

Diversity of arbuscular mycorrhizal fungi in pasture areas in the *Serra do Itajaí* National Park

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ABSTRACT: As obligate biotrophs, arbuscular mycorrhizal fungi (AMF) (Phylum Glomeromycota) may be directly affected by deforestation. The present study evaluated the effect of forest conversion into pastures on AMF diversity within the *Serra do Itajaí* National Park (Santa Catarina, Brazil). Soil samples were collected during two seasons (spring and autumn) from four areas: native forest (NF); two pastures undergoing recovery through application of brushwood (PB) and perch (PP) techniques, respectively; and an untreated pasture (UP). AMF spores were extracted from soil through wet sieving and decanting. Species were identified based on the morphology of the spores. In total, 33 AMF species were observed, distributed into six families and 11 genera. The best-represented genera were *Acaulospora* and *Glomus*, with 14 and 8 species, respectively. Conversion of forests into pastures did not affect mean species richness of AMF between the areas, nor between the seasons. However, the total number of species was higher in the native forest, indicating the negative effect of deforestation on AMF community diversity in the evaluated pasture areas. AMF species diversity was also greater during autumn.

Key words: biodiversity; deforestation; Glomeromycota; pasture

Diversidade de fungos micorrízicos arbusculares em áreas sob pastagens no Parque Nacional da Serra do Itajaí

RESUMO: Os fungos micorrízicos arbusculares (FMA) (Filo Glomeromycota) são biotróficos obrigatórios podendo ser diretamente afetados pelo desmatamento. O presente estudo analisou o efeito da conversão de florestas em pastagens na diversidade de FMA em áreas do Parque Nacional da Serra do Itajaí (Santa Catarina, Brasil). Amostras compostas de solo foram coletadas em duas épocas (primavera e outono) e quatro áreas: floresta nativa (FN); duas pastagens sob recuperação através das técnicas de galharia (PG) e poleiro (PP); e uma pastagem sem tratamento de recuperação (PS). Os esporos de FMA foram extraídos do solo por peneiramento úmido e decantação, e as espécies identificadas com base na morfologia dos mesmos. No total, 33 espécies de FMA foram observadas, distribuídas em seis famílias e 11 gêneros. Os gêneros melhores representados foram *Acaulospora* e *Glomus*, com 14 e 8 espécies, respectivamente. A conversão de florestas em pastagens não afetou a riqueza média de espécies de FMA entre as áreas, ou estações. Porém, o número total de espécies foi maior na FN, indicando um efeito negativo do desmatamento sobre a diversidade de FMA nas pastagens estudadas. A diversidade de FMA foi também maior durante o outono.

Palavras-chave: biodiversidade; desmatamento; Glomeromycota; pastagens

Introduction

Conversion of forests into agricultural land is accepted as the main driver of forest loss, accounting for 80% of deforestation worldwide (Kissinger et al., 2012). A recent study summarized the main drivers of forest loss in South America considering data from the period between 1990 and 2005 (De Sy et al., 2015). The study indicated that during this period, agriculture was responsible for 88.5% of deforested areas, of which pasture represented 71.2%. Particularly, in the Brazilian territory, about 80% of deforestation was associated with pastures planted for cattle grazing.

Historically considered one of the principal forests in the Americas, the Atlantic Forest is listed as a global biodiversity hotspot, with high levels of diversity and endemism (Leão et al., 2014). However, 88.27% of the Atlantic forest has been lost and only 11.23% of it is still conserved, mainly, in the form of small forest fragments (<50 ha) (Ribeiro et al., 2009). Consequently, this ecosystem and its biodiversity are among the most threatened in the world.

Arbuscular mycorrhizal fungi (AMF) (Phylum Glomeromycota) establish a mutualistic symbiosis with the roots of several plants, having a direct impact on the absorption of nutrients from the soil (Stürmer & Siqueira, 2013). These fungi also contribute to alleviating the effect of several biotic and abiotic stresses on host plants, such as the presence of pathogens and water deficits, for example (Stürmer & Siqueira, 2013).

AMF require a physiologically active root to complete their life cycle, which characterize them as obligate biotrophs (Stürmer & Siqueira, 2013). Additionally, although the mycorrhizal symbiosis is not specific, a certain degree of preference between AMF and plant species has been reported (Torrecillas et al., 2012; Van Geel et al., 2016). Therefore, many authors have suggested a direct relationship between above and below ground diversities. In the context of extensive Atlantic Forest deforestation, the present research aimed to evaluate the effect of conversion of forests into pasture areas within the *Serra do Itajaí* National Park, Santa Catarina, Brazil.

Materials and Methods

The present study was conducted at the *Serra do Itajaí* National Park, an Integral Conservational Unit within the Brazilian Atlantic Forest. The Park is located in the valley of *Itajaí*, state of Santa Catarina, Brazil. In the present study,

four sampling areas were selected for evaluation of AMF diversity. These were: native forest (NF); two pasture areas undergoing restoration processes through the introduction of perches (PP) and brushwood (PB), respectively; and an untreated pasture (UP), not undergoing any restoration technique.

The native forest vegetation belongs to the phytoecological region of *Floresta Ombrófila Densa* (Ombrophylus Dense Forest), *Montana* formation. The principal plant species are: *Alchornea triplinervia* (Spreng.) Müll. Arg (Euphorbiaceae), *Alsophila setosa* Kaulf. (Cyatheaceae), *Cedrela fissilis* Vell. (Meliaceae), *Cyathea phalerata* Mart. (Cyatheaceae), *Dicksonia sellowiana* Hook. (Dicksoniaceae), *Guatteria australis* A. St. – Hil. (Annonaceae), *Ocotea puberula* (Rich.) Nees (Lauraceae) and *Vernonanthura discolor* (Spreng.) H. Rob. (Asteraceae).

The perch restoration technique was installed in May, 2013 in a 12 ha pasture area. The perches consisted of *Eucalyptus* stacks measuring 3.5 m, buried 0.5 m in the soil. In the upper part of the area, bamboo stacks, measuring 1.5 m, were placed for birds to alight on. In total, 240 perches were randomly installed (20 perches ha⁻¹).

The brushwood transposition technique was also installed in May, 2013 in a two-hectare pasture area. This method consists of the installation of brushwood modules composed of vegetation remnants that form a biodiversity nucleus providing shelter, nesting sites and food sources for several animal species. In total, 16 modules were installed (80 modules ha⁻¹). The distribution was equidistant, with approximately 11 m between each one. The area occupied by each module was of about 2 m x 2 m (4 m²), with a total of 320 m²ha⁻¹ (3.2%). For the brushwood formation, logs and branches from exotic plant species present in the areas of the Park were used. These included *Pinus* spp. and *Eucalyptus* spp. adult plants, without any cones or fruits. The material was air dried before installing the brushwood technique.

Soil sampling for evaluation of AMF communities was performed in each of the four previously described areas of the Park (Native forest – NF; pasture with perches – PP; pasture with brushwood – PB; and untreated pasture – UP) in spring/2014 and autumn/2015. Five soil samples, each made up of five sub-samples, were collected at each of the sites, per season, at a depth of up to 20 cm from the surface of the soil. Therefore, in total, 40 soil samples were used to evaluate AMF diversity.

AMF spores were extracted from 50 g of soil through wet sieving (Gerdemann & Nicolson, 1963) followed by a

Table 1. Soil chemical characterization of the sampled areas within the *Serra do Itajaí* National Park, Santa Catarina, Brazil. NF: native forest; PB: pasture with brushwood; PP: pasture with perches; and UP: untreated pasture.

Sampled area	Mg	K	H+Al			pH water	Corg	P
			(cmol _c dm ⁻³)					
NF	1.0	0.35	17.6	3.0	4.15	3.9	3.43	24
PB	0.7	0.14	11.9	8.5	1.83	4.6	1.81	20
PP	0.2	0.13	13.4	7.0	1.03	4.2	2.04	20
UP	0.9	0.16	15.6	6.8	1.85	4.5	1.94	20

sucrose gradient (Jenkins, 1964). Spores were differentiated in morphotypes under a dissecting microscope. Following that, they were mounted in permanent microscopy slides using polyvinyl lactoglycerol (PVLG) and PVLG + Reagent of Melzer. Identification of AMF species was performed under a compound microscope based on the morphology of the spores.

Species richness was defined as the number of species found per study area and/or season. Frequency of isolation (F_i %) was expressed as the number of samples from which a certain species was isolated in a particular area and season in relation to the total number of samples (Zhang et al. 2004). Using the F_i values, the dominance of AMF species in each of the studied sites and seasons was estimated, following the classification proposed by Zhang et al. (2004): dominant ($F_i > 50\%$), very common ($30\% < F_i \leq 50\%$), common ($10\% < F_i \leq 30\%$) and rare ($F_i \leq 10\%$) species. In addition, AMF species were classified as generalists (being present in the four areas), intermediate (being present in two or three areas) and exclusive (being present in just one area).

Normality and homogeneity of variance were analyzed using the Shapiro-Wilk and Bartlett statistical tests, respectively. Mean values of AMF species richness were compared using the Scott Knott Test ($p \leq 0.05$). The software used for data processing was R version 3.4.1 (R Core Team, 2013).

Results and Discussion

Considering the two sampling seasons in the four studied areas, 33 AMF species, distributed in six families and 10 genera, were identified (Table 2). This is equivalent to 22% of the total number of AMF species reported for Brazil, and 42% of the number reported for the Atlantic Forest (Zangaro & Moreira, 2010; LBM, 2018). Around 296 AMF species have been described worldwide, of which 153 have already been reported in Brazil. Particularly, in the Atlantic Forest, 78 AMF species have been verified.

Although the number of species reported in the present study might be considered high, comparison of the AMF species richness found in the present study with other publications is difficult. This is partly due to different vegetation and soil characteristics in the study areas, as well as variations in the sampling techniques between studies. Bonfim et al. (2016) recently evaluated the AMF community associated with a Brazilian Atlantic Forest topo sequence in the state of São Paulo (SP). The authors identified 58 AMF species, which is higher than the species richness found in the present study (33). However, Bonfim et al. (2016) collected 35 soil samples in each of three subareas located at different altitudes and in four seasons of the year (autumn, winter, spring and summer). Silva et al. (2016) reported the occurrence of 26 AMF species in four areas under secondary vegetation and agricultural use in part of the Atlantic Forest within the state of Rio de Janeiro (RJ), Brazil. The authors collected three soil samples in each of the four

areas and during two seasons of the year (wet and dry), and complemented the diversity study with trap cultures for each of the areas.

Of the species verified in the present study, 13 were only identified up to the genera level. The family Acaulosporaceae, with 14 species, presented the highest species richness. The latter was responsible for 42% of the total number of species identified. The second most represented family was Glomeraceae with nine species (33% of the total). On the other hand, families with the lowest number of representatives were Ambisporaceae and Archaesporaceae, with just one species detected for each one (Table 2).

In the genera level, *Acaulospora* and *Glomus*, accounted for the highest number of species, with 14 and 8 species, respectively. These two genera have been found to be the best represented in other AMF communities studied. Bonfim et al. (2016) reported these genera as having the highest number of species in Atlantic Forest areas in the *Serra do Mar* State Park, SP, with 16 and nine species detected for *Acaulospora* and *Glomus*, respectively. Silva et al. (2016) in the Atlantic Forest, in *Rio de Janeiro*, Brazil, also reported a similar pattern.

No significant differences in the mean species richness between the sampled areas were detected (Scott Knott $p \leq 0.05$) (Figure 1). On the other hand, considering the same area, mean values of this variable also showed no statistical difference between the seasons.

However, the cumulative species richness in the analyzed soil samples was different between the areas (Figure 1). The highest AMF diversity was observed in the native forest, with 23 species. The latter ecosystem was followed in this ecological index by the perches, brushwood and untreated pastures with 16, 14 and 14 AMF species, respectively (Figure 1). Although the inverse has also been proposed, plant communities have been suggested as regulators of AMF diversity (Castillo et al., 2016). According to van der Heijden et al. (1998), AMF regulate and are regulated by the plant community, with diversity of both groups being positively correlated. In this manner, the results observed in the present study might be explained by the more diverse plant community in the native forest, compared to the other three areas (brushwood, perches and untreated pastures).

On the other side, perturbed environments might have lower AMF species richness, in general, as was the case of the untreated pasture, and the areas under the brushwood and perch restoration techniques. Sites where native vegetation and/or the superficial soil layer have been removed tend to be characterized by a significant loss of AMF propagules (Mergulhão et al, 2009).

The highest number of exclusive AMF species was observed in the native forest. In this area, nine exclusive species were identified, with these being: *Acaulospora tuberculata*, *Acaulospora* sp1, *Glomus heterosporum*, *Glomus* sp2, *Glomus* sp3, *Glomus* sp4, *Glomus* sp5, *Racocetra verrucosa* and *Glomus coremioides*. In the

Table 2. Frequency of occurrence of the arbuscular mycorrhizal fungal (AMF) species in four sampled areas of the Serra de Itajaí National Park, Santa Catarina, Brazil. NF: native forest; PP: pasture with perches; PB: pasture with brushwood; UP: untreated pasture (* species exclusively during spring; **species exclusively during autumn). D: dominant; VC: very common; C: common; R: rare.

AMF Family / Species	Frequency of occurrence (Fi%) and Dominance category							
	Spring, 2014*				Autumn, 2015**			
	NF	PP	PB	UP	NF	PP	PB	UP
Acaulosporaceae								
<i>Acaulospora alpina</i> Oehl, Sykorova & Sieverd**	-	-	-	-	20 C	-	-	40 VC
<i>Acaulospora foveata</i> Trappe & Janos	20 C	40 VC	-	-	20 C	60 D	-	40 VC
<i>Acaulospora gedanensis</i> Blaszk.*	20 C	40 VC	-	-	-	-	-	-
<i>Acaulospora herrerae</i> Furrázola, B.T. Goto, G.A. Silva, Sieverd. & Oehl	-	-	20	-	-	-	20 C	-
<i>Acaulospora lacunosa</i> J.B. Morton	40 VC	-	-	-	60	20 C	60 D	20 C
<i>Acaulospora laevis</i> Gerd. & Trappe	-	80 D	-	40 VC	20 C	100 D	60 D	60 D
<i>Acaulospora mellea</i> Spain & N.C. Schenck	20 C	60 D	-	60 D	100 D	100 D	40 VC	-
<i>Acaulospora morrowiae</i> Spain & N.C. Schenck	-	-	20 C	20 C	40 VC	40 VC	-	20 C
<i>Acaulospora scrobiculata</i> Trappe	20 C	-	-	-	20 C	-	20 C	60D
<i>Acaulospora tuberculata</i> Janos & Trappe	20 C	-	-	-	40 VC	-	-	-
<i>Acaulospora verna</i> Blaszk. **	-	-	-	-	-	20 C	-	-
<i>Acaulospora walker</i> Kramad. & Hedger*	-	-	20 C	-	-	-	-	-
<i>Acaulospora</i> sp1**	-	-	-	-	20 C	-	-	-
<i>Acaulospora</i> sp2	20 C	20 C	40 VC	-	-	-	20 C	-
Ambisporaceae								
<i>Ambispora leptoticha</i> (N.C. Schenck & G.S. Sm.) C. Walker, Vestberg & A. Schüssler	-	20 C	20 C	-	20 C	40 VC	20 C	-
Archaeosporaceae								
<i>Archaeospora trappei</i> (R.N. Ames & Linderman) J.B. Morton & D. Redecker**	-	-	-	-	-	20 D	-	-
Diversisporaceae								
<i>Diversispora</i> sp1	40 VC	60 D	40 VC	-	-	20 C	60 D	20 C
<i>Diversispora</i> sp2	20 C	-	-	60 D	60 D	60 D	20 C	60 D
Gigasporaceae								
<i>Cetraspora pellucida</i> (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd.*	-	-	-	20 C	-	-	-	-
<i>Gigaspora decipiens</i> I.R. Hall & L.K. Abbott	-	-	-	40 VC	-	20 C	-	20 C
<i>Gigaspora</i> sp1**	-	-	-	-	-	20 C	-	-
<i>Racocetra verrucosa</i> (Koske & C. Walker) Oehl, F.A. Souza & Sieverd.	20 C	-	-	-	20 C	-	-	-
<i>Racocetra</i> sp1**	-	-	-	-	-	-	-	20 C
<i>Scutellospora</i> sp1**	-	-	-	-	60 D	-	-	60 D
Glomeraceae								
<i>Glomus coremioides</i> Berk. & Broome**	-	-	-	-	20 C	-	-	-
<i>Glomus heterosporum</i> G.S. Sm. & N.C. Schenck**	-	-	-	-	20 C	-	-	-
<i>Glomus</i> sp1	100 D	100 D	60 D	100 D	100 D	100 D	100 D	100 D
<i>Glomus</i> sp2**	-	-	-	-	20 C	-	-	-
<i>Glomus</i> sp3**	-	-	-	-	20 C	-	-	-
<i>Glomus</i> sp4*	40 VC	-	-	-	-	-	-	-
<i>Glomus</i> sp5*	40 VC	-	-	-	-	-	-	-
<i>Glomus</i> sp6**	-	-	-	-	-	-	20 C	-
<i>Rhizophagus clarus</i> (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüssler	-	20 C	20 C	-	-	20 C	20 C	-

pasture area treated with the perch restoration technique *Acaulospora verna*, *Archaeospora trappei* and *Gigaspora* sp1 were the exclusive species. The pasture area undergoing the brushwood restoration technique also presented three exclusive species, *Acaulospora herrerae*, *Acaulospora walkeri* and *Glomus* sp6. The untreated pasture area (not under restoration) had two exclusive species: *Cetraspora pellucida* and *Racocetra* sp1.

The generalist AMF species, which occurred in all four sampled areas, were: *Acaulospora lacunosa*, *Acaulospora laevis*, *Acaulospora mellea*, *Acaulospora morrowiae*,

Diversispora sp1, *Diversispora* sp2 and *Glomus* sp1. The intermediate AMF species were those which occurred in two or three of the sampled areas. Nine species were classified in this category: *Acaulospora alpina*, *Acaulospora foveata*, *Acaulospora gedanensis*, *Acaulospora scrobiculata*, *Acaulospora* sp2, *Ambispora leptoticha*, *Gigaspora decipiens*, *Scutellospora* sp1 and *Rhizophagus clarus*.

In general, more than 50% of the generalist and intermediate species identified in the present study belonged to the genera *Acaulospora*. This suggests high ecological plasticity and easier adaptation to diverse environments

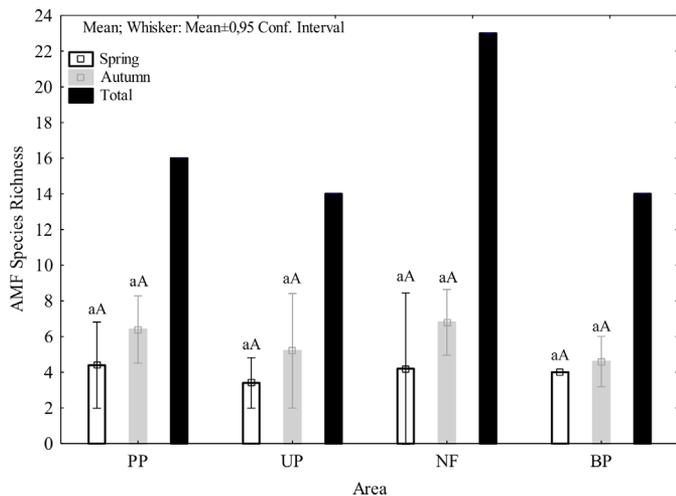


Figure 1. Species richness (Mean values \pm 0.95 confidence intervals – white and gray bars; Total species richness – black bars) of the arbuscular mycorrhizal fungal (AMF) community in four sampled areas of the *Serra do Itajaí* National Park, Santa Catarina, Brazil (PP: pasture with perches; UP: untreated pasture; NF: native forest; PB: pasture with brushwood) in two seasons (spring, 2014 and autumn, 2015). Lowercase letters compare species richness in the different areas within the same season; Capital letters compare the species richness within the same area between the two sampled seasons. Scott Knott Test 5%.

of some species within this genera (Zangaro & Moreira, 2010). Another possible explanation for this pattern is the acid pH characteristic of the four studied areas (Table 1). Previous studies have indicated the genera *Acaulospora* as predominant in ecosystems with low pH values (Stürmer et al., 2006). Additionally, *Acaulospora* species have previously been reported as generalists in other studies in the Atlantic Forest and surrounding areas (Trufem et al., 1994; Silva et al., 2006). According to Sieverding (1991) decreases in fertility favour species from this genera. This might have been the case in the pasture areas included in the present study which showed low K, P and organic C levels, when compared to the native forest (Table 1).

The conditions of the native forest might have favoured the higher number of exclusive species (nine). The pasture areas, under restoration techniques or not, presented only two or three exclusive species each, indicating a loss of species when compared to the native forest. This suggests that mainly species capable of adapting to vegetation variations and other environmental perturbations persist in these ecosystems. At the same time, these results might be considered indicators of AMF host preference representing a case of how below ground AMF diversity is affected by above ground plant diversity. Recent studies have indicated host preference of AMF species to colonize certain plant species (Torrecillas et al., 2012; Van Geel et al., 2016).

On the other hand, species such as *Archaeospora trappei*, *Cetranspora pellucida*, *Gigaspora decipiens*, *Racocetra* sp1,

Glomus sp6, and *Rhizophagus clarus* were not observed in the native forest, only being found in the pasture areas. This may indicate a preference by these species for disturbed environments. It may also be the result of variations in the sporulation patterns of species already present in the soil, when variations in the plant community structure occurs.

Evaluation of the data by season indicated the occurrence of 21 AMF species during the spring of 2014 in the four sampled areas of the Park (Table 2). Species such as *Acaulospora gedanensis*, *Acaulospora walkeri*, *Acaulospora pellucida*, *Glomus* sp4 and *Glomus* sp5, were found exclusively during spring (Table 2). In the autumn of 2015, a total of 28 species was verified. Therefore, 12 species were considered exclusive to this season: *Acaulospora alpina*, *Acaulospora verna*, *Acaulospora* sp1, *Archaeospora trappei*, *Gigaspora* sp1, *Racocetra* sp1, *Scutellospora* sp1, *Glomus heterosporum*, *Glomus* sp2, *Glomus* sp3, *Glomus* sp6 and *Glomus coremioides*. However, no significant difference in the AMF community was detected between the seasons (Scott Knott - $p < 0.05$).

The general pattern previously described for all the sampled areas in the Park, is representative of that observed for each of the areas independently. Figure 2 illustrates the number of species per area, distributed as a function of their occurrence during just one or both of the seasons. Although no statistical difference was detected between the seasons within each area (Figure 1), AMF species richness in the autumn showed a tendency to be higher than in the spring in all cases. In the NF, 39% of all the identified species were common to both seasons, being equivalent to the percentage of species that were exclusively found during autumn, but higher than the number of species occurring

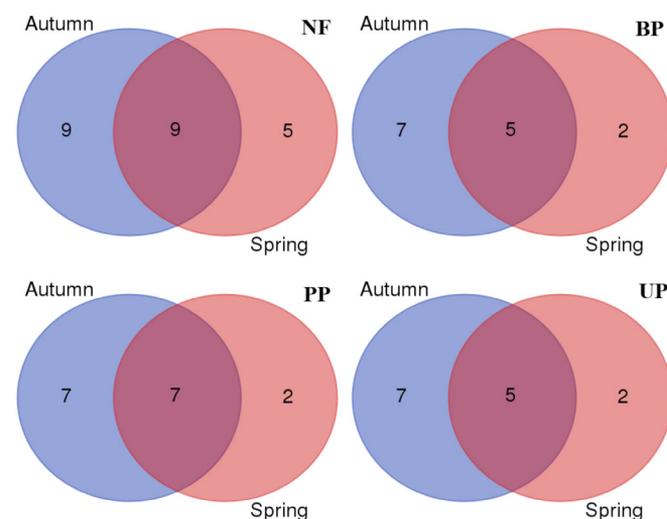


Figure 2. Number of species of arbuscular mycorrhizal fungi exclusively observed during spring, 2014 and autumn, 2015, and common to both seasons, in each of the sampled areas within the *Serra do Itajaí* National Park, Santa Catarina, Brazil. NF: native forest; PB: pasture with brushwood; PP: pasture with perches; UP: untreated pasture.

exclusively during spring (22%). In the PP 44% of the species were verified during both seasons. While in the latter area the proportion of species exclusively detected in the autumn was also 44%, in the spring only 12% of the total number of species identified for this area were observed. The BP and UP showed a very similar pattern between them. In these two areas, 36% of the species were present in both seasons, while 50% and 14% were exclusive to autumn and spring, respectively.

Seasonal variations in AMF communities have been previously reported for various ecosystems worldwide. Although this stills represents a contentious issue, some authors suggest more humid seasons as having, in general, higher AMF plant colonization (Halder et al., 2015) and lower sporulation (Santos et al., 2014). Therefore, AMF diversity studies using spore morphology as an identification method may detect lower numbers of species during the wet period of the year. This was the case in the present study, with the autumn (dry period) accounting for the greatest species richness compared to spring (wet season). Autumn also showed more exclusive species than spring. Different AMF species also have variations in their sporulation patterns, which might explain the occurrence of some species exclusively during autumn and others during spring. These results highlight the importance of sampling in at least more than one period of the year when seeking to evaluate AMF diversity by the morphology of the spores present in the field, as Bonfim et al. (2016) also indicated.

In all, nine species were classified as dominant ($F_i > 50\%$) in at least one of the studied areas in one or both of the sampled seasons (Table 2). The species *Glomus* sp1 was the most frequent of all, being dominant in the four areas and in the two seasons included in the study. However, in general, only a few species maintained the dominant category in the same sampling area in the two seasons. Among these were *Acaulospora laevis*, *Acaulospora mellea* (PP) and *Diversispora* sp2 (UP). Other species such as *Acaulospora foveata* and *Acaulospora scrobiculata* were only dominant during autumn, in the perches and untreated pastures, respectively. None of the identified species were found to be only dominant during spring.

The season with the lowest number of dominant species, independently of the area, was spring, presenting five species. In this period, AMF communities in the NF and PB had only one dominant species (*Glomus* sp.1). There were no rare species in any of the seasons or areas evaluated. Independently of the season, the studied areas were found to have AMF communities with a predominance of common and mostly common species, with frequency ranging from 20 to 40%.

Conclusions

Conversion of forests into pastures within the *Serra do Itajaí* National Park did not affect the mean species richness of AMF between the areas. Considering average values,

this ecological index also showed no variation between the studied seasons in any of the areas. However, the total number of species verified during the sampled seasons was higher in the native forest, suggesting a possible negative effect of deforestation on AMF community diversity within the evaluated pasture areas. AMF species diversity also showed a tendency to be higher during autumn, with lower total number of species observed during the spring.

Acknowledgments

The authors would like to thank the *Restaurar* Project for its financial support and Professor PhD Sidney L. Stürmer for his contribution with the AMF species identification.

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