create indefinitely large numbers of fungal linkages connecting together many plants in a community (Newman, 1988; Giovannetti et al., 2006). Changes in vegetation as a result of environ-mental changes may affect the spread of native AMF. This suggests that AMF formation could be an important element of plant succession in ecosystems (Bellgard and Williams, 2011). Consequently, AMF presents an increasingly interest for agriculture, agroforestry, altered lands restoration and natural ecosystems conservation. Observations on plant succession patterns in semiarid regions reported that AMF could play an important role in the composition and stability of plant communities. However, there are still no conclusive results on the relationship between mycorrhizal colonization and the spatial heterogeneity of ecosystems (Boerner et al., 1996).

Mycorrhizal fungi have a widespread presence in all environments and especially within the tropics (Fuchs and Haselwandter, 2004; Moreira et al., 2007; Makoi and Ndakidemi, 2009) although most of the knowledge regarding the effect of soil management on mycorrhizae comes from temperate regions (Raviv, 2010). In particular, little is known about the possible relationship between infectivity capacities of native AMF from a dry forest type called "Mata Seca" (in Portuguese), in the *caatinga* biome. The *caatinga* is of considerable importance because this type of tropical dry forest is the only biome of exclusively Brazilian distribution (Queiroz, 2006) and an important part of the world's biological richness is found in that ecosystem. The uniqueness and threat level of this vegetation type was reviewed by Espírito-Santo et al. (2009). The objective of this work was to evaluate, under controlled conditions in pots, the spread of indigenous mycorrhization, the contribution of genera and abundance of AMF spores on soils collected from Brazilian Mata Seca ecosystems with different successional stages.

MATERIALS AND METHODS

Study sites

Soil samples were collected from the Dry Forest State Park, in the north part of Minas Gerais State, Brazil (43° 97′02′′S -14° 64′ 09′′W and 44° 00′ 05 ′′ S -14° 53′ 08′′ W). The climate is tropical wet (Aw), the mean annual maximum temperature is 32°C, annual average 18 °C, annual rainfall is 660 mm (SIMGE, 2008). The region is characterized by a well marked dry season (May-October; rainfall less than 60 mm/month, temperature 24 to 26°C). Rains are irregular and concentrated in the summer (December-March).

Soil sampling at field sites and preparation of trap cultures for AMF

Soil samples (0-10 and 10-20 cm depth) were collected during the wet season (February, 2008) in a pasture area with 5 years without anthropogenic interference, covered mainly by *Panicum maximum* (= *Urochloa maxima* (Jacq.) Webster RD) grasses and in three areas with different stages of plant succession: (a) Initial (area with 8 years of spontaneous regeneration process), (b) Intermediate (area with 17 years without anthropogenic interference) and (c) Late (area without any sign of anthropogenic interference). For each area of study, plots of 1000 m^2 were delimited. For each area, soil samples (1500 g each) were collected with five replications. Any individual sample (from each area and depth) was composed by nine subsamples of (0-10 and 10-20 cm depth). Field soil was sieved through a 1 cm mesh, large root fragments were cut in pieces of 1 cm, they were returned to the soil and air dried in darkness at 10°C until use one month later.

The experiments were conducted at the Embrapa Agrobiologia, Seropédica-Universidade Federal Rural do Río de Janeiro, Seropédica, Rio de Janeiro, Brazil. Trap cultures of AMF in each area were made by using experimental units (n=60) which consisted of white plastic pots of 0.8 kg (used for plants growing until 45 days after planting-DAP) or 1 kg (used for plants growing until 90 DAP). All pots were filled with a substrate composed of not autoclaved homogenized soil from the field and twice autoclaved

Table 1. Soil characteristics at sites selected from the Dry Forest State Park. OC= organic carbon.

Site	pH (water)	P (mg kg^{-1})	OC (g kg^{-1})	Al (cmol$_c$ kg^{-1})	H (cmol$_c$ kg^{-1})
Initial	7.02 a	0.61 b	22.03 a	< 0.00	0.86 b
Intermediate	5.06 b	0.20 b	18.01 b	0.58	3.61 b
Late	6.62 a	0.44 b	21.90 a	< 0.00	1.92 a
Pasture	6.42 a	1.87 a	14.42 b	< 0.00	1.89 b

Means within the same column followed by the same letter are not significantly different (LSD,P<0.05).

river sand in a proportion of 2:1. The experiment was set up with 5 replications, 4 areas (soil from 0-10 cm depth of Initial, Intermediate, Late and Pasture areas) and 2 harvest (45 DAP and 90 DAP). Additionally, 0.8 kg pots were filled with soil from the same areas at 10 to 20 cm depth with 5 replications and harvested at 45 DAP. To avoid preferences of mycorrhizal infection for a certain host plant, experiment was done using only *Braquiaria* grasses as host plant in all treatments. Seeds were sterilized with 4% sodium hypochlorite solution for approximately 5 min, rinsed with distilled-autoclaved water, pregerminated in sterile vermiculite and grown in a growth chamber during 5 days. Homogeneous-sized pre-germinated plants were planted in pots (4 plants pot^{-1} in 45 DAP pots, and 3 plants pot^{-1} in 90 DAP pots). Plants were grown in glasshouse under natural light conditions, daily watered with distilled water and the substrate was maintained at water holding capacity (65% w/s). Hoagland solution (P 30%) was supplied at 30 and 60 DAP.

Trap cultures harvest: Plant growth and mycorrhizal colonization assess

After 45 DAP and 90 DAP plants were harvested from 0.8 and 1 kg pots, respectively. Shoots were cut and plant material was oven dried at 60°C for shoot dry matter (SDM) estimation. As a measure of the infection potential of field samples, initial mycorrhizal root colonization was determined in the trap cultures (Oehl et al., 2004) after 45 and 90 DAP. Root material was washed out free of soil and collected on sieve (0.5 mm). Immediately, roots were cut into 1 cm pieces, thoroughly mixed, cleared with KOH (10%, 30 min, 90°C), acidified (HCl, 0.1 N), and stained with trypan blue (0.05% vol^{-1}, 5 min, 100°C) in lactoglycerol (1:1:1 lactic acid, glycerol, distilled water) according to the modified method described by Phillips and Hayman (1970). Presence of mycorrhizal infection was assessed by microscopic examination (40X) of the root system. A segment was considered infected if contained hyphae plus arbuscules, coils or vesicles. Mycorrhizal colonization was estimated as AMC (%) and percentage of arbuscules (Ar) of the root cortex, according to the Trouvelot et al. (1986) method.

AMF spore isolation and identification

AMF spores of the trap cultures were extracted by wet sieving and sucrose density gradient centrifugation (Daniels and Skipper, 1982). The procedure included passage of 50 cm^3 of harvested trap culture substrate through 500, 125, 53 and 37 μm sieves. The contents of the 125, 53 and 37 μm sieves were centrifuged at 800 × g for 3 min, the supernatant was discarded, and the pellet was layered onto a water-sucrose solution (80% [wt/vol]) gradient and centrifuged at 900 × g for 2 min. The resulting supernatant was passed through the 37 μm sieve, washed with tap water. Quantification of spore number (spores, spore clusters, and

sporocarps) was carried out in 9 cm diameter Petri dishes with a gridline of 1 cm per side under a stereoscopic microscope at 50X (Lugo and Cabello, 2002). Ten divisions were counted and related to the total spores number (SN) (Schalamuk et al., 2006). Taxonomic identification was made at the Microbiology Laboratory-Estación Experimental Agropecuaria INTA, Balcarce (Argentina). For that, 80% of spores of each sample were mounted onto slides with the permanent mounting medium for fungi polivinil alcohol PVA (Omar et al., 1979) with or without Melzer's reagent to assist with the identification of AMF spores (Morton, 1988). About 70% of the spores fixed on slides could be identified at the genus level. Dead and decaying spores, lacking clear morphological features were not included in the number quantification or the genus identification. The spores' identification was based on current species descriptions and identification manuals (Schenck and Pérez, 1990; International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi http://invam.caf.wvu. edu/Myc_Info/Taxonomy/species.htm; Schüßler and Walker, 2010).

Statistical analysis

Data were analyzed by ANOVA (SAS 9.2 Institute Inc., 2002-2008). Means among treatments were compared with the least significant difference (LSD) test (P < 0.05). All presented data are means of untransformed values.

RESULTS AND DISCUSSION

Soil physicochemical attributes varied significantly among sites (Table 1) and the soil from the Intermediate area was the most different in the quantified characteristics in comparison to the other areas. In that sense, both the most acidic condition and highest Al and H contents were found in those samples. Furthermore, the lowest soil P content was determined at the Intermediate site whereas the highest soil P was found in the Pasture soil. Initial and Late soils showed the highest organic carbon contents.

The SDM, AMC, Ar and SN significantly varied among the different areas where the soil was collected (Table 2). SDM of *Braquiaria* was highest in plants growing in intermediate and pasture soils at 45 DAP (Figure 1). However, at 90 DAP plants growing in Intermediate soils showed lowest SDM production. This may be due in part to the lower available soil P and Ca content in Intermediate soils, which were two or three times lower than in the other areas, and also to the low pH, which

Table 2. Analysis of variance of successional areas (SA) and soil depth (SD) on shoot dry matter (SDM) production, arbuscular mycorrhizal colonization (AMC), percentage of arbuscules (Ar) of *Braquiaria* plants and spore numbers (SN) of soils at 45 days after planting.

Source of variation	Degree of freedom	SDM	AMC	Ar	SN
Replications	4	0.09	0.74	0.83	0.01
Successional area (SA)	3	0.05	0.01	0.10	<0.0001
Soil depth (SD)	1	<0.001	<0.0001	<0.0001	0.41
A×SD	3	<0.01	0.28	0.03	0.94
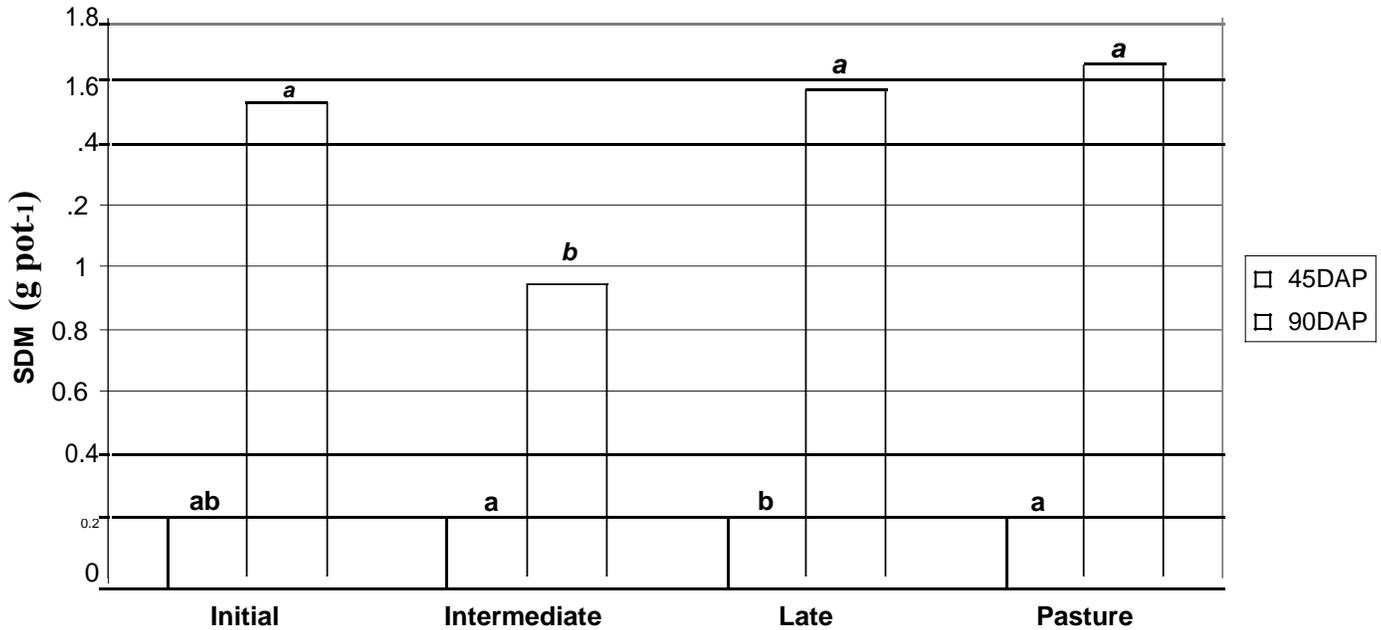
Figure 1. Shoot dry matter production (SDM) of *Brachiaria* at 45 and 90 days after planting (DAP) growing in soils from different successional ecosystems. For each sampling date (standard letters for 45 DAP, italics for 90 DAP), columns with the same letters show no significant differences in SDM among soils (LSD, P <0.05).

was about 1.5 times lower than in the other soils. With increasing soil depth, we found decreases in the percentage of roots colonized by AMF and in plant productivity. AMC and Ar of roots were significantly higher at 45 DAP in pots filled with the field soil of 0 to 10 cm depth comparing with 10 to 20 cm depth samples. This could indicate higher soil propagules content in the most superficial soil depth. Also Schwab and Reeves (1981), and Cooke et al. (1993) reported decreases in mycorhizal colonization through soil depth profiles. However, Covacevich et al. (2006) did not found differences in colonization between 0 to 10 and 10 to 20 cm of soil samples collected in agricultural field grasses of Argentina. On the other hand Neville et al. (2002) found that AM hyphae and total fungal colonization of *Populus tremuloides* Michx were significantly lower in the top 5 cm of the soil than in the 5 to 10 cm. Furthermore, at our experiment, the SN was not significantly different when comparing these 0 to 10 and 10 to 20 soil depths (Table

3). Ohel et al. (2005) found either differences or no differences in the SN between 0 to 10 and 10 to 20 cm of a Calcaric Regosol (pH 7, P 10.9 mg P kg^{-1}) and a Haplic Luvisol (pH 6.2, P 21.8 mg P kg^{-1}) soil samples collected in grassland fields from Upper Rhine Valley (Germany), respectively. It is an indicative that SN and AMF infective propagules are not always well correlated. Some incongruities among results indicate that AMF are differentially partitioned at different soil depths and it could be accounted by extreme differences in propagule richness related to soil characteristics and differences in time of germination of spores of different soil environments. Highest SN were found in soils from Pasture followed by Initial area while the lowest SN were found in pots with Intermediate area soil (Table 4). Silva Santos Santos (2010) working with field samples collected in the same areas as in this study also found that Pasture had highest SN (about 600 spores ×100 g soil $^{-1}$) followed by the Initial area (about 420 spores × 100 g soil $^{-1}$).

Table 3. Effect of soil depth on shoot dry matter (SDM) production, arbuscular mycorrhizal colonization (AMC), percentage of arbuscules (Ar) of *Braquiaria* roots and spore numbers (SN) at 45 days after planting.

Soil depth (cm)	SDM	AMC	Ar	SN (spores × 100 g soil^{-1})
0-10	0.308 a	44.7 a	30.9 a	87 a
10-20	0.232 b	25.1 b	15.2 b	80 a

Means within the same column followed by the same letter are not significantly different (LSD,P<0.05).

Table 4. Spore number (SN) and contribution of representative genera of arbuscular mycorrhizal fungi found in soils (0-10 cm) of different successional areas with *Braquiaria* plants grown until 45 and 90 days after planting (DAP).

Harvest	Site	SN (spores 100 g soil^{-1})	Genera (%)				
			Glomus	*Acaulospora*	*Archaeospora*	*Gigaspora*	*Scutellospora*
45 DAP	Initial	116 b	61	21	--	8	10
	Intermediate	10 c	26	32	2	10	30
	Late	51 c	48	36	--	9	7
	Pasture	172 a	42	33	5	9	11
90 DAP	Initial	133 b	52	29	1	9	9
	Intermediate	38 c	27	42	1	5	25
	Late	95 b	41	40	--	10	9
	Pasture	237 a	39	36	4	11	10

Means within the same DAP followed by the same letter are not significantly different (LSD,P<0.05).

However, the SN quantified at our study was about 3.5 to 6 folds lower than the amounts reported by Silva Santos Santos (2010). In the present study, the lowest SN of soil at the trap culture was found in soils collected at the Intermediate area. Silva Santos (2010) determined that the Late area had the lowest SN under field conditions (about 240 spores × 100 g soil^{-1}) and pointed that lowest SN could be an indicative of a highest environmental stability of soils. The most abundant genus was *Glomus*, followed by *Acaulospora* that showed the highest frequency in Intermediate soils at 45 and 90 DAP and reached the same proportion of *Glomus* in Late soils at 90 DAP. Silva Santos (2010) also found that *Acaulospora* presented the highest frequencies at the Intermediate site under field conditions. The results obtained in the present study are agree with other studies that reports that *Acaulospora* could be found preferably under moderately acid soil-conditions at pH < 6.2 (Stürmer, 1999) and preferably within the range 3.5-5.8 (Trufem, 1995). Interestingly, no spores belonging to the genus *Archaeospora* were found in the pots filled with the Initial soils at 45 DAP nor in the Late soils during the two harvest of the experiment. When *Archaeospora* spores were found, their contribution to the total recorded genera was the lowest. Silva Santos (2010) did not found any spores belonging to the *Archaeospora* at the Late site under field conditions. On the other hand, *Gigaspora* and *Scutellospora* were always present at Intermediate rates from 5 to 25% (Table 4).

Silva Santos (2010) analyzed SN and mycorrhizal diversity of soil samples collected in the field. The present paper analyzed SN and most representative genera from soils collected at the same areas and at the same moment like Silva Santos (2010) in order to establish AMF-trap cultures using *Braquiaria* as host plants. Although the overall response patterns were similar between our results and those of Silva Santos (2010), some differences were found (for example in the overall amount of the SN). Differences between our results in pots and those found by Silva Santos (2010) under field conditions could be attributed to the fact that AMF sporulation varies with host and AMF species and also with growing conditions (affecting fungus directly and indirectly through host physiology). Many studies have demonstrated that not necessarily the same AMF species are found in a field site and in greenhouse cultures with soil from the same site and growing in "trap cultures" (Jansa et al., 2002; Oehl et al., 2003, 2004), although there is usually a considerable overlap between the same species groups. The period of time during the cultures are grown appears to be important. Sporulation often occurs within 3 to 4 weeks after onset of mycorrhizal colonization under almost any conditions except the high availability of soil P, which could depress or inhibits all fungal growth phases (http://invam.caf.wvu.edu/fungi/taxonomy/concepts/lifecycle.htm). Declerck et al. (2001) found that sporulation under controlled conditions increased from 4 to 5 weeks
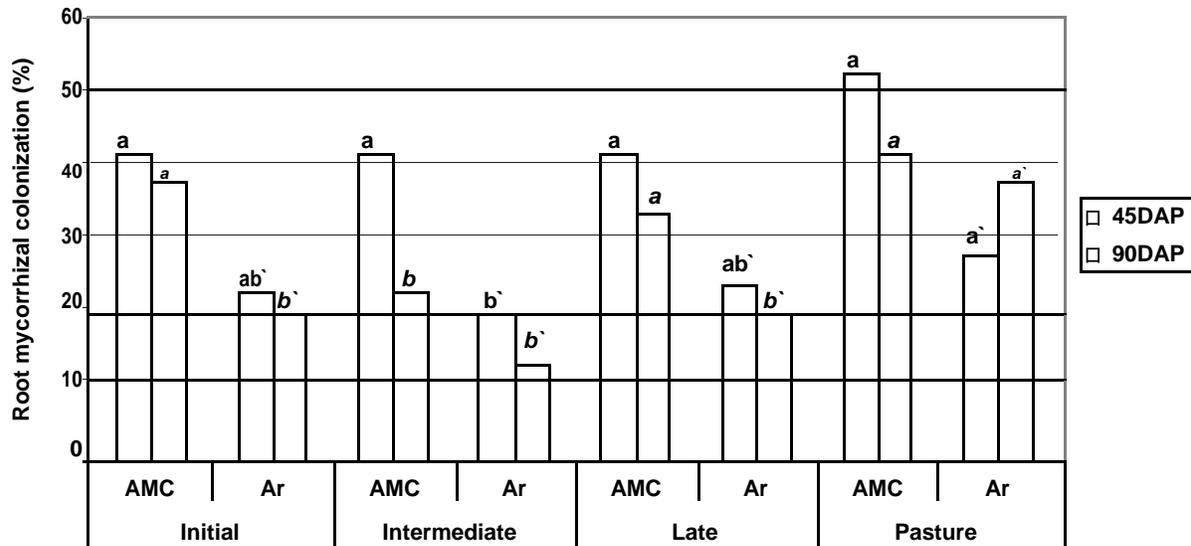
Figure 2. Arbuscular mycorrhizal colonization (AMC) and percentage of arbuscules (Ar) of *Brachiaria* roots at 45 and 90 days after planting (DAP) grown in soils from different successional ecosystems. For each sampling date (standard letters for 45 DAP, italics for 90 DAP) and value of root mycorrhizal colonization (without apostrophe for AMC, with apostrophe for Ar) columns with the same letters show no significant differences in mycorrhizal colonization among soils (LSD, P <0.05).

to approximately 10 weeks; at this time sporulation was stabilized. Oehl et al. (2004) found that there is a wide time range (4 to 20 months) in which AMF could sporuLate.

Mycorrhizal development of *Braquiaria* roots decreased from 45 to 90 DAP (Figure 2). It is likely that most of the photosynthates were allocated in the aerial part at 90 DAP and therefore less C was available in the roots to maintain the mycorrhizal symbiosis. AMC and Ar were highest in roots growing in pots filled with Pasture soil followed by the Initial and Late soils (Figure 2). The lowest formation of symbiosis was detected on plants grown in the Intermediate soil. This could be probably associated with a lower pH than in the other sites. Soil acidity is a factor that normally affects the development of mycorrhizal symbiosis (Sieverding, 1991; Bartolome-Esteban and Schenck, 1994; Fuchs and Haselwandter, 2004; Kittiworawat et al., 2010), mainly when the soil presents high Al^+ and H^+ contents, which are toxic to AMF (Porter et al., 1987) and sometimes interferes with roots colonization, because P adsorption makes it unavailable to the plant. It was mentioned that highest Al^+ and H^+ contents were found in Intermediate soils, which could explain the lowest presence of AMC in this site. However, Siqueira et al. (1990) mentioned that root colonization of *Brachiaria* was not greatly different between Brazilian soils of pH 4.51 to 6.0. But in a previous study, Siqueira et al. (1986) also showed that the ability to colonize roots grown on soil ranging in pH 4.5 to 6.3 was different among AMF species and that, in general, mycorrhiza formation of maize increased gradually as soil pH increased to pH 5, and decreases at

the highest soil pH of 6.3. If in the present study the pH was not the cause of lower mycorrhiza formation at the Intermediate soil, it is likely that the lower amount of C in roots as a consequence of the symbiosis maintenance could result in a lower plant growth This is because AMF are obligate symbionts of plants that consume photosynthates from roots (Smith and Read, 2008).

Mycorrhizal root colonization and SN of soils followed, in general, the same pattern among sites with the lowest mycorrhizal development in soils from the Intermediate area. Changing environments can induce the predominance of Intermediate-sized spores, such as in *Glomus* species that in general moduLate the production of spores directly according to C availability, showing a direct reproduction investment as expected for r-strategists (Ijdo et al., 2010), as well as a reduction in AMF abundance and in richness species. However, Clapp et al. (1995) mentioned that AMC as well as SN could not necessarily reflect the actual AMF populations in the soils and Ahulu et al. (2006) showed that species with an abundant production of spores in the field or in the greenhouse do not always dominate the AMF community in the field roots. This could be explained because freshly formed spores can often not be readily distinguished from spores formed earlier in the season (Lee and Koske, 1994). Pianka (1970) reported that AMF that sporuLates early in trap cultures could potentially be representatives of the r strategy because they could dominate resource-rich uncolonized habitats in early successional stages of the fungal community. Thus, in the present study, *Glomus* also seems to have shown an r-strategy and could be found also in highest proportion in soils coming

from early and Late successional stages. Sýkorová et al. (2007) also found that some *Glomus* phylotypes showed an r-strategist behavior, rapidly colonizing ruderal habitats in early successional stages in French grasslands. They also mentioned that although AMF r-strategists typically invest heavily into their reproduction, it is possible that some of them are not prolific sporulators. The k-strategists would follow the opposite strategy showing slow growth rates under resource-limited conditions and occurrence in Late successional areas (Pianka, 1970). In that sense because *Acaulospora* increased in soils coming from Intermediate and Late sites (Table 4), it probably followed a k-strategy. However, it is difficult to draw direct conclusions about the life history strategy of the AMF detected by their spores.

It is known that belowground diversity of AMF is a major factor contributing to the maintenance of plant biodiversity and to ecosystem functioning; however, the increase/decrease of SN with plant succession is controversial. In Brazil, Carrenho et al. (2001) developed a plant restoration study along the river Moji-Guaçu, using a mixture of pioneer, secondary (early and Late) and climax plant species. They found that the rhizosphere of pioneer species (*Croton urucurana*) had the lowest number of spores, and this increased progressively until the occurrence of climax species such as *Genipa americana*. They concluded that the number of AMF spores increases with the stage of succession. Contrarily, Zangaro et al. (2007, 2008) found that fast-growing native woody species in southern Brazil showed higher levels of AMC compared to slow growing ones. The last authors suggested that the occurrence of mycorrhiza and the density of AMF spores decreased along the succession, and it could be probably due to morphological root traits in fast-growing species that favored the contact with AMF propagules in the soil. Additionally, studies by Pagano et al. (2008, 2010) also in semiarid fields of Minas Gerais State, showed higher mycorrhizal sporulation in disturbed sites.

In this study the highest SN was found both at Initial like (Carrenho et al., 2001) and Pasture soils and it was in accordance to the highest mycorrhizal development of roots. Moreover, it was previously showed that *Braquiaria* and *Panicum* are mycorrhizal plants (Wang and Qiu, 2006) and that AMF enhance the growth and P uptake of these grasses (Siqueira et al., 1990; Roos and Allsopp, 1997).

Recently, Oehl et al. (2010) reported that soil type strongly affect AMF composition and the occurrence of many species. Our results also suggest that the physicochemical characteristics of soils influenced AMF formation more than the successional stages of the sites.

Regardless the origin and consequence of the disturbance (which sometimes can be a consequence of physicochemical differences among sites), ecosystems tend to recover naturally. It is suggested that this adaptation is mediated largely by soil microorganisms whose activity depends on the presence of indigenous AMF (Johansson et al., 2004; Artursson et al., 2006; Jin et al., 2010)

However, unexpected disruptions may occur in natural sites due to human interventions that can also accelerate natural processes of disturbance due to climatic change.

When a soil loses its natural condition (mainly by human action or when agricultural practices are installed) changes in the physical, chemical and biological soil properties undoubtedly occur. According to Lejon et al. (2005) these are the main factors controlling the transformation of organic matter and nutritional status in soils. In this sense, the monitoring of microbial populations (particularly AMF) (Pagano et al., 2011) and the physicochemical properties of soils can be useful tools to determine the state of disturbance in different sites. Studies suggest that establishment and maintenance of plant diversity in communities relies on the presence of a wide diversity of AMF (van der Heijden et al., 1998). Further, plant productivity is in general greater when a large array of AMF is associated with plants. In this regard, our results showed that Intermediate soils showed the lowest plant productivity and it was associated with the lowest mycorrhiza formation. Presumably, each AMF has a degree of specificity with hosts and it is also affected by the environment, resulting in a complex interaction whereby better combinations of fungal populations-plant-environment could contribute to plant growth and environmental sustainability. For a better understanding of the dynamics of AMF communities associated with different stages of environmental disturbance it will be necessary to classify AMF phylotypes according to their life history strategies and to monitor indigenous AMC. The present study provides the first steps in this direction. Our findings also emphasize that, in a short-term greenhouse experiment (3 month), only a certain subset of AMF genera, preferably comprising r-strategists, colonized roots of trap plants. Colonization and spore number appeared to be more reLated to soil physicochemical characteristics and host plant development than soil successional stage. However, mechanisms regulating and maintaining AMF species composition, spore density and fungus infectivity (as a measure of activity) among sites with different successional stages are not well understood. Therefore, more studies need to be aimed to identify involved mechanisms in order to ensure a successful management for the conservation and restoration of our diverse and threatened natural ecosystems.

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