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Effect of the inoculation and distribution of mycorrhizae in *Plathymenia reticulata* Benth under monoculture and mixed plantation in Brazil

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Abstract This paper investigates the distribution of arbuscular mycorrhizal fungi (AMF) spores and AMF colonization in a field study in southeastern Brazil. Response to AMF and rhizobial inoculation was studied in monocultures of *Plathymenia reticulata* and mixed plantations with both *Tabebuia heptaphylla* and *Eucalyptus camaldulensis* in a sandy soil during two consecutive years. *P. reticulata* height and diameter and mycorrhizal colonization and AMF diversity were measured in dry and rainy periods. The inoculated treatment of *E. camaldulensis*, *T. heptaphylla* and *P. reticulata* mixed plants showed higher height and diameter growth of *P. reticulata* used as well as increased root colonization and AMF spore numbers. Spore populations were found to belong to five genera: *Acaulospora*, *Entrophospora*, *Glomus*, *Gigaspora* and *Scutellospora*, with *Glomus* dominating. Agroforestry practices including use of leguminous tree *P. reticulata* effectively maintained AMF spore numbers in soils and high AMF colonization levels compared with monocultures, proving an efficient system for productivity and sustainability.

Keywords Arbuscular mycorrhizal fungi · Diversity · *Plathymenia* · *Tabebuia* · *Eucalyptus* · Dry forest · Agroforestry · Tree legume

Introduction

In the semi-arid region of the Minas Gerais state in Brazil, the dominant vegetation is woody Caatinga species which attain a height of 30 m and have intense leaf loss during the dry season. Caatinga, which means white forest, because only the stems of drought deciduous trees can be seen in the dry season, is a biome inserted under high levels of solar

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radiation, annual average temperature and evaporation. Also, low and irregular precipitation is limited to a very short period (Prado 2003).

After forest clearing, the "Carrasco" vegetation invades the area, and early-successional tree (attaining up to 5 m height) and small shrub species with thin stems occur (Rizzini 1997). The latter abounds and significantly retards or stops the natural succession in northwest (NW) of Minas Gerais.

The Jaíba Project, one of the most important irrigation programs in Brazil using the São Francisco River, was established in the NW of Minas Gerais, aiming at promoting the region's agricultural and social development. Demand for wood in this area has become a continuous threat to the preserved area, one of the largest protected areas of woody Caatinga or Dry Deciduous Forest (Rizzini 1997).

With the objective of catering for wood demand in the Jaíba Project an agroforestry system was installed at the Jaíba region to study the growth of intercropped species, *Eucalyptus* plants and native species of woody Caatinga (*Plathymenia reticulata* and *Tabebuia heptaphylla*). *Plathymenia reticulata* is able to associate symbiotically with rhizobia and/or mycorrhizal fungi, and the use of legumes increases soil fertility, biomass production (DeBell et al. 1985; Parrota 1999) and nutrient cycling, especially the N and P cycling in litter (Binkley et al. 1992). *Eucalyptus* species, due to their fast growth, show a good adaptation to different soils and climatic conditions, and high timber value, being increasingly used for reforestation (DeBell et al. 1985; Khanna 1997; May and Attiwill 2003). *Eucalyptus* spp. have the capacity to form two types of mycorrhizas, arbuscular (AM) and ectomycorrhiza (ECM) (Zambolim and Barros 1982). Almost all studies report a temporal replacement, initially dominated by arbuscular mycorrhizal fungi (AMF) and then by ECM (Carrenho et al. 2008). Nevertheless, Adjoud-Sadadou and Halli-Hargas (2000) reported that the replacement of AM by ECM in *Eucalyptus* is not a general phenomenon in this genus under natural conditions in Algeria. Thus *Eucalyptus* growth responses to inoculation with AMF remain controversial.

Tree species, such as *Leucaena leucocephala* (Lam.) de Wit (Manjunath et al. 1984), *Albizzia lebbeck* (L.) Benth. (Faria et al. 1995), *Centrolobium tomentosum* Guill. ex Benth. (Marques et al. 2001) and *Anadenanthera peregrina* Speg. (Gross et al. 2004; Pagano et al. 2007, 2008), are legumes that had their growth and nodulation improved when inoculated with rhizobia and/or AMF, and show that rehabilitation of tropical land has proved more successful if plants are inoculated with AMF (Cuenca et al. 1998; Marques et al. 2001).

Mycorrhizas improve plant nutrient cycling (Smith and Read 1997), soil structure (Miller and Jastrow 1992; Wright and Upadhyaya 1998) and biological soil quality (Cardoso and Kuyper 2006), not only regulating the functional populations in the rhizosphere (Matsumoto et al. 2005), but also decomposing the litter (Aristizábal et al. 2004; Scotti and Correa 2004). Therefore, transfer of nitrogen and phosphorus of decomposed leaves to host plants is improved (Hodge et al. 2001). The traditional taxonomy of AMF is based on spore morphology; however, in some cases difficulties have been found in AMF studies regarding morphological identification (Kirk et al. 2004). Plants growing under stress conditions show higher mycorrhizal dependence, especially because AM symbiosis improves water and nutrient supply, increasing their survival (Augé et al. 1987; Subramanian et al. 1997), particularly in semi-arid and arid environments (Varma 1995; Nouaim and Chaussod 1996). Mixed species plantations usually allow a larger diversity and/or abundance of AMF than monocultures (Cardoso et al. 2003; Cardoso and Kuyper 2006; Muleta et al. 2008). There are few reports showing AMF occurrence, distribution and diversity in the Caatinga region, most of them in cultivated soils (Maia and Trufem 1990; Yano-Melo et al. 1997, 2003). However, Albuquerque (2008) evaluated three natural areas

in the Caatinga biome and verified that diversity varied according to environmental conditions, mainly plant cover and soil properties.

The present paper aims to: (a) examine the intercropping effect among *Eucalyptus camaldulensis* and *T. heptaphylla* over the growth of native species *P. reticulata* and over soil biology and fertility, (b) estimate root colonization and fungal diversity (density and richness) of AMF spores during the rainy and dry periods, and (c) evaluate the response to inoculation with *Rhizobium* and AMF on native *P. reticulata* species plant growth, in pure and mixed stand, as a productive model for revegetation of local degraded areas. The results provide the first indication of the diversity and species composition of AM fungi in the dry forest of Minas Gerais, Brazil.

Materials and methods

Study areas

The experimental study area was at the Jaíba Project of Irrigation (15°09′03″S 43°49′26″W), between São Francisco River and Verde Grande River, in the north region of Minas Gerais, Brazil. The climate is semi-arid BSh according to Köppen's classification (Silva et al. 2006). Annual precipitation is 1 mm (July) to 217 mm (December), mean temperature varying between 14.8°C (July) and 34.0 (October), and the annual mean of potential evaporation being 4.88 mm day^{-1} (Codevasf 2004). Annual mean precipitations (871 mm) are concentrated in the November–March months (Rodrigues et al. 2001). Rainfall at this site was recorded throughout the study (2003–2004; Fig. 1).

Soil sampling and analysis

Rhizospheric soil samples were collected from the top 20 cm. Three samples/block for each plant species and for each treatment were collected, totalizing 54 samples. The samples were analyzed for chemical and physical properties. The soil analysis was performed by the Brazilian company of agricultural research (Embrapa 1979). Soil pH (H_2O), cation exchange capacity (CEC) and base saturation (BS) were determined. The texture of the soil is sandy loam (Embrapa 1999), its physical and chemical characteristics being shown in Table 1.

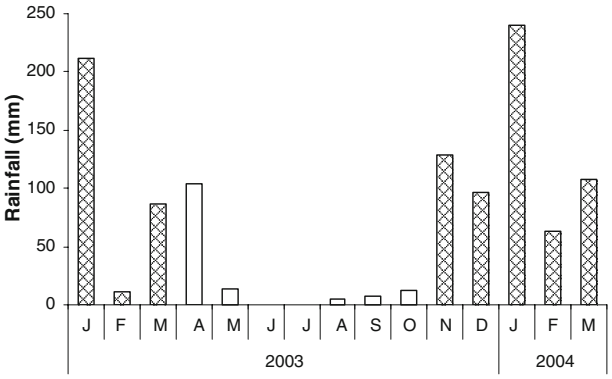

Fig. 1 Monthly rainfall as measured at Jaíba, Minas Gerais. Dry season (*white*), rainy season (*hatched*)

Table 1 Chemical and physical characteristics of the soil sites at the different treatments in the experimental area

T	Chemical properties										Texture			
	pH (H_2O)	H + Al ($cmol_c\ dm^{-3}$)	Ca^{2+} ($cmol_c\ dm^{-3}$)	Mg^{2+} ($cmol_c\ dm^{-3}$)	P ($mg\ dm^{-3}$)	OM (%)	SS ($cmol_c\ dm^{-3}$)	CEC ($cmol_c\ dm^{-3}$)	BS (%)	Al S (%)	Clay (%)	Silt (%)	Coarse sand (%)	Fine sand (%)
1	6.4[a]	1.53	2.32	0.72	3.02	0.84	2.99	4.4	67	0	13	1	51	35
2	5.9	2.04	2.5	0.61	3.29	1.01	2.74	4.3	63	0	13	1	51	35
3	6	2.13	2.46	0.7	4.09	0.98	2.8	4.5	61	0	16	0	50	34
4	6.1	1.91	2.27	0.57	3.96	0.72	2.81	4.5	62	0	16	0	48	36
5	6	2.06	1.96	0.55	3.42	0.88	3.2	4.9	66	0	17	0	44	39
6	6	2.04	2.13	0.56	3.22	0.89	3.4	4.9	69	0	15	2	50	33

T, treatments: 1, *P. reticulata* monoculture; 2, *P. reticulata* monoculture (*Rhizobium* + AMF); 3, *Eucalyptus* monoculture; 4, *Eucalyptus* monoculture (AMF); 5, mixed plantation of *P. reticulata* + *E. camaldulensis* + *T. heptaphylla*; 6, mixed plantation of *P. reticulata* (*Rhizobium* + AMF) + *E. camaldulensis* (AMF) + *T. heptaphylla*

OM, organic matter; SS, soil saturation; CEC, cation exchange capacity

[a] Mean of two measures from one composite sample. Particle size distribution: coarse sand 2–0.2 mm, fine sand 0.2–0.02 mm, silt 0.02–0.002 mm and clay <0.002 mm

Field experimental design

The experimental site (<1 ha) was cleared of "Carrasco" vegetation and seedlings were transplanted after the rainy season (February) in 2003. *Plathymenia reticulata* Benth (Leguminosae), *E. camaldulensis* Dehnh (Myrtaceae) and *T. heptaphylla* (Vell.) Tol. (Bignoniaceae) were cultivated in mixture and in monocultures.

The experimental design consisted of three replicate blocks (each 24 × 12 m for single plantations, and 24 × 18 m for mixed ones). A randomized block design with six treatments was used. Within the mixed plot, the seedlings were planted in columns of three species each, 48 plants per plot randomized by species within the plot with a 3-m spacing between individual seedlings.

The treatments were: 1—monoculture of *Plathymenia reticulata* with complete fertilization; 2—monoculture of *P. reticulata* with 80% fertilization + inoculation with *Rhizobium* and AMF; 3—monoculture of *E. camaldulensis* with complete fertilization; 4—monoculture of *E. camaldulensis* with 80% fertilization + inoculation with AMF; 5—mixed plantation of *E. camaldulensis*, *P. reticulata* and *T. heptaphylla* with complete fertilization; 6—mixed plantation of *E. camaldulensis*, *P. reticulata* and *T. heptaphylla* with 80% fertilization + inoculation with *Rhizobium* and AMF. Complete fertilization consisted in triple superphosphate (500 kg ha^{-1}), KCl (382 Kg ha^{-1}), $MgSO_47H_2O$ (50 kg ha^{-1}), $ZnSO_47H_2O$ (46.8 kg ha^{-1}), $Mo7O_24H_2O$ (1.76 kg ha^{-1}), urea (222 kg ha^{-1}) following Somasegaran and Hoben (1985) and was applied at the beginning of the plantation.

Inoculation

The leguminous *P. reticulata* was inoculated with *Rhizobium* and AMF. The *Rhizobium* strain BHCB-AGRh2, which has slow growth, was previously isolated from nodules of *P. reticulata*, collected at the forest reserve, and pre-screened for its effectiveness under greenhouse, nursery and field conditions (Scotti and Correa 2004). The bacterial inocula were provided at 1 ml per pot (10^7 cfu ml^{-1}) according to Somasegaran and Hoben (1985).

Arbuscular mycorrhizal inoculation was accomplished by placing into each pot 1 ml of suspension composed by 150 spores ml^{-1} in a total of three species: *Gigaspora margarita* Becker and Hall, *Scutellospora heterogama* (Nicol. and Gerdemann) Walker and Sanders, and *Glomus brohultii* Sieverding and Herrera isolated from pot cultures with *Brachiaria decumbens* Stapf. The AMF strains used were from the Biological Science Institute, Belo Horizonte (BHCB) culture collection. The plant species *T. heptaphylla* was not inoculated and *E. camaldulensis* was inoculated with AMF.

Analysis of plant growth under field conditions

Plant height and diameter of *P. reticulata* were measured in field over 2 years. The sampling took place within the dry period (May–October 2003), the rainy period (November 2003–March 2004), and the dry period (May–October 2004). Growth data in different treatments were compared by Tukey's test ($P < 0.05$) following ANOVA.

Root colonization

Roots of *P. reticulata*, *T. heptaphylla* and *E. camaldulensis* were collected at experimental site by excavating from the trunk to the lateral root system of each tree. Root samples were

harvested around each species tree from 10 to 40 cm depth, from three trees at each block. Roots were fixed in FAA solution (5 ml of formaldehyde, 5 ml of acetic acid and 90 ml of ethyl alcohol) until samples could be processed. Roots were stained and assessed for mycorrhizal infection as follows. Roots were cleared and stained with Trypan Blue (Phillips and Hayman 1970). Roots were cut into 1 cm segments and 31 cm root fragments were examined per sample for their AM status under a compound microscope (100×). If at least one root segment was found to contain fungal mycelia, arbuscules or vesicles, then the sample was considered as an AM plant, recorded as "+". Plants were recorded as non-mycorrhizal ("−") when neither arbuscules/vesicles nor fungi form mycelia were detected in their root cortical cells. Quantification of mycorrhiza colonization was done according to McGonigle et al. (1990), and results were expressed as percentage of colonized segments. *Eucalyptus* root samples were checked for natural ectomycorrhizal colonization and quantified by a line intercept method (Brundrett et al. 1996). Fine roots with a fungal mantle and Hartig net were scored as ectomycorrhizal colonization. Dual mycorrhizae (AM and ECM) were observed and individually recorded for calculation. Percent colonization was arcsin $(x/100)^{1/2}$ transformed. The data were subjected to one-way ANOVA using the MINITAB software version 13.2 and means were compared by Tukey's test ($P < 0.05$).

AM fungal communities distribution

Three soil sub-samples were harvested around each tree species and mixed together, because spores can be aggregate distributed (Picone 2000). Composite samples were collected from three trees at each block in August 2003 (dry period) and March (rainy period) 2004 and October (dry period) 2004. *Plathymenia reticulata* and *E. camaldulensis* rhizospheric soils were collected at 10 cm depth for analysis of AMF spores. AMF spores were recovered from 100-g triplicate sub-samples of soil of each treatment in the field, separated from the soil by wet sieving and decanting (Gerdemann and Nicolson 1963) and sucrose centrifugation (Walker et al. 1982); analysed data were expressed as number of spores/100 g of dry soil. Healthy spores were counted. Each spore type was mounted sequentially in PVLG (polyvinyl alcohol–lactic acid–glycerol) (Koske and Tessier 1983) and a mixture of PVLG and Melzer's reagent (Morton 1988) to identify and to obtain permanent slide specimens.

The morphological properties and subcellular structures were observed under a light microscope at 100× to 1000× magnifications. Identification was based on spore colour, size, surface ornamentation and wall structure, with reference to the descriptions provided by Schenck and Pérez (1988), International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM, West Virginia, USA, http://invam.caf.wvu.edu) and the original species descriptions. Spore numbers were square rooted transformed and statistically analyzed. The data were subjected to one-way ANOVA using MINITAB software version 13.2, and means were compared by Tukey's test ($P < 0.05$).

The frequency of occurrence of each AMF species was computed with the formula: $X_i/X_0 \times 100$, where $X_i =$ the number of spores for an individual species and $X_0 =$ the total number of spores. The frequency of occurrence of each species was used to calculate the Shannon diversity index (H), species richness (S) and evenness (E), according to Magurran (1988). Differences in AM diversity among treatments were determined with ANOVA using the MINITAB software version 13.2, and means were compared by Tukey's test ($P < 0.05$).

Results

Soil and climate characteristics

The sandy loam soil at the research site was acid with a pH ranging between 5.9 and 6.4 in the topsoil (Table 1). Soil pH was similar among the experimental area soils (~6). Textural composition was as follows: coarse sand = 49%, fine sand = 35.4%, silt = 0.6% and clay = 15%. The highest levels of silt were detected in the inoculated mixed model. CEC was high in the experimental area and the percentage of BS was medium. Soil phosphorus content was higher in *E. camaldulensis* monocultures than in the other treatments and reference sites.

The higher pluviometric index (Fig. 1) was found in January in contrast to the drought observed between March and October.

Plant growth

When inoculated with *Rhizobium* and AMF, both in monoculture and in mixed stands with *E. camaldulensis* and *T. heptaphylla*, the legume *P. reticulata* showed higher height growth than non-inoculated plants (Fig. 2a). With respect to *P. reticulata* diameter growth, the inoculated monoculture differed from uninoculated mixed plantation (Fig. 2b). Thus, after 2 years of cultivation, there was an effect of inoculation on *P. reticulata* height growth, both in monoculture and mixed plantation.

Root colonization by AM fungi

Plants in monocultures presented lower hyphae, vesicles and arbuscular colonization than in mixed plots (Fig. 3).

Plathymenia reticulata did not show significant differences in hyphal colonization. During the rainy period an increase in vesicles production was observed in mixed plantations. The percentage of arbuscules in *P. reticulata* roots increased significantly in the

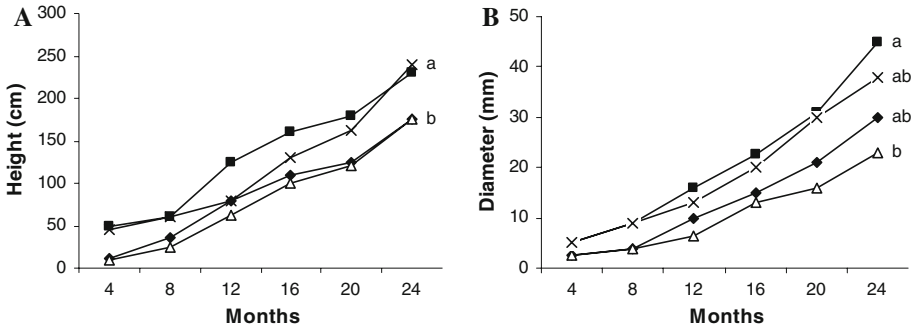

Fig. 2 Height (**a**) and diameter (**b**) growth of *Plathymenia reticulata* under different treatments after 2 years of field plantation at Jaíba. Treatments: ◆ single plantation of *P. reticulata*, ■ single plantation of *P. reticulata* inoculated with Rhizobia and AMF, △ mixed plantation of *P. reticulata* + *E. camaldulensis* + *Tabebuia heptaphylla*, × mixed plantation of *P. reticulata* (Rhizobia + AMF) + *E. camaldulensis* (AMF) + *T. heptaphylla*. *Different letters* indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$)

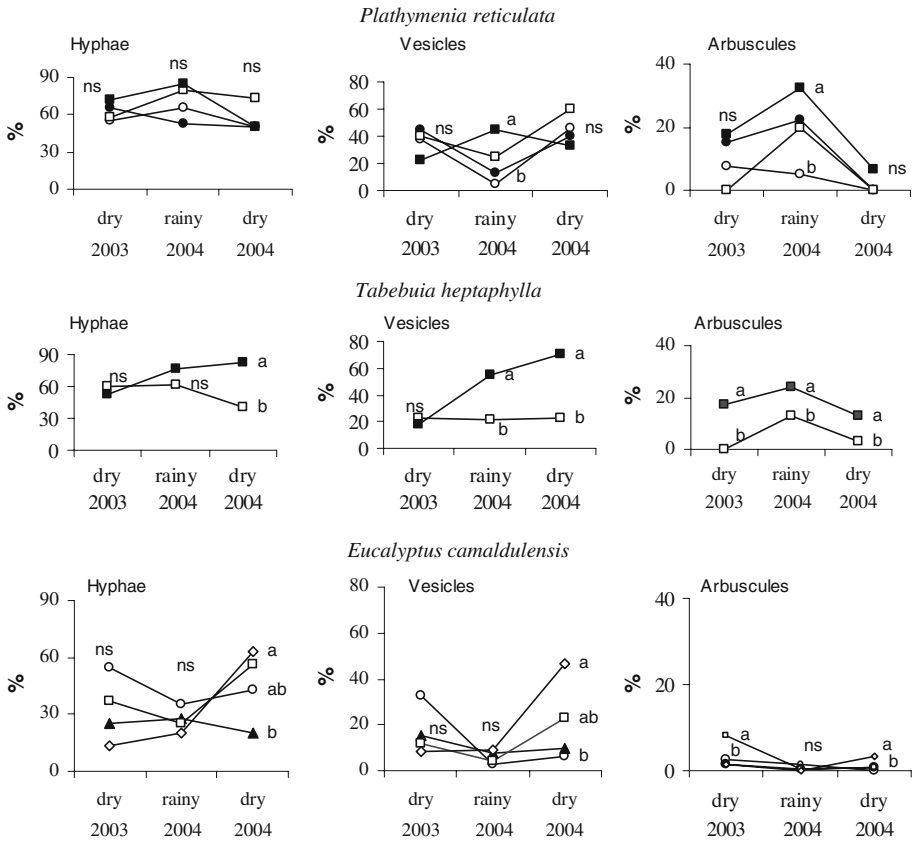

Fig. 3 Mycorrhizal colonization during dry and rainy periods at monocultures and mixed plantations at Minas Gerais, Brazil. Treatments: ○ monoculture, ● monoculture (*Rhizobium* + AMF), □ mixed plantation, ■ mixed plantation (*Rhizobium* + AMF), ▲ *E. camaldulensis* monoculture (AMF), ◇ mixed plantation (AMF). Dry 2003 and 2004 (dry seasons), and rainy 2004 (rainy season). *Different letters* indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$)

rainy season, especially in mixed and inoculated plants, compared to the non inoculated monoculture (Fig. 3).

The roots of *T. heptaphylla* (which was not inoculated with AMF) showed significantly higher hyphal colonization levels (83.33%) when intercropped with inoculated plants of *P. reticulata* and *E. camaldulensis* in the last dry season (Fig. 3). Hyphae, vesicles and arbuscules increased during the rainy season and continued to increase till the last dry period. In general, *T. heptaphylla* mixed with inoculated plants presented significantly higher values of root colonization in comparison with plants mixed with non-inoculated intercropping plants.

Plants inoculated (*P. reticulata*) and noninoculated (*T. heptaphylla*) had higher percentage of vesicles and arbuscules than those of noninoculated treatments in the rainy season but no significative difference was seen in the second dry season for the legume.

Eucalyptus camaldulensis presented both types of root colonization, by AMF and ECM. During the rainy season the AMF colonization decreased in all treatments, in monocultures as well as mixed plantations inoculated or not (Fig. 3), while the native ECM colonization

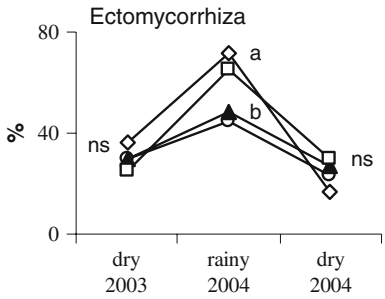

Fig. 4 Ectomycorrhizal colonization of *E. camaldulensis* monocultures and mixed plantations at Minas Gerais, Brazil, during dry and rainy periods. Treatments: ○ monoculture, ▲ monoculture (AMF), □ mixed plantation, ◇ mixed plantation (AMF). *Different letters* indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$)

levels improved (attaining 72%; Fig. 4). In contrast, in the dry seasons ECM was reduced up to 16.6% and AMF colonization increased, especially vesicle colonization, which reached 46.6% in the inoculated intercropping plot during the last dry season (Fig. 3).

Spore number and species richness of AM fungi

Among AMF species in the studied areas a total of 14 taxa was found. Of these, one belongs to the genus *Glomus*, eight to *Acaulospora*, three to *Gigaspora* and two to *Scutellospora* (Table 2).

Mean spore number was found to be significantly higher in the rainy season compared with the dry season (Fig. 5; Table 2).

The dominant Glomeromycota was Glomeraceae with only one species, *Glomus brohultii*, found during all samplings, in all treatments (Fig. 5; Table 2). During the dry season the spore numbers of this species did not differ; whereas during the rainy season, the highest spore density was found in the mixed plantation inoculated with *Rhizobium* and AMF (treatment 6).

Species of Gigasporaceae (Fig. 5), especially *Gigaspora margarita* (Table 2), were dominant in the rhizosphere of *P. reticulata* in the mixed plantation inoculated with AMF and *Rhizobium* in both studied seasons: rainy and dry.

Acaulosporaceae did not show significant differences among treatments in either of the studied seasons (Fig. 5), presenting the highest species richness (seven) despite not being inoculated (Table 2).

The AMF spore number was high in the *P. reticulata* inoculated plants in monoculture as well as in mixed plantations. This pattern, however, was not observed in the *E. camaldulensis* monoculture inoculated with AMF.

In the rhizospheres of *Eucalyptus*, sporulation was observed to increase in the rainy season when compared to the dry period (Table 2). During the rainy season, the total spore numbers in treatments 3 and 4 (*E. camaldulensis* and *E. camaldulensis* + AMF) were higher than in other treatments, except in the inoculated mixed plantation; these treatments, however, presented the lowest species richness and diversity.

Species richness ranged from three to eight. The highest species richness was found in the mixed plantations. The lowest species richness was found in *Eucalyptus* monocultures, presenting only three to six species (Table 2). The rooting-soil of *P. reticulata* showed

Table 2 Spore number (per 100 g soil) from each of the arbuscular mycorrhizal fungi (AMF) species distinguished in the soil samples from the different treatments

	AMF species	Treatments[a]					
		1	2	3	4	5	6
Rainy period	Gigasporaceae						
	Gigaspora margarita Becker and Hall	4.2b	ND	ND	0.86b	11.4ab	55.89a
	Gigaspora sp. 1	ND	2.5	ND	ND	ND	ND
	Scutellospora coralloidea (Trappe, Gerd. and Ho) Walker and Sanders	ND	0.87	ND	ND	ND	ND
	Acaulosporaceae						
	A. delicata Walker, Pfeiffer and Bloss	ND	1.72 NS	ND	ND	0.85	ND
	A. laevis Gerdemann and Trappe	ND	1.71 NS	ND	ND	2.56	ND
	A. mellea Spain and Schenck	2.55b	2.60b	2.57b	ND	16.27a	3.44b
	A. rhemii Sieverding and Toro	1.74ab	0.85b	1.71ab	ND	3.85ab	9.43a
	A. scrobiculata Trappe	8.73ab	ND	ND	9.5ab	0.85b	14.57a
	Acaulospora sp. 1	5.97 NS	0.85	5.14	5.12	9.53	5.05
	Acaulospora sp. 2	0.87b	ND	ND	ND	ND	4.28a
	Glomeraceae						
	Glomus brohultii Sieverd. and Herrera	140.18ab	95.43b	203.41a	198.63a	117.94ab	217.97a
	Total spore number (*N*)	164.24ab	106.53b	212.83ab	214.11ab	163.25ab	310.63a
	Species richness	6	7	3	4	8	8
	Evenness	0.40 NS	0.27	0.22	0.17	0.52	0.42
	Diversity	0.68ab	0.45ab	0.36ab	0.26b	1.05a	1.26a
Dry period	Gigasporaceae						
	Gigaspora margarita Becker and Hall	2.5b	ND	2.5b	ND	2.5b	32.5a
	Gigaspora sp. 1	ND	2.5 NS	ND	2.5	ND	ND
	Scutellospora cerradensis Spain and Miranda	ND	ND	ND	ND	ND	ND
	Scutellospora coralloidea (Trappe, Gerd. and Ho) Walker and Sanders	ND	ND	ND	ND	2.5	ND

Table 2 continued

AMF species	Treatments[a]					
	1	2	3	4	5	6
Acaulosporaceae						
A. delicata Walker, Pfeiffer and Bloss	2.5 NS	ND	ND	ND	2.5	ND
A. laevis Gerdemann and Trappe	2.5 NS	2.5	ND	ND	8.75	ND
A. mellea Spain and Schenck	10a	5b	13.8a	ND	9.17a	ND
A. rhemii Sieverding and Toro	ND	ND	ND	ND	ND	2.5 NS
A. scrobiculata Trappe	15.83b	22.5b	20b	35.83ab	22.5b	43.13a
Acaulospora sp. 1	3 NS	ND	3	ND	3	ND
Acaulospora sp. 2	ND	2.5 NS	ND	ND	ND	5
Entrophosporaceae						
Entrophospora infrequens (I. R. Hall) R. N. Ames and R. W. Schneid. emend. Oehl and Sieverd.	ND	ND	7.5 NS	ND	ND	10
Glomeraceae						
Glomus brohultii Sieverd. and Herrera	125b	148.3a	123.3b	93.33b	75.83b	146.9a
Total spore number (*N*)	161.33b	183.3ab	170.1ab	129.16ab	126.75ab	240.03a
Species richness	7	6	6	3	8	6
Evenness	0.44 NS	0.41	0.57	0.64	0.60	0.65
Diversity	0.91b	0.77b	0.98b	0.85b	1.34a	1.33a

Values are mean of three field plot replicate. Data within a row followed by the same letter indicates that the values do not differ significantly by one-way ANOVA and Tukey's HSD test ($P \leq 0.05$)

ND, not detected

[a] Treatments: 1, *P. reticulata* monoculture; 2, *P. reticulata* monoculture (*Rhizobium* + AMF); 3, *Eucalyptus* monoculture; 4. *Eucalyptus* monoculture (AMF); 5, mixed plantation; 6, mixed plantation (*Rhizobium* + AMF)

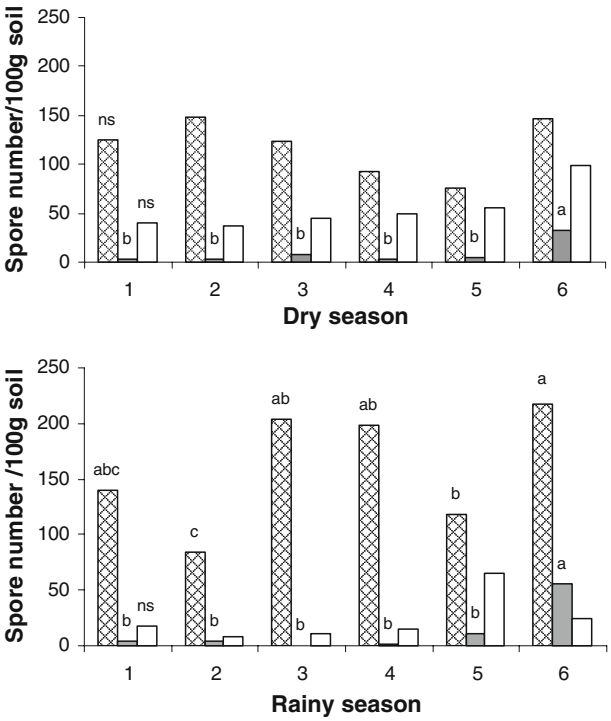

Fig. 5 Frequency of occurrence for the members of Acaulosporaceae, Gigasporaceae and Glomeraceae in rhizosphere of *P. reticulata* in treatments (1, 2, 5, 6), and in rhizosphere of *E. camaldulensis* (3 and 4) at Minas Gerais, Brazil. Treatments: (1) *P. reticulata* monoculture, (2) *P. reticulata* monoculture (*Rhizobium* + AMF), (3) *Eucalyptus* monoculture, (4) *Eucalyptus* monoculture (AMF), (5) mixed plantation, (6) mixed plantation (*Rhizobium* + AMF). Glomeraceae (*hatched*), Gigasporaceae (*grey*) and Acaulosporaceae (*white*). *Different letters* indicate significant differences as determined by Tukey's HSD test ($P \leq 0.05$)

more AMF species richness than *Eucalyptus* (Table 2) and AMF diversity was higher in mixed plantations (Table 2).

Discussion

The soil analyses revealed low organic matter content (mean 0.8%) in the experimental area. These levels are comparables to those of foredunes (0.8%) in Brazil (Córdoba et al. 2001). The low soil fertility seems to account for the plants having a high AM dependency which minimizes hydric stress and nutrient deficit as found by Tarafdar and Praveen-Kumar (1996). Lower CEC results from the sandy texture and low soil clay content.

Plathymenia reticulata plants grew better when inoculated with *Rhizobium* and AMF as reported by Manjunath et al. (1984), Faria et al. (1995), Marques et al. (2001), Gross et al. (2004), and Pagano et al. (2007). It is known that dual inoculation generally increases plant growth to a greater extent than inoculation with only one symbiont (Chalk et al. 2006), and we suggest that *P. reticulata* be inoculated with *Rhizobium* and AMF for growth improvement. The lowest growth of *P. reticulata* uninoculated under mixed plantation is in agreement with other reports (Marques et al. 2001; Tian et al. 2003).

The dominance of hyphae and vesicles in the roots of *T. heptaphylla* in inoculated treatment suggests a nurse effect of inoculated plant species, like *P. reticulata*, on the non-inoculated *T. heptaphylla.* Leguminous plants have a much greater infectivity potential (Tarafdar and Praveen-Kumar 1996). Carneiro et al. (1998) found high root colonization in the same *Tabebuia* species and in *Tabebuia serratifolia* Rolfe, which was reported as responsive to P when mycorrhizal, presenting high arbuscular and vesicular colonization

(Siqueira and Saggin-Júnior 2001). Similar AM colonization in *Tabebuia roseo-alba* (Ridl.) Sand. seedlings in greenhouse was showed by Zangaro et al. (2003).

We note that there was a seasonal effect related to mycorrhizal colonization and AM species diversity in the study area.

The increase in the percentage of arbuscules in the rainy season (in the native plants) may be related to the improvement of soluble nutrients released through litter decomposition (Scotti and Correa 2004). Mycorrhizal root colonization was always higher in *P. reticulata* plants inoculated in the mixed model than in other treatments, and the presence of arbuscules was observed in the three sampled periods.

Eucalyptus camaldulensis showed a lower colonization (hyphae, vesicles and arbuscules) during the rainy season, unlike the colonization of the other two plant species analyzed. However, during the rainy season, ECM increased. This may be explained by the periodical death of ECM when water is limited and the demand by the trees is high, which have to form again when drought ends before being able to resume nutrient uptake (Courty et al. 2006).

This suggests that *Eucalyptus* could obtain nutrients mainly from litter decomposition through ECM effect during the rainy season. This corroborates results from Santos et al. (2001), who observed an increase in ECM colonization and AMF reduction in *E. camaldulensis*. However, few arbuscules were found in *E. camaldulensis* roots as also confirmed by Santos et al. (2001), probably due to the presence of tannin, which interferes with arbuscules and vesicles observation. *Eucalyptus* usually presents a 40–50% ECM on root apical zones (Marschner and Dell 1994). The decrease of AMF colonization of *E. camaldulensis* in the rainy season and the continuous increasing of AMF colonization on *T. heptaphylla* suggests that the native species could help the AMF re-colonization of *E. camaldulensis*. *Eucalyptus* plants supply the reduction of ECM in the dry periods, increasing AMF colonization. This indicates the importance of AMF in this semi-arid region. The AM colonization of the *Eucalyptus* species studied is in accordance with data showed by Santos et al. (2001) and Dhar and Mridha (2006), who found a similar percentage of colonization in *E. camaldulensis.*

AMF spores were recovered at varying numbers from all treatments investigated, regardless of the inoculation treatment with AMF. The overall AMF spore number in the individual plants ranged from 106 to 214 during the rainy period and 129–183 in the dry season in single plantations reaching up to 310 in mixed and inoculated plantations during the rainy season, while in the dry season 240 spores were found. The number of spores was similar to those previously found. Souza et al. (2003), in the Caatinga region, documented a spore number varying between 34 and 850 spores/100 g soil. Yano-Melo et al. (1997) recorded similar results of 44–271 spores/100 g soil in the São Francisco valley. Albuquerque (2008) identified 29 species of AMF in three areas of Caatinga with different physiognomies (Serra Talhada-Sertão with a hyperxerophytic Caatinga; Caruaru—Agreste with hyperxerophytic Caatinga; Araripina—Chapada with Cerrado). The number of species ranged from 14 to 21, with *Acaulospora* being the most diversified genus. Also, variations were observed on spore number and richness promoted by seasonal changes. Dry periods favored the sporulation of a great number of species, increasing the populations of AMF. The results suggest that AMF spore number depends on the seasonal effect and on the diversity of vegetal species cover. Also, interspecific differences in the quality of litter produced by individual plant species can influence the mycorrhizal community (Conn and Dighton 2000), increasing its diversity.

In diverse and stable plant ecosystems Gigasporaceae occurs at low spore densities and high species richness (Siqueira et al. 1989; Lovelock et al. 2003; Zhao et al. 2003). In our

study, we found three to four species of Gigasporaceae, whereas an increased spore number of *Gigaspora* in the mixed plantation inoculated with AMF and *Rhizobium* were found in both studied seasons. This can be attributed to the fact that *G. margarita* was included in the inoculum and also the preference of *P. reticulata* for this AMF species.

Within a regional context, AMF species richness at our sites was lower in comparison to natural Caatinga areas. The lowest AMF species richness was found in *E. camaldulensis* stands followed by *P. reticulata.* The highest species richness was found in mixed plantations. These values are considerably lower than those reported by Souza et al. (2003), who found 24 taxa in Caatinga, and Yano-Melo et al. (1997, 2003), who recorded similar results of 14–15 AMF species in the São Francisco valley (Brazil).

Glomeraceae was dominant in our field experiments with a single species *Glomus brohultii*. This predominance is frequently observed under semi-arid and arid habitats (Stutz et al. 2000; Kennedy et al. 2002; Muthukumar and Udaiyan 2002; Panwar and Tarafdar 2006). *Glomus* has been considered as the best adapted genus for habitats subjected to drought (Haas and Menge 1990). *Glomus fasciculatum* proved to be the most effective AMF under arid conditions (Tarafdar and Praveen-Kumar 1996). *Glomus* promoted the growth of *Eucalyptus dives* and *Eucalyptus viminalis* (Adjoud et al. 1996). Moreover, Chen et al. (2007) found mostly *Glomus* species from 155 different eucalypt species plantations.

The literature shows that *Glomus* and *Acaulospora* are more competitive in the infection process because they are able to colonize the roots from all inoculum types (spores, colonized root fragments and hyphae), whereas *Gigaspora* and *Scutellospora* do it mainly from spores (colonization strategy) and poorly by extraradical hyphae (Klironomos and Hart 2002). This would explain in part the predominance of these two genera (*Glomus* followed by *Acaulospora*) observed in this work. On the other hand, these genera may be selected by host tree species effect.

In this study, *Eucalyptus* monocultures, especially in the rainy period, decreased AMF diversity, enhancing the dominance of *Glomus brohultii*. Carrenho et al. (2008) stated that *Eucalyptus* is ecologically damaging to many native plant species and its litter inhibits the annual understory vegetation. Our findings support the hypothesis that ECM develop more intensively in the organic residues of their autotrophic partners (Read 1991) and degrade the recalcitrant litter. The composition and properties of plant litter are essential controlling factors for organic matter formation and soil humification (Kögel-Knabner 2002).

The different colonization patterns of *Eucalyptus* during both rainy and dry periods may be explained in part by the lesser tolerance of ECM to dry conditions. Soil moisture is an important control of soil microbial activity through osmosis or controlling nutrient supply (Killham 1994). On the other hand, AMF usually increase host growth rates during drought by affecting nutrient acquisition and possibly hydration (Giri et al. 2003); however, colonization by different fungi affects water use efficiency differently (Simpson and Daft 1990). Moreover, according to other authors, the AMF-ECM succession is simply related to spatial competition for infection sites (Chilvers et al. 1987).

In this study, mixed plantations inoculated with rhizobia and AMF promote the growth of *P. reticulata*, supporting the observation that mycorrhizal plants acquire more nutrients, and are able to share them via an underground network of hyphal connections linking individuals within and between species (Simard et al. 2003). Co-occurring plant species respond differently to each AMF species and plant biomass increases due to their presence (van der Heijden 2003). Moreover, AMF are efficient in improving growth and nitrogen (N) content in legumes (Barea et al. 2002), which can transfer N to eucalypt plants by AMF hyphae interconnection (Rodrigues et al. 2003).

Our findings corroborate the hypothesis that vegetal community influences the community of AMF (van der Heijden et al. 1998) and mixed plantations that increase plant diversity also increase AMF spore number and diversity. On the other hand, mycorrhizal associations are potential factors determining diversity in ecosystems (Read 1990) that warrant ecosystem sustainability (Hart and Klironomos 2003).

Conclusion

The present study constitutes a first evaluation of mycorrhizal distribution for the successful establishment of the native species *P. reticulata* on cutaway dry forest in Brazil.

It demonstrated that the introduction of *P. reticulata* and *E. camaldulensis* inoculated and mixed with *T. heptaphylla* promotes a higher AMF diversity, percentages of AMF colonization and native plant growth regardless of the season. Stem height was found a better indicator of inoculation response than diameter for *P. reticulata* plants.

This study also contributed to our understanding of the basis for observed response of different plant species to the same site conditions, showing that AM and ECM colonization of *E. camaldulensis* varies with seasons.

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