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Species richness of arbuscular mycorrhizal fungi in forests with *Araucaria* in Southern Brazil

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ABSTRACT - (Species richness of arbuscular mycorrhizal fungi in forests with *Araucaria* in Southern Brazil). This study aimed to characterize species richness of arbuscular mycorrhizal fungi (AMF) in three distinct forests with *Araucaria angustifolia* (native forest, reforestation with *A. angustifolia* and plantation with *Pinus taeda*) in the "Floresta Nacional de São Francisco de Paula", State of Rio Grande do Sul, also accounting for seasonal variation. Six soil samples from each forest were collected from *Araucaria angustifolia* rhizosphere in four seasons during a one-year period, for identification of the AMF species. Spores were extracted by wet-sieving followed by centrifugation in water and 50% sucrose solution. The greatest richness (16 species) was found in the *Araucaria* reforestation and the lowest in the native forest (eight species). The season with higher species richness was different in each site. The most representative genera in all forests were *Acaulospora* and *Glomus*. Historical aspects and plant species composition can account for some of the differences among sites.
Key words: AMF, *Araucaria angustifolia*, *Pinus taeda*, reforestation

RESUMO - (Riqueza de espécies de fungos micorrízicos arbusculares em florestas com *Araucaria* no sul do Brasil). Este estudo teve como objetivo caracterizar a riqueza de espécies de fungos micorrízicos arbusculares (FMA) em três florestas distintas com *Araucaria angustifolia* (mata nativa, reflorestamento com *A. angustifolia* e plantação com *Pinus taeda*), em função da variação sazonal, na Floresta Nacional de São Francisco de Paula, Rio Grande do Sul. Seis amostras de solo foram coletadas da rizosfera de *Araucaria angustifolia*, em cada floresta, nas quatro estações, durante um ano, para identificação das espécies de FMA. Os esporos foram extraídos através de peneiramento úmido e centrifugação em água e solução de sacarose 50%. A maior riqueza (16 espécies) foi encontrada na plantação de *Araucaria* e a menor na floresta nativa (oito espécies). Os gêneros que mais ocorreram foram *Acaulospora* e *Glomus*. A estação com maior riqueza de espécies foi diferente em cada local. Atribuiu-se ao histórico das áreas e à composição das espécies vegetais as diferenças encontradas entre os três locais de coleta.
Palavras-chave: *Araucaria angustifolia*, FMA, *Pinus taeda*, reflorestamento

Introduction

Araucaria angustifolia (Bert.) O. Ktze. (Araucariaceae) is one of the few gymnosperms in Brazil, and it occurs as an emergent tree component of the mixed ombrophyllous forests (Teixeira *et al.* 1986) or, simply, *Araucaria* forests. Its geographical distribution includes Argentina and Brazil, where it is concentrated in the southernmost States of Paraná, Santa Catarina and Rio Grande do Sul. It is long known that the species establishes a mutualistic association with arbuscular mycorrhizal fungi (AMF) (Milanez & Monteiro 1950, Oliveira & Ventura 1952), but few studies have been conducted in order to characterize the AMF species associated to the species under natural conditions. The first AMF survey in *Araucaria* rhizosphere was made on individuals growing in a botanical garden in São Paulo (Bononi *et al.* 1990), and 15 species were found. Ten years later, Breuninger *et al.* (2000) compared the richness of species between an area of *Araucaria* forest and an adjacent grassland in the northern highlands of Rio Grande do Sul, and found a two-fold difference in the number of species of AM fungi: 13 in the former and six in the latter. When comparing a native forest with *Araucaria* and a reforested area with both *Araucaria* and *Pinus* in the State of São Paulo, Moreira-Souza *et al.* (2003) found a greater number of AMF spores in the native forest (21 species) than

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in the reforested area (14 species). Recently, Moreira *et al*. (2007b) found 25 AMF species three *Araucaria* ecosystems: a native forest (15 species), an *Araucaria* reforestation (13 species) and a burned *Araucaria* reforestation (13 species).

The slow growth rate of *Araucaria angustifolia*, its common association with AMF (*e.g*., Oliveira & Ventura 1952, Muchovej *et al*. 1992, Breuninger *et al*. 2000, Moreira-Souza *et al*. 2003, Moreira *et al*. 2006), and its strong growth response to inoculation with AM fungi (Moreira-Souza & Cardoso 2002, Zandavalli *et al*. 2004) suggest that the success of any plantation effort, aiming either the conservation or the sustainable use of the species, might greatly depend on ensuring that the young plants are colonized by the fungal partners.

In order to know which AMF species can be inoculated and to characterize the benefits of the fungus species to *Araucaria angustifolia* seedlings, we need to survey the AMF species that are associated with *A. angustifolia* in different locations. In this study, we aimed to briefly characterize species richness of arbuscular mycorrhizal fungi (AMF) associated to *A. angustifolia* in the "Floresta Nacional de São Francisco de Paula", State of Rio Grande do Sul, also accounting for the seasonal variation of such richness.

Material and methods

The study was conducted at a National Forest (29°24'S and 50°22'W, 912 m above sea level) located in the city of São Francisco de Paula, Rio Grande do Sul, Brazil. The annual rainfall is around 2,469 mm, and an annual dry season is not present (Mota *et al*. 1970). The mean temperature is about 14.5 °C, and the means of the highest and lowest temperatures are 20.3 °C and 9.9 °C, respectively. According to Fernandes & Backes (1998), the soils in the region are Cambisols, characterized by fine texture, low pH and high levels of exchangeable Al and organic matter. A native forest and both an *Araucaria* and a *Pinus* plantation were chosen for this study. Selected soil characteristics for each specific site are shown in table 1. The distance between any two sites varied between 2.5 and 3.0 km. The *Pinus taeda* (Pinaceae) plantation is approximately 40 years old, and shows a pronounced occupation by several native tree species, including *A. angustifolia* and some species of Aquifoliaceae, Myrtaceae, Lauraceae, Podocarpaceae and Sapindaceae. The *Araucaria* reforestation is approximately 50 years old, with the upper canopy dominated by *A. angustifolia* and others species belonging to the Aquifoliaceae, Lauraceae, Myrsinaceae, Myrtaceae, Rutaceae and Sapindaceae. The upper canopy of the native forest is comprised by *A. angustifolia* and by other species of the same families found in the *Araucaria* reforestation.

Six soil samples were obtained from each of the three forest sites, in August and November 1999 and in February and May 2000. Each sample (500 g) consisted of four sub-samples, taken from the rizosphere of mature individuals of *Araucaria*, after litter removal, at a depth of 0-20 cm. Samples

Table 1. Chemical characteristics of the soil in the three forest sites at the "Floresta Nacional de São Francisco de Paula", Rio Grande do Sul, Brazil.

	Pinus plantation	*Araucaria* reforestation	Native forest
Clay (%)	31.5	26.0	27.7
pH (in water)	4.22	4.15	4.65
Organic matter (%)	6.57	6.05	5.32
Cation exchange capacity (cmol l^{-1})	22.9	22.5	19.6
Base saturation (%)	5.00	18.7	51.5
Al saturation (%)	33.9	22.8	5.35
Al+H (cmol l^{-1})	21.7	18.2	9.25
N (%)	0.18	0.17	0.15
P (mg l^{-1})	3.62	2.90	2.35
K (mg l^{-1})	48.2	117	132
Al (cmol l^{-1})	7.77	5.17	1.05
Ca (cmol l^{-1})	0.55	2.97	7.50
Mg (cmol l^{-1})	1.80	0.87	2.25

were stored in polyethilene bags, under 4 °C temperature. All six samples from each site were pooled and homogenized. From this mix, 100 g soil sample was used to extract the spores. Spores were extracted by wet sieving (Gerdemann & Nicolson 1963), followed by centrifugation in water and 50% sucrose solution (Jenkins 1964). The material were mounted on permanent slides using PVLG (polyvinyl alcohol, lactic acid, glycerol) and PVLG mixed with Melzer's reagent (iodine, chloral hydrate, potassium iodide and water), and identified by comparison to "vouchers" in slides. Active cultures and reference culture were obtained on INVAM (2000). The Jaccard index was used to evaluate species similarity between sites, and was calculated as $c / (a + b - c)$, where a and b are the species richness of each of the two sites being compared and c is the number of species they have in common.

Results and Discussion

The AMF species identified in each forest and season are listed in table 2. The native forest had the lowest overall species richness, with only eight AMF taxa recorded: four *Acaulospora* and four *Glomus* species. Nine taxa were found in the *Pinus* plantation, distributed into three genera: six *Acaulospora*, two *Scutellospora* and one *Glomus*. The highest number of species and genera was found in the *Araucaria* reforestation, with 16 taxa, distributed into five genera: eight *Acaulospora*, three *Glomus*, three *Scutellospora*, one *Entrophospora* and one *Gigaspora*. While both the *Pinus* plantation and the *Araucaria* reforestation had several species which were detected in all seasons, only *Glomus sinuosum* was identified in all seasons in the native forest.

Table 2. Arbuscular mycorrhiza fungal (AMF) taxa found in three forest sites at the "Floresta Nacional de São Francisco de Paula", RS, Brazil. Seasons: Sp = spring, Su = summer, Fa = fall and Wi = winter.

AMF taxa	*Araucaria* reforestation				*Pinus* plantation				Native forest			
	Sp	Su	Fa	Wi	Sp	Su	Fa	Wi	Sp	Su	Fa	Wi
Acaulospora bireticulata Rothwell & Trappe		X						X				
Acaulospora foveata Trappe & Janos		X		X	X		X	X				
Acaulospora koskei Błaszk					X	X	X	X				
Acaulospora lacunosa Morton		X	X		X	X	X	X				
Acaulospora mellea Spain & Schenck	X	X	X	X	X	X	X	X			X	X
Acaulospora scrobiculata Trappe	X	X	X	X						X	X	
Acaulospora spinosa Walker &Trappe	X	X	X	X								
Acaulospora tuberculata Janos & Trappe		X	X								X	X
Acaulospora sp.1												X
Acaulospora sp.2		X										
Acaulospora sp.3					X							
Entrophospora infrequens (Hall) Ames & Schneider	X											
Gigaspora decipiens Hall & Abbott		X										
Glomus ambisporum Smith & Schenck	X	X	X	X						X	X	X
Glomus clarum Nicolson & Shenck										X		
Glomus pansihalos Berch & Koske										X		
Glomus sinuosum (Gerd. & Bakshi) Almeida & Schenck	X	X	X	X	X		X	X	X	X	X	X
Glomus sp. 1	X											
Scutellospora fulgida Koske & Walker		X										
Scutellospora pellucida (Nicolson & Shenck) Walker & Sanders				X								
Scutelospora verrucosa (Koske & Walker) Walker & Sanders				X								
Scutellospora sp.1					X							
Scutellospora sp.2					X							

In mature, undisturbed forests, spores may be relatively less important than other vegetative propagules, and the colonization of new roots is achieved primarily by the soil hyphal networks (Jasper *et al*. 1989). As a result, native forests, which are older and less productive than younger forests, usually have small spore populations and high mycorrhizal colonization levels (Muthukumar *et al*. 2003). The native forest surveyed in this study is older and probably less productive than both the plantation and the reforestation, and apparently with lowest regeneration of tree species in general, which could explain the low number of AMF taxa.

Spore number in the native forest (16.49 spores.g-1) was also low when compared to the *Araucaria* reforestation (40.12 spores g-1), but similar the *Pinus* plantation (13.74 spores g-1). Although these data might be overestimated, due to the possible inclusion of non-infective and dead spores in the count, the comparison among sites is still valid. In contrast to Moreira-Souza *et al*. (2003) and Moreira *et al*. (2007a), but in agreement with Moreira *et al*. (2006), we found greater spore abundance in the *Araucaria* reforestation than in the native forest. These contrasting observations are probably related to historical and structural differences between the examined forests.

Previous studies by Duarte *et al*. (2002) have reported a significantly higher growth rate of *Araucaria angustifolia* seedlings in the same *Pinus* plantation and *Araucaria* reforestation surveyed in the present study. Also, daily mean values of irradiance were higher in the *Pinus* plantation than in the other two sites, suggesting a greater productivity in this forest plantation, compared to the other two forests. It is important to point out that, despite the favorable environment offered by the *Pinus* plantation to the recruitment and growth of young individuals of *A. angustifolia*, this forest site has its overstory dominated by *Pinus taeda*, a characteristically ectomycorrhizal species (Hacskaylo & Vozzo 1971), which may explain the much lower count of AMF spores in this site, compared to the reforestation with *A. angustifolia*.

The *Araucaria* reforestation was the only site where the genera *Entrophospora* and *Gigaspora* were represented in the soil samples. Also, the taxa *Acaulospora* sp. 2, *A. spinosa*, *Glomus* sp. 1, *Scutellospora fulgida*, *S. pellucida* and *S. verrucosa* were exclusively recorded in this site. The variety of native plant species and the relative young age of the *Araucaria* reforestation might be related to its high number of AMF species. According to Mosse & Bowen (1968) and Muthukumar *et al*. (2003), a continuous root grow is associated with a large potential of sporulation.

Acaulospora and *Glomus* accounted for the majority of the taxa associated with the rhizophere of *Araucaria angustifolia*, and were the only two genera sampled in the native forest. On the other extreme, *Gigaspora* spores were poorly represented in all three sites. The soils of the region are acidic (table 1) and, according to Stürmer (1999), *Acaulospora* species are frequently found in such soils, while *Gigaspora* is associated to alkaline ones (Melo *et al*. 1997). The genera *Acaulospora* and *Scutellospora* were more abundantly represented in the two sites with the lowest pH, *i.e*. *Araucaria* reforestation and *Pinus* plantation. *Glomus*, on the other hand, was represented by a larger number of species in the site with the highest pH, *i.e*. the native forest. The greatest overall fertility (table 1) of the native forest probably generated a weaker stimulus to host plant mycorrhizal colonization and, consequently, lower spore abundance and reduced number of taxa. Considering that P availability was low in all three sites, it does not seem that the availability of this specific nutrient is governing the observed differences. One must have in mind, however, that correlating soil characteristics to AMF occurrence is not an easy task, because several other variables may influence fungal sporulation.

The number (23) and identity of the taxa reported in the present study were similar to those of previous surveys of AMF species associated with *Araucaria angustifolia* (Bononi *et al*. 1990, Breuninger *et al*. 2000, Moreira-Souza *et al*. 2003). Recently, Moreira *et al*. (2007b) found a total of 25 taxa in a survey of three *A. angustifolia* ecosystems in the State of São Paulo. Also, in all the before-mentioned studies, *Acaulospora* and *Glomus* were the genera with the greatest number of species.

The Jaccard index of similarity between the native forest and the *Araucaria* reforestation was 0.20, while the value between these two forests compared to the *Pinus* plantation was lower (0.14 in both cases). This is not surprising, due to the similarity in plant species composition between the two *Araucaria* forests (native forest and *Araucaria* reforestation). Although sharing some of the tree species with the other two forests (though most of them in the seedling stage), the *Pinus* plantation, as

already said, is dominated by an ectomycorrhizal tree species, *Pinus taeda*.

The season of the year with the greatest number of taxa varied among areas (table 2): summer for the *Araucaria* reforestation (12 taxa), spring for the *Pinus* plantation (eigh taxa), and summer, fall and winter for the native forest (five taxa). *Glomus sinuosum* was absent only in one season (summer) in the *Pinus* plantation site, and *Acaulospora mellea* just in the spring and summer in the native forest. These two species seem to be the most generalist ones. The fact that some AMF species were found only in a specific season may be due to the fact that different AMF have different sporulation times, a response which has been interpreted as a strategy to reduce direct competition (Gemma *et al.* 1989). The number of taxa found in a site also depends on sample size. In some of the sites and/or seasons, the number of taxa may have been underestimated, due to the assincronic and clumped sporulation of the species (Gemma *et al.* 1989, Friese & Koske 1991). The seasonal sampling was then of particular importance to reach for a more complete survey of the AMF species.

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