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THE EFFECTS OF PHOSPHOROUS FERTILIZATION ON THE MYCORRHIZAL COLONIZATION OF NATIVE FORAGE GRASSES IN THE PAMPA BIOME

Efeito da adubação fosfatada na colonização micorrízica de gramíneas forrageiras nativas no bioma Pampa

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Abstract: Most of natural grasslands of Southern Brazil have low availability of soil phosphorus (P), and under such conditions, the association with arbuscular mycorrhizae is an adaptation to the nutrient supply. However, one of the alternatives to improve the productivity of these grasslands is P fertilization, which can alter mycorrhizal colonization. This study aimed to evaluate the effect of fertilization on mycorrhizal colonization and to characterize the mycorrhizal colonization of native grass species of different growth rate. Treatments consisted of (i) 50 mg kg^{-1} of P (+ P); and (ii) a control without addition of P (-P). The grasses used in the experiment were *Axonopus affinis* and *Paspalum notatum* (fast growing species), and *Andropogon lateralis* and *Aristida laevis* (slow growing species). The plant species were planted in pots with 5 kg of soil and arranged in a randomized block design with four replications. For the fast growing species (*A. affinis* and *P. notatum*), the addition of P reduced mycorrhizal colonization by an average of 55%. In relation to slow-growing species (*A. lateral* and *A. laevis*), -P had a similar mycorrhizal colonization to that of +P. Spore production was higher in slow growing species compared to fast growing species, regardless of treatments. These results indicate the key role slow growing species have in this environment. Therefore, even if fertilization reduces the colonization in the root system of fast-growing species, slow-growing species allow arbuscular mycorrhizal fungi to remain present in this environment. This type of characteristic becomes important for the coexistence of the species in natural grasslands.

Keywords: Natural grasslands, symbiosis, mycorrhizal fungi, mycorrhizal spores.

Resumo: A maioria das pastagens nativas do Sul do Brasil tem baixa disponibilidade de fósforo do solo (P), e sob tais condições, a associação com micorrizas arbusculares é uma adaptação para o fornecimento de nutrientes. No entanto, uma das alternativas para melhorar a produtividade dessas pastagens é adubação com P, que pode alterar a colonização micorrízica. Este estudo teve como objetivo avaliar o efeito da adubação na colonização micorrízica e caracterizar a colonização micorrízica de espécies de gramíneas nativas de diferentes velocidades de crescimento. Os tratamentos consistiram de (i) 50 mg kg^{-1} de P (+P); e (ii) um controle sem adição de P (-P). As gramíneas usadas no experimento foram *Axonopus affinis* e *Paspalum notatum* (espécies de crescimento rápido), e *Andropogon lateralis* e *Aristida laevis* (espécies de crescimento lento). As espécies vegetais foram plantadas em vasos com 5 kg de solo e dispostas em delineamento em blocos casualizados com quatro repetições. Para as espécies de crescimento rápido (*A. affinis* e *P. notatum*), a adição de P reduziu a colonização micorrízica, em média 55%. Em relação as espécies de crescimento lento (*A. lateral* e *A. laevis*), o -P teve uma colonização micorrízica semelhante ao da aplicação de + P. A produção de esporos foi maior nas espécies de crescimento lento em comparação com as espécies de crescimento rápido, independentemente dos tratamentos. Esses resultados indicam o papel fundamental que as espécies de crescimento lento possuem

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neste ambiente. Por isso, mesmo que a fertilização reduza a colonização no sistema radicular de espécies de crescimento rápido, as espécies de crescimento lento permitem que os fungos micorrizicos arbusculares permaneçam presentes neste ambiente. Esse tipo de característica torna-se importante para a coexistência das espécies em pastagens naturais.

Palavras-chave: Pastagem nativa, simbiose, fungos micorrízicos, esporos de micorrizas.

1 INTRODUCTION

From the southern half of the state of Rio Grande do Sul (RS), in Brazil, to Argentina and Uruguay, natural vegetation consists of natural grasslands. This vegetation is mostly comprised of C_4 grass species. In this type of ecosystem, most of the soils are acid, with low availability of nutrients such as phosphorous (P) (RHEINHEIMER et al., 1994; RHEINHEIMER et al., 2008).

In that region, plant growth is limited by diverse factors such as water availability and soil nutrients. Thus, one of the plant´s adaptive alternatives to low soil fertility is the ability to associate with microorganisms that help them take up nutrients, such as the arbuscular mycorrhizal fungi (AMFs) (BRUNDRETT, 1991). AMFs are microorganisms belonging to the phylum *Glomeromycota*, which perform a mutually beneficial interaction (SMITH et al., 2011), through which the plant provides photoassimilates to the fungus, while the fungus provides nutrients to the plants, such as P, and water (GRANT et al., 2005). The increased nutrient uptake enabled by the AMFs is mainly due to the absorption surface and the soil volume explored by hyphae (GRANT et al., 2005; SMITH et al., 2011).

Considering animal raising in the south Brazilian native grasslands, one of the management tools that can be used to maximize forage production is the application of fertilizers, mainly phosphate (TIECHER et al., 2013). However, an increased availability of P in the soil generally causes a reduction of mycorrhizal colonization, although Rheinheimer and Kaminski (1995) found that in *Paspalum notatum,* the addition of small doses of P triggered mycorrhizal colonization. In contrast, increasing P availability with high doses result in reduced AMFs colonization.

Thus, P fertilization may alter the mycorrhizal colonization in the root system of forage grasses that make up natural grasslands, thus reducing the importance of such ecological process and making these species more dependent on external inputs. However, the process of colonization on the roots of native grasses of the Pampa biome by AMF remain uncertain, especially when subjected to fertilization with high amounts of P.

The botanic composition of these grasslands comprises approximately 450 grass species (BOLDRINI, 2009). Aiming to the rational use of these grasslands, Cruz et al. (2010) used leaf traits such as dry matter content (LDMC) and specific leaf area (SLA) to group the grass species into four plant functional types (PFTs). Such PFTs in general can be differentiated into two groups, species of rapid growth (PFTs A and B) and species of slow growth (PFTs B and C). Plants with higher SLA, low tissues density and high nutrient concentration tend to have rapid resources capture and high growth rate, that allow those plants to be dominant in moist and fertile areas. Opposite traits define species with efficient resources conservation, allowing the plant to minimize nutrient loss and increase competitive abilities in dry and nutrient poor environments.

As the characteristics of both groups are associated with the growth rate, it is believed that colonization by AMF have different patterns depending on the different growth rates and fertilization. For this reason, the present study was designed with the objectives of: (i) assessing the effect of fertilization with high phosphorus content on mycorrhizal colonization and (ii) characterizing mycorrhizal colonization in four native grasses of different plant functional types. Understanding these points is important to determine if the symbiosis formed between AMF and plant roots have the potential to influence ecosystem processes in natural communities. However, AMF possess an undetermined role in the diversity of plants because AMF can induce a wide variety of responses in the growing plant species that coexist in natural environments.

2 MATERIAL AND METHODS

The study was conducted from May 2012 to January 2013 in the greenhouse of the Soil Department of the Federal University of Santa Maria (UFSM), Rio Grande do Sul. A sample of a Typic Hapludalf soil was collected at the 0-20 cm layer in a native grassland without history of fertilizer addition. After being collected, the soil sample was dried, sieved in a 4 mm mesh sieve and transferred to pots of 5 kg. The soil physical and chemical characteristics are as follows: 180g kg^{-1} of clay; 14.5

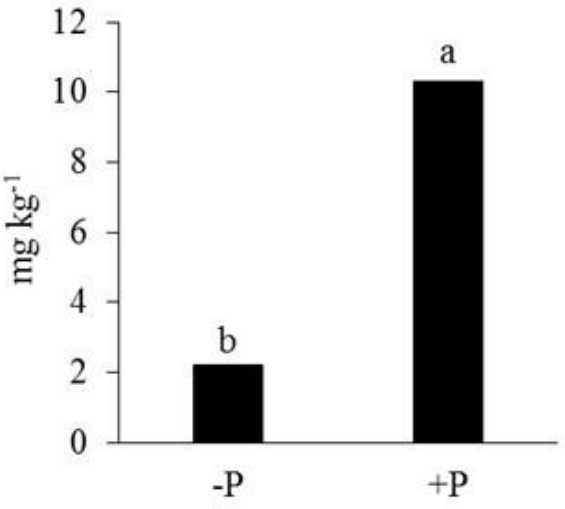
g kg^{-1} of organic carbon; pH (H_2O) (1:1) of 4.6; 1.9 $cmol_c$ dm^{-3} of Al; 26.5% of base saturation; 6.2 $cmol_c$ dm^{-3} of effective CEC; 2.8 $cmol_c$ dm^{-3} of Ca; 1.4 $cmol_c$ dm^{-3} of Mg; 3 mg kg^{-1} of P (Mehlich-1); and 76 mg kg^{-1} of K (Mehlich-1).

Four of the most common forage grass species found in native grasslands in Rio Grande do Sul were used: *Axonopus affinis*, *Paspalum notatum*, *Andropogon lateralis* and *Aristida laevis.* Each grass species was classified as functional types (PFT) A, B, C or D, respectively, according to Cruz et al. (2010). The species were collected in May from the same native grassland from which the soil sample was collected, and the tillers were transplanted to the pots (five tillers per pot). *A. affinis* and *P. notatum* were selected as fast-growth species, while *A. lateralis* and *A. laevis* as slow-growth species. *A. affinis* is a stolon growth species and is mostly found in marshes with high soil moisture. *P. notatum* is the most common species found in the grasslands of that region and has rhizome-based growth. *A. lateral*is is a species with erect growing habit, forming clumps. *A. laevis* is a grass with erect growing habit, forming clumps, often found in well-drained areas and low grazing sites.

From May to September 2012, the species passed through a period of acclimation. On Sep. 12, treatments were set up, and the plants cut uniformly 5 cm aboveground. The species were arranged in a randomized block design with four replications. Treatments consisted of the application of 50 mg kg^{-1} of P (+P) and one control, without addition of P (-P). P was applied in the form of potassium phosphate (KH_2PO_4). The potassium level in the control sample was corrected with potassium chloride (KCl) in amounts equivalent to those applied to the other treatments. Soil moisture was maintained at 70 % of field capacity, which was calculated by weighing the pots daily. The species were collected on Nov 27, 2012, 76 days after the beginning of the experiment.

The plants were separated into two parts, shoots and roots. Soil was screened out from the roots and then washed with distilled water. The plant´s shoot and root material was kept in paper bags and dried in an oven with air circulation at 65 ^oC for 72 hours. After weighed, the material was ground by a Willey mill to 1 mm mesh size, for determination of P in the dry matter (DM). Analysis of P in the DM was performed as Tedesco et al. (1995). In the dried soil, the P content was estimated by the extraction method using anion exchange resin (AER) with AR 103 membrane and 434 QDP plate, and was saturated with sodium bicarbonate ($NaHCO_3$) (Figure 1).

Figure 1 - P extracted by anion exchange resin (P resin) in soil under soil with low (- P) and high (+P) phosphorus content.



When the plants were removed from the pots, root samples were taken and stored in a 70% alcohol solution, as well as wet soil samples, and both were kept under refrigeration at 4 °C, for determination of the percent of mycorrhizal colonization and spores count, respectively. To determine the percent of colonization the methodology of Phillips e Hayman (1970) was utilized. The colonization rate was assessed with the aid of a magnifying glass, in conformity with Giovannetti and Mosse (1980).

Spore count followed the methodology described by Gerdemann and Nicolson (1963). Fifty mL of a moist soil sample were centrifuged in water at 3000 rpm for three minutes. The supernatant was discarded, and the residual material was centrifuged again at 2000 rpm for two minutes in a 45% sucrose solution. The supernatant was poured onto a 0.053 mm mesh sieve, the material retained by the sieve was transferred to a ribbed plate, and count was performed under a magnifying glass.

Each variable was tested for normality of errors and variance homogeneity, when necessary, using logarithmic transformation. The bi-factor statistical model was used, Species × Fertilization, and when interactions were not significant, the discussion was performed based on the means of the main factors. When the treatment effects were significant at 5% probability level, the differences between the means were compared by Tukey's test. Multivariate analyses of clustering and principal coordinate ordination (PCoA) were used to test the hypotheses about the rate of mycorrhizal colonization and the other determined variables using MULTIV software.

3 RESULTS

The mycorrhizal colonization for *A. lateralis* (PFT C) and *A. laevis* (PFT D) did not decrease with +P (Table 1). For *A. affinis* (PFT A) and *P. notatum* (PFT B), the increase of P availability diminished the mycorrhizal colonization on average 50% compared to –P. In the – P, mycorrhizal colonization was higher for *A. affinis* (PFT A) when compared to the other species. The spore number in the ryzosphere soil did not show difference among adition or not of P (Table 1); for *A. lateralis* (PFT C) and *A. laevis* (PFT D), the spore number was 86% higher than the mean value for *A. affinis* (PFT A) and *P. notatum* (PFT B).

Table 1 - Mycorrhizal colonization, spore number and total P extracted by anion exchange resin (P resin) in soil cultivated with four native grass species of the Pampa biome under soil with low (-P) and high (+P) phosphorus content.

Species	-P	+P	Mean
	Mycorrhizal colonization (%)		
Axonopus affinis	42.2 aA	23.1 aB	26.7
Paspalum notatum	26.2 bA	10.9 bB	17.4
Andropogon lateralis	14.1 cA	14.0 bA	12.4
Aristida laevis	27.5 bA	22.7 aA	24.2
Mean	27.5	17.7	
	Spore number (spores 50 g^{-1} soil)		
Axonopus affinis	831.0	498.4	643.1 b
Paspalum notatum	553.1	351.4	4502 b
Andropogon lateralis	1.202.2	1.048.1	1.133.2 a
Aristida laevis	866.5	971.6	902.5 a
Mean	863.4 A	717.1 A	

Means followed by the same lower case letter in the column and the same upper case letter on the rows are not statistically different by Tukey's at 5% probability level.

Regarding the mean value of the four species, the DM of the shoot was 37.2% higher in +P compared to the -P (Table 2). In the treatments with +P, *A. affinis* (PFT A) and *P. notatum* (PFT B) showed, on average, 67.5% more DM in the shoot than the other species. The average production of root DM achieved by the species, when submitted to fertilization with P, corresponded to increases of 36% compared to the -P (Table 2).

For the four species, P accumulated in the shoot DM was greater in the +P treatment compared to the -P (Table 2). In the shoot DM +P treatment, *A. affinis* (PFT A) and *P. notatum* (PFT B) accumulated more P in the shoots than *A. lateralis* (PFT C) and *A. laevis* (PFT D). P availability in the soil extracted by AER was on average 4.5 times greater in the +P treatment compared to the –P (Figure 1).

For the multivariate analysis (Figure 2), PCoA could represent 86% of the results variation. The clustering analysis separated the species in treatments into four distinct groups (P<0.1). The mycorrhizal colonization was correlated negatively with increased root DM (- 0.57), P in the shoots DM (-0.53), and P in roots DM (-0.51). All native species cultivated in the -P were grouped into one distinct group. This group was associated with a higher rate of mycorrhizal colonization and lower P in the shoots of the plants. The group composed of *A. affinis* (PFT A) and *P. notatum* (PFT B) cultivated in the +P treatment was correlated with greater DM in the shoots compared to the other groups, and lower mycorrhizal colonization.

4 DISCUSSION

The mycorrhizal colonization percent of the four species ranging from 9.1% to 42.2 %, as found in this work, are in agreement with the findings of Rheinheimer et al. (1994), Hartnett & Wilson (2002), and Lugo et al. (2012) (Table 1). However, considering P accumulated in the shoots DM (Table 2), the higher colonization rate by native mycorrhizal fungi in the control was not a

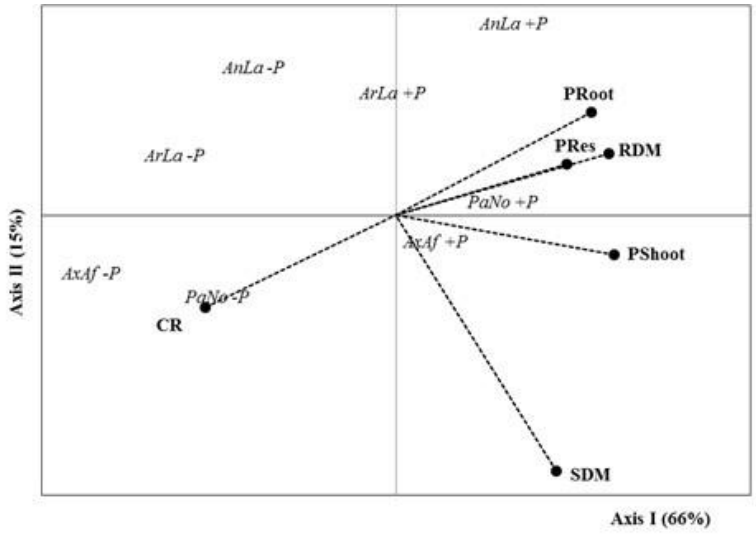
determinant factor for higher P concentration in the plants, when compared to the treatments with P application. The greater P concentration by the fast-growing species *A. affinis* (PFT A) and *P. notatum* (PFT B) (Table 2), is associated with the response ability to fertilization, as well for DM production (Table 2), thus reducing colonization (Table 1).

Table 2 - Production of shoot dry matter (SDM) and root dry matter (RDM), phosphorous in the shoots of four native grass plants of the Pampa biome as a result of soil with low (-P) and high (+P) phosphorus content.

Species	-P	+P	Mean
	SDM (g pot^{-1})		
Axonopus affinis	11.7 bB	21.1 aA	22.2
Paspalum notatum	19.7 aA	23.3 aA	27.1
Andropogon lateralis	7.1 bB	14.1 bA	13.5
Aristida laevis	8.3 bA	12.4 bA	13.4
Mean	11.7	17.6	
	RDM (g pot^{-1})		
Axonopus affinis	5.6	6.5	6.8 b
Paspalum notatum	10.4	12.1	12.6 a
Andropogon lateralis	9.5	15.6	13.4 a
Aristida laevis	5.7	8.5	8.6 b
Mean	7.8 B	10.7 A	
	P shoot (g pot^{-1})		
Axonopus affinis	3.6 aB	18.7 aA	18.1
Paspalum notatum	5.6 aB	18.4 aA	21.7
Andropogon lateralis	3.7 aB	10.5 bA	10.7
Aristida laevis	4.0 aB	9.1 bA	9.6
Mean	4.2	14.2	

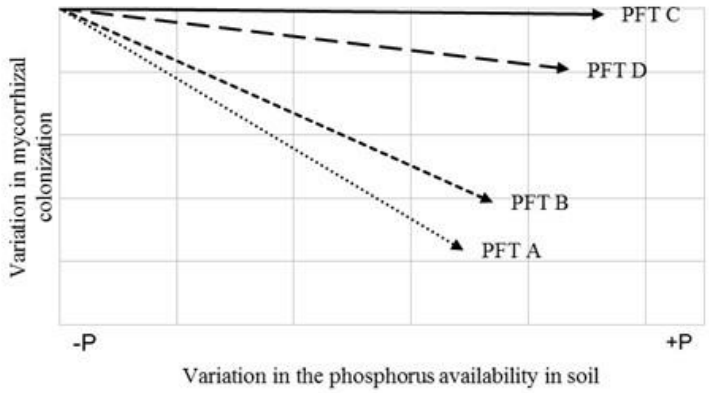
Means followed by the same lower case letter in the column and upper case letters on the rows are not statistically different by Tukey's at 5% probability level.

Figure 2 - Ordination diagram of the species *Andropogon lateralis* (AnLa), *Aristida laevis* (ArLa), *Axonopus affinis* (AxAf), *Paspalum notatum* (PaNo), and the control, with a natural phosphorus content (-P), and with the addition of a high phosphorus content (+P), as a function of the following variables: colonization rate (CR), root phosphorous (PRoot), shoot phosphorous (PShoot), soil phosphorous extracted by AER (PRes), shoot dry matter (SDM), and root dry matter (RDM).



According to the conceptual graph of the variation in mycorrhizal colonization in functional types of plants depending on the content of P in the soil (Figure 3), observing a variation between species. The fast-growth species, TFP A and B, showed a great variation on mycorrhizal colonization due to the increased availability of P. The slow growing species, TFP C and D, showed less variation in mycorrhizal colonization.

Figure 3 - Conceptual graphic of variation in the mycorrhizal colonization of different plant functional types (PFT) from grasslands of southern Brazil, as a function of soil P.



Such behavior observed for *A. affinis* (PFT A) and *P. notatum* (PFT B) is as expected, because the increased DM and P accumulation diminish mycorrhizal activity, which can be seen through the negative correlation shown in Figure 2. Thus, the ability that fast-growing species have to respond to fertilization by increasing tissue P concentration and DM production contributes to a reduction in colonization (Figure 3). While in a natural fertility condition, colonization with AMFs is a mechanism used by all species, for both the slowgrowing species *A. lateralis* (PFT C) and *A. laevis* (PFT D), mycorrhizal colonization is maintained even under enhanced soil fertility (Figure 3), as highlighted by Brundrett (1991).

The response of fast-growing species to reduction in mycorrhizal colonization is linked to the ability to respond to soil nutrient availability, presenting a greater plasticity. Based on what Brundrett (1991) suggests, the change that colonization by AMFs presents after fertilization is associated with the growth stimulus that the plant receives. Therefore, as the plant takes up more soil nutrients, it alters the growth morphology and such morphological changes prevent the formation of structures that enable association in the roots, determining altered colonization rates in the root system.

What it is suggested is that a larger nutrient availability, such as P, triggers production of new tissues, thus demanding larger amounts of photoassimilates. However, two factors can be related with such response. One is the species growth rate, i.e. species that have a natural fast growing rate, under fertilization demand a larger amount of photoassimilates to form new tissues, compared to species that grow slowly. Therefore, photoassimilate translocation is reduced and, consequently, the stimulus to colonization decreases. In this regard, Graham et al. (1997) show that in high nutrient supply, the mycorrhizal association may diminish the plant growth, due to the higher carbon drain by colonized roots.

For the species under study, the rapid growing rate feature is assigned to the species *A. affinis* (PFT A) and *P. notatum* (PFT B) (MACHADO et al., 2013); therefore, the larger DM by these species (Table 2) possibly caused reduced mycorrhizal colonization (Table 1; Figure 3) in view of a higher demand of photoassimilates to produce tissues. The species *A. lateralis* (PFT C) and *A. laevis* (PFT D), which have a slow growing rate (MACHADO et al., 2013), had hight DM content, when compared to *A. affinis* (PFT A) and *P. notatum* (PFT B). Because they require a hight amount of photoassimilates to produce tissue, they do not alter the mycorrhizal colonization possibly because they do not reduce the translocation of photoassimilates to the roots and C deposition in the rhizosphere.

The behavior of *A. lateralis* (PFT C) and *A. laevis* (PFT D) in maintaining mycorrhizal colonization (Table 1; Figure 3) even in a condition of greater P availability after fertilization, may indicate that this mechanism is more important for these species than

to *A. affinis* (PFT A) and *P. notatum* (PFT B). Such interaction between AMFs and slow-growing species may help those species growing in poorer soils, as suggested by Tiecher et al. (2013) for *A. laevis* (PFT D). A similar trend of AMF colonization in slow-growing grasses was highlighted by Martins et al. (1999) for *Aristida setifolia*, a native and pioneering grass, which in high acid and low P availability had a considerable effect on growth through AMFs colonization, contributing significantly to the establishment of this species in native and degraded soils. Thus, we can say that for grass species C4, which has a cespitose growth habit (clump formation) and slow-growing rate, AMFs are a key mechanism for nutrients uptake from low fertility soils, as suggested by Brundrett (1991), Wilson and Hartnett (1997), and Lugo et al. (2012).

The larger number of spores found associated to *A. lateralis* (PFT C) and *A. laevis* (PFT D), as compared to *A. affinis* (PFT A) and *P. notatum* (PFT B) (Table 1), indicates the key role that the slow growing species have in this environment. Thus, even with the application of fertilizers in native grasslands, which are characterized by the coexistence of species of slow and species of fast growth, *A. lateralis* (PFT C) and *A. laevis* (PFT D) maintain the propagule bank for other species. Therefore, even though fertilization reduces colonization in the root system of fast-growing species, slow-growing species allow AMFs to be present in this environment, a major trait that allows species to coexist in natural environments, as suggested by Chen et al. (2005).

5 CONCLUSION

The slow growing species *A. laevis* and *A. lateralis* did not alter the percent of mycorrhizal colonization with increased P availability. P fertilization enhances P concentration and reduces the percent of mycorrhizal colonization of the fast-growing species *A. affinis* and *P. notatum*. The slow-growing species had larger amount of mycorrhizal spores in the rhizospheric soil, when compared to the fast-growing species.

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