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Mycorrhizal inoculation and phosphorus fertilization show contrasts on native species of the Brazilian Atlantic Forest and Cerrado

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ABSTRACT: Restoration of degraded sites by using native plants like *Plathymentia reticulata* Benth. and *Melanoxylon brauna* Schot. is advisable. However, seedlings of both species, when raised on commercial substrates, may present low survival. This study aimed to evaluate the growth of seedlings under inoculation with arbuscular mycorrhizal fungi (AMF). The seedlings were raised on soil sampled from near an adult plant of the same species. The *P. reticulata* and *M. brauna* seedlings were grown with or without the inoculation of a mix of three species of AMF (*Rhizophagus clarus*, *Claroideoglossum etunicatum*, and *Gigaspora albida*), and five doses of P (0, 50, 150, 300, and 450 mg dm⁻³ of P). All seedlings were inoculated with *Bradyrhizobium* sp., isolated from each species. After 90 days, *P. reticulata* inoculated with AMF showed higher growth and nutrient content than those uninoculated, and the maximum plant growth was obtained when received 160 to 280 mg dm⁻³ of P. On the other hand, no effects of AMF inoculation or phosphate fertilization were observed on *M. brauna*. For both species, no effect of inoculation was observed on mycorrhizal colonization, and *M. brauna*, unlike *P. reticulata* presented a considerable number of nodules. We conclude that inoculation with AMF and P fertilization improves the growth of *P. reticulata* seedlings but does not promote the growth of *M. brauna*; presenting the necessity to investigate each species.

Keywords: *Plathymentia reticulata*, *Melanoxylon brauna*, AMF, seedling production, *Bradyrhizobium*.



INTRODUCTION

The Atlantic Forest and Cerrado have high rates of endemic species and are priority areas for conservation in Brazil (Myers et al., 2000; Beech et al., 2017). However, these biomes are threatened because there is significant anthropogenic pressure (Rockström et al., 2009; IBA, 2017; Guerra et al., 2020). The original size of the Atlantic Forest and the Cerrado has been reduced, respectively, to less than 12 % (Ribeiro et al., 2009) and 45 % (Machado et al., 2004), which leads to the extinction of several species (Brooks et al., 2002) and, consequently, the loss of ecosystem services. It is important to restore areas to reverse this scenario by reintroducing native species (Duarte et al., 2015; Shaw, 2019; Asmelash et al., 2021) for directing the revegetation process.

Some native forest species, such as *Plathymeria reticulata* Benth., have desirable characteristics for reforestation (Lacerda et al., 2001; Lacerda et al., 2002), as well as the commercial value because of the durability and quality of the wood (Lorenzi, 1992; Carvalho, 2009). This species belongs to the Fabaceae family, Mimosoideae (synonym of *P. foliolosa* Benth.), found in the Atlantic Forest and Cerrado as distinct ecotypes (Warwick and Lewis, 2003; Morim, 2020). *Melanoxylon brauna* Schot. (Fabaceae, Caesalpiniodeae) is threatened with extinction (IBAMA, 2018). This species native in the Atlantic Forest and Cerrado biomes, as an early to late secondary species (Lorenzi, 1992; Rando et al., 2020), was intensively exploited due to its commercial characteristics (Gibson et al., 2019). Besides deforestation and the absence of replanting reduced the population and area of occurrence, *M. brauna* individuals are growing isolated in small forest fragments, mainly in those in an advanced stage of regeneration (Silva et al., 2003; Carvalho et al., 2007; Crepaldi and Peixoto, 2010; Versieux et al., 2011).

Seedling production of *P. reticulata* and *M. brauna* has been limited due to the low survival rate in the nursery when grown on commercial substrates. This limitation may be associated with the absence or lower level of symbiotic microorganisms, like diazotrophic bacteria and arbuscular mycorrhizal fungi, in commercial substrates (Goetten et al., 2016; Prates Júnior, 2018; Asmelash et al., 2020; Karthikeyan and Arunprasad, 2021). The symbiotic dependency can be an adaptive strategy that allows for greater efficiency in the absorption and use of nutrients for nutritionally oligotrophic and acid soil in their natural occurrence area (Fonseca et al., 2010; Freitas et al., 2017), allowing greater efficiency in the absorption and use of nutrients.

Information on the nutritional requirements of native forest species is scarce due to the different ecophysiological requirements (Wright et al., 2018; Barbosa et al., 2019), and there may be variations among pioneer, secondary or climax species. Thus, these plants may respond differently to the nutrients supply provided by fertilization and promote the increase or decrease in plant growth (Cruz et al., 2010; Rossa et al., 2015; Berghetti et al., 2020). *P. reticulata* has a positive response to fertilization with 175 and 45 mg dm⁻³ of K and S, respectively (Duarte et al., 2015), as well as fertilization with 300 mg dm⁻³ of P (Freitas et al., 2017). For *M. brauna*, there is a record of response to Basacote® controlled-release fertilizer 8000 mg dm⁻³ in N-P-K formulation 13-6-16 (Gibson et al., 2019). However, there is a lack of information related to fertilization and inoculation with arbuscular mycorrhizal fungi (AMF) for both species.

Arbuscular mycorrhizal fungi promotes plant growth, decreases seedling formation time, improves nutritional status, and decreases mortality in the field (Goetten et al., 2016). Plants associated with AMF explore higher soil volume (Smith and Read, 2008), present greater nutrient uptake, such as N and P (Parniske, 2008), increase the tolerance to biotic (Johnson et al., 2010) and abiotic stress (Sikes et al., 2009; He et al., 2019), and increase the density of bacteria in the rhizosphere or mycorrhizosphere (Revillini et al., 2016). Arbuscular mycorrhizal fungi may contribute to increasing the number of nodules and legume growth (Dias et al., 2012; Karthikeyan and Arunprasad, 2021), due to the

increase in P uptake, which is one of the indispensable prerequisites for nitrogen biological fixation (Scheublin et al., 2004).

The plant-soil feedback (PSF) model, however, may well explain positive interferences when the soil under the influence of a given plant favors the growth of seedlings of the same or other species (van der Putten et al., 2013). Soil can be considered as part of the extended phenotype of a plant (van Breemen and Finzi, 1998) and a source of inoculum of beneficial microorganisms and may present locally adapted AMF and rhizobia species (Revillini et al., 2016), capable of favoring the survival, growth, and the better use of nutrients.

This type of study is essential to assist in producing seedlings in a nursery and contributing to revegetation studies with the planting of these species, expanding the understanding of symbiotic relationships with AMF. Therefore, we assume that the soil from adult plants of the same species inoculated with mycorrhizal fungi and phosphate fertilization favors the growth of *P. reticulata* and *M. brauna* seedlings. This study aimed to evaluate the growth of *P. reticulata* and *M. brauna* seedlings under different doses of phosphates and inoculation with AMF in soils collected from adult plants, of each species separately, to understand the benefits of the microorganism-plant interaction and enhance seedling production.

MATERIALS AND METHODS

Seeds and substrates

Seeds of *P. reticulata* and *M. brauna* were obtained from the seed bank of the Sociedade de Investigações Florestais (SIF), Viçosa-MG, Brazil. They were superficially disinfected with ethanol 70 % for 30 s, sodium hypochlorite (2.5 %, v:v) for 10 min and with successive washes in sterile water under aseptic conditions. The seeds were transferred to Petri dishes containing moistened filter paper with sterile deionized water, then incubated for seven days at room temperature. After germination, seedlings of similar size were transplanted to 1 L plastic pots containing 650 g of soil.

Previous assays indicated that both species were plant-soil feedback (PSF) positive, which implied carrying out the experiments on soil from an adult plant of the same species (Prates Júnior, 2018). The experiments were conducted independently of each other for each forest species. For the experiment with *P. reticulata*, pots were filled with soil collected near an adult *P. reticulata* plant, growing in the fragment of the native forest of Viçosa-MG, Brazil (20° 39' 14" S, 42° 59' 05" W, at an altitude of 630 m), and was taken within a radius of 2 m from the trunk of the tree at a depth of 0.00-0.20 m, at randomly distributed points. For the experiment of *M. brauna*, the pots were filled with soil collected near an adult *M. brauna* plant, growing in the grazing area of the municipality of Leopoldina-MG, Brazil (21° 33' 7.08" S, 42° 36' 0.03" W, at an altitude of 425 m), within a radius of 2 m from the trunk of the tree at a depth of 0.00-0.20 m, at randomly distributed points. The soil of both plants was sieved in the field (2 mm), homogenized, transported, and stored until filling in the pots. The samples obtained in the field were used for chemical and granulometric characterization (Table 1).

Microbial characterization and obtaining AMF and rhizobium inoculants

Soil collected near an adult *P. reticulata* plant presented 258 spores in 100 mL, and soil collected near an adult *M. brauna* plant presented 789 in 100 mL. The isolates of AMF, *Claroideoglossum etunicatum* RJN101A, *Rhizophagus clarus* RJN102A, and *Gigaspora albida* PRN201A were obtained from the International Collection of Glomeromycota Culture (CICG, www.furb.br/cicg) belonging to the Fundação Universidade Regional de Blumenau (FURB), Santa Catarina, Brazil. The isolates were multiplied in *Urochloa brizantha* Hochst Stapf in a mixture of soil and sand (1:1, v:v).

Table 1. Chemical and granulometric properties of the soil samples collected near an adult plant of *Plathymeria reticulata* Benth. and *Melanoxydon brauna* Schot

pH(H ₂ O)	P	K	Ca ²⁺	Mg ²⁺	Al ³⁺	SB	CEC (T)	CEC (t)	m	P-rem	Textural class
	— mg dm ⁻³ —		cmol _c dm ⁻³						%	mg L ⁻¹	
<i>Plathymeria reticulata</i> Benth.											
4.25	3.9	53	0.41	0.18	1.58	0.73	12.93	2.31	68.4	22.3	Sandy clay
<i>Melanoxydon brauna</i> Schot.											
4.39	5.8	128	2.55	0.89	0.69	3.77	12.57	4.46	15.5	26.6	Clay

P and K extracted with Mehlich 1; pH in water at a soil:solution ratio of 1:2.5; SB: sum of exchangeable bases; CEC (T): cation exchange capacity at pH 7.0; CEC (t): cation exchange capacity; m: saturation index by aluminum.

Isolates of *Bradyrhizobium* sp., identified molecularly, after the sequencing of the 16S rRNA gene using primer pairs 27F (AGAGTTTGACCTGGCTCAG) and 1492R (GGTTACCTGTTACGACTT) (Lane, 1991), were isolated from a nodule of each species of seedling obtained in the nursery of the Department of Forest - UFV, using YM agar medium with Red Congo. The inoculum was produced in YM broth.

Experimental design and inoculation

The experiments were conducted separately in a completely randomized design, in a factorial arrangement (2 × 5), with seedlings inoculated or not with a mixture of AMF: *Rhizophagus clarus* (100 spores per pot), *Claroideoglossum etunicatum* (120 spores per pot), and *Gigaspora albida* (6 spores/pot); and five doses of KH₂PO₄ were tested (0, 50, 150, 300, and 450 mg dm⁻³ of P), with seven replicates.

Previously germinated, the seeds were soaked in YM broth with *Bradyrhizobium* sp. separately for 10 min. The *Bradyrhizobium* sp. (Brad 1) obtained and inoculated in *P. reticulata* had 7.5 × 10⁶ CFU, and *Bradyrhizobium* sp. (Brad 2) obtained and inoculated in *M. brauna* had 2.7 × 10⁶ CFU. Then, the seeds of each species were transplanted into 1 L plastic pots containing 650 g of soil harvested near an adult of each species separately.

The seedlings were irrigated with tap water to maintain moisture near field capacity, for *P. reticulata* about 50 % and *M. brauna* about 60 %. For every 15 days, 50 mL of a solution containing micro and macronutrients without P (Clark, 1975) were added to each pot.

Plant growth, nutrient content, nodulation, and responsiveness to inoculation

After a 90-days growing period, the plant's height was evaluated together with its stem diameter at 1.5 cm from the soil, shoot fresh matter (SFM), shoot dry matter (SDM), number and dry matter of nodules (DMN), and mycorrhizal responsiveness. The SDM and DMN were determined after drying until constant weight at 70 °C in a forced ventilation oven.

Nutrient contents were determined after evaluating growth measures. As *M. brauna* showed no difference for any measurement of growth, nutrient content was performed only for *P. reticulata*. The dried shoots of *P. reticulata* were processed in a Willey mill with a sieve of 40 mesh and submitted to nitric-perchloric acid digestion (Johnson and Ulrich, 1959). The nutrient contents K, Ca, Mg, and S were determined by Emission Spectrometry Inductively Coupled Plasma Optics. The N was determined by the Kjeldahl method (Silva, 2009), and the P content was determined colorimetrically by the vitamin C method as modified by Braga and Defelipo (1974).

Mycorrhizal responsiveness (MR) was calculated as suggested by Janos (2007), using the equation adapted proposed by Plenchette et al. (1983): MR = [(total shoot dry matter of inoculated plants - total shoot dry matter of uninoculated plants)/total shoot

dry matter of inoculated plants] \times 100. Data on mycorrhizal responsiveness were considered: $>75\%$ = extremely high responsiveness, $50\text{--}75\%$ = high responsiveness, $25\text{--}50\%$ = moderate responsiveness, $<25\%$ low responsiveness.

Mycorrhizal colonization

Fine roots (about 0.2 to 1.4 mm in diameter) were randomly sampled for evaluation of mycorrhizal colonization. After being washed under tap water, the roots were stored in FAA solution (formaldehyde, alcohol, and acetic acid, 5:90:5, v:v:v). These roots were washed in tap water one more time for removal of the FAA, and diaphanized with KOH 10% (w:v), by heating in a water bath at 90 °C for 1.5 h, followed by immersion in H₂O₂ 30% (10 min). Immediately afterward, they were immersed in KOH 10% (w:v) for 12 h, with successive washes in water, and subsequent acidification with HCl 2% (w:v) for 5 min, and, then stained with trypan blue 0.05% in lactoglycerol (w:v) for 12 h at room temperature (adapted from Phillips and Hayman, 1970). After staining, the roots were stored in a solution of lactoglycerol (Brundrett et al., 1996). The percentage of mycorrhizal colonization was estimated by the grid-counting method under a stereoscopic microscope (Giovannetti and Mosse, 1980).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) at a level of 1, 5, and 10%. Quantitative data were subjected to regression analysis, and the regression coefficients were analyzed by Student's t-test, using a free version (14.5) of SigmaPlot software.

RESULTS

The growth of *P. reticulata* and nutrient content of inoculated seedlings with AMF showed higher values compared to uninoculated ones (Figures 1 and 2). A quadratic effect with maximum points between 160 and 280 mg dm⁻³ of P was observed for plants inoculated with AMF in height, diameter, fresh (FDM), and shoot dry matter (SDM), while for the uninoculated plants, this effect was linear (Figure 1). At doses below 300 mg dm⁻³, AMF-inoculated seedlings showed higher growth than uninoculated ones (Figure 1). Seedlings inoculated with AMF showed increases varied from 60 to 73% in height, from 55 to 75% in diameter, from 30 to 45% in FDM, and 24 to 39% in SDM, depending on the dose of P applied to the soil concerning the uninoculated seedlings. For the highest dose of P tested (450 mg dm⁻³), there was no difference between the plants with or without AMF (Figure 1).

There was an interaction between doses of P and inoculation of AMF in *P. reticulata* to increase the nutrients contents up to 300 mg dm⁻³, varying from 59 to 73% for N; 68 to 86% for P; 58 to 79% for K; 52 to 75% for Ca; 55 to 77% for Mg; 61 to 78% for S; 30 to 72% for Zn; 40 to 64% for Fe; 50 to 77% for Mn; and 69 to 87% for Cu (Figure 2). There was a quadratic effect with maximum points at 150 mg dm⁻³ of P for the inoculated plants, whereas in the uninoculated plants, a linear effect was observed with nutrient quantity growing as the doses increased (Figure 2). For the micronutrient Zn and Cu, the regression was significant when the plants were inoculated with AMF, and the absence of inoculum did not affect the absorption of these elements, regardless of the doses of P (Figures 2e and 2h). For the absorption of Fe, the non-inoculated plants showed significant regression, and inoculation with AMF did not influence the absorption of this nutrient, regardless of the dose of P (Figure 2j).

Seedlings of *Melanoxylon brauna* inoculated or not with AMF or fertilized with different doses of P (0, 50, 150, 300, and 450 mg dm⁻³) showed no difference to growth measures (Figure 3). Regarding the percentage of mycorrhizal colonization, there were no differences between seedlings inoculated or not with the AMF, along with the dose of P, for both *P. reticulata* and *M. brauna* (Figures 4a and 4b).

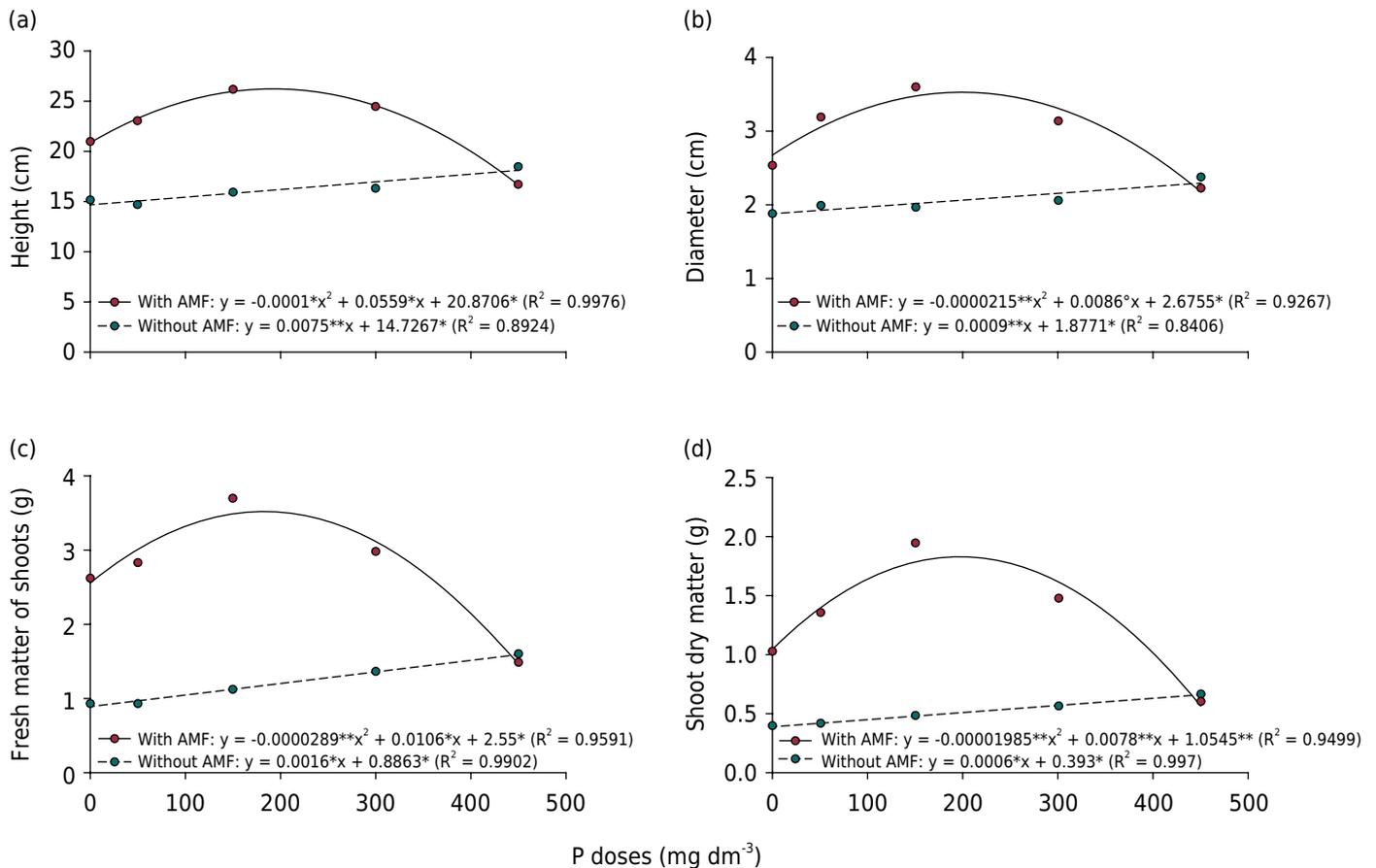


Figure 1. The response of *P. reticulata* seedlings to different doses of P (0, 50, 150, 300, and 450 mg dm⁻³ of soil), inoculated or uninoculated with a mix species of arbuscular mycorrhizal fungi (*Claroideoglossum etunicatum*, *Rhizophagus clarus*, *Gigaspora albida*), after 90 days of cultivation: (a) height, (b) diameter, (c) fresh, and (d) shoot dry matter. The indicative levels of significance of the regression coefficients in the equations were presented as *, ** and ° corresponding to 0.01, 0.05, and 0.10 probability, respectively.

Plathymentia reticulata did not present nodules due to inoculation with *Bradyrhizobium* sp. in any of the treatments. *Melanoxylon brauna* showed between zero to 344 nodules and between zero to 0.353 mg DMN, not differing from inoculation with AMF or even by the increasing doses of P (Figures 4c and 4d).

Mycorrhizal responsiveness to inoculation for *P. reticulata* was high (>60 %) at doses of P between 0 and 300 mg dm⁻³ and did not change with a dose of 450 mg dm⁻³, in which the growth of the inoculated plants was equivalent to that of the uninoculated ones (Table 2). *M. brauna* did not show any mycorrhizal responsiveness, being the shoot dry matter equal for the treatments (Figure 3).

DISCUSSION

Inoculation with AMF and phosphate fertilization favors the growth of *P. reticulata* seedlings but did not favor the growth of *M. brauna* in soil with rhizospheric microbiota of each species. These data partially support our hypothesis, which implies different fertilization strategies during the production of seedlings of tropical species (Freitas et al., 2017; Wright et al., 2018; Gibson et al., 2019). While *P. reticulata* is a median-growing plant categorized as early secondary (Lorenzi, 1992; Morim, 2020) and responds to fertilization and inoculation with FMA, *M. brauna* is a slow-growing plant categorized as late secondary (Lorenzi, 1992; Rando et al., 2020) and does not respond to fertilization with P and inoculation with AMF.

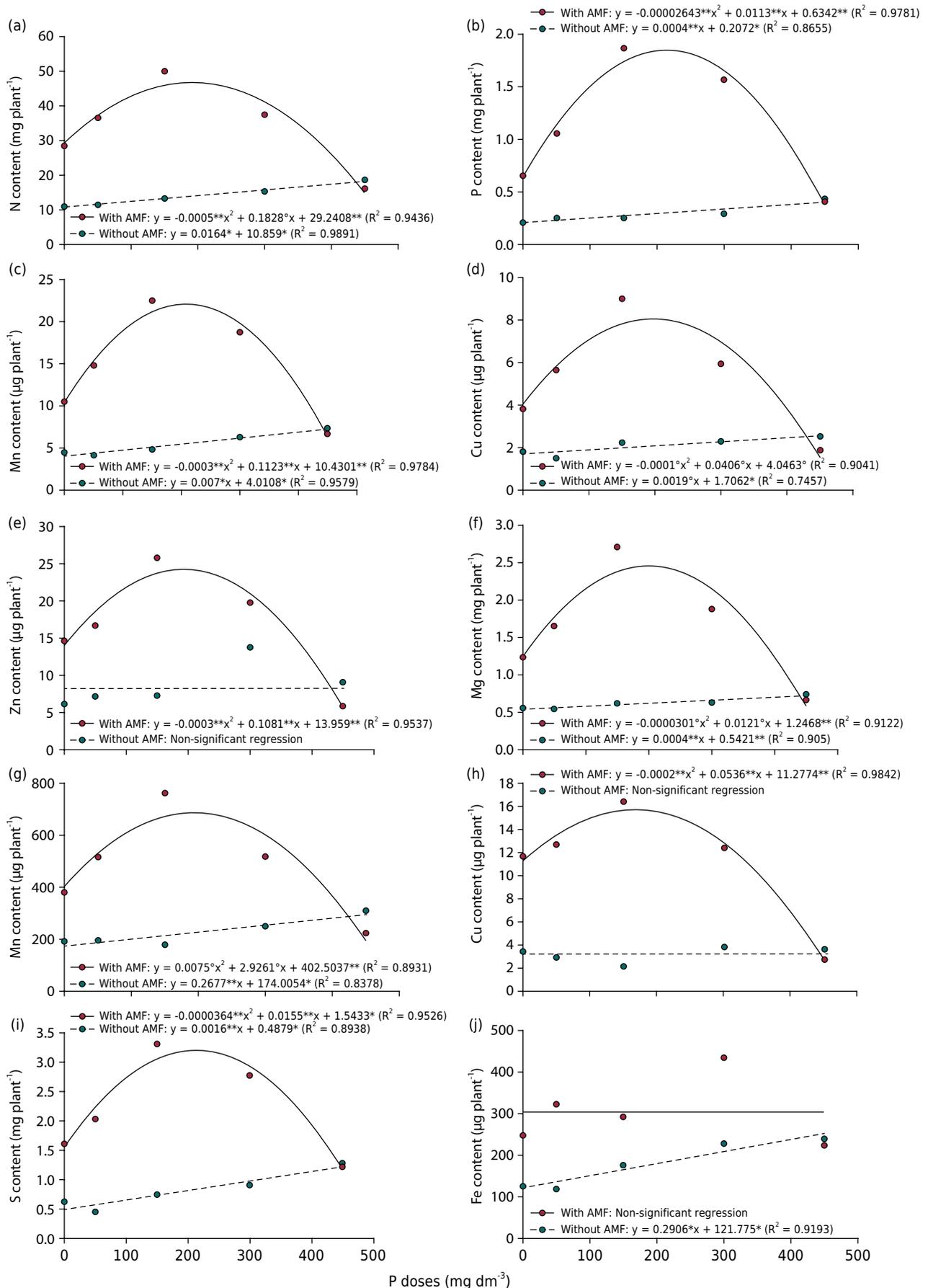


Figure 2. Nutrient content (a) N, (b) P, (c) K, (d) Ca, (e) Zn, (f) Mg, (g) Mn, (h) Cu, (i) S, and (j) Fe of *P. reticulata* seedlings after 90 days of cultivation, fertilized with P (0, 50, 150, 300, and 450 mg dm⁻³) and inoculated or uninoculated with species of arbuscular mycorrhizal fungi (*Claroideoglomus etunicatum*, *Rhizophagus clarus*, *Gigaspora albida*). The indicative levels of significance of the regression coefficients in the equations were presented as *, **, and ° corresponding to 0.01, 0.05, and 0.10 probability, respectively.

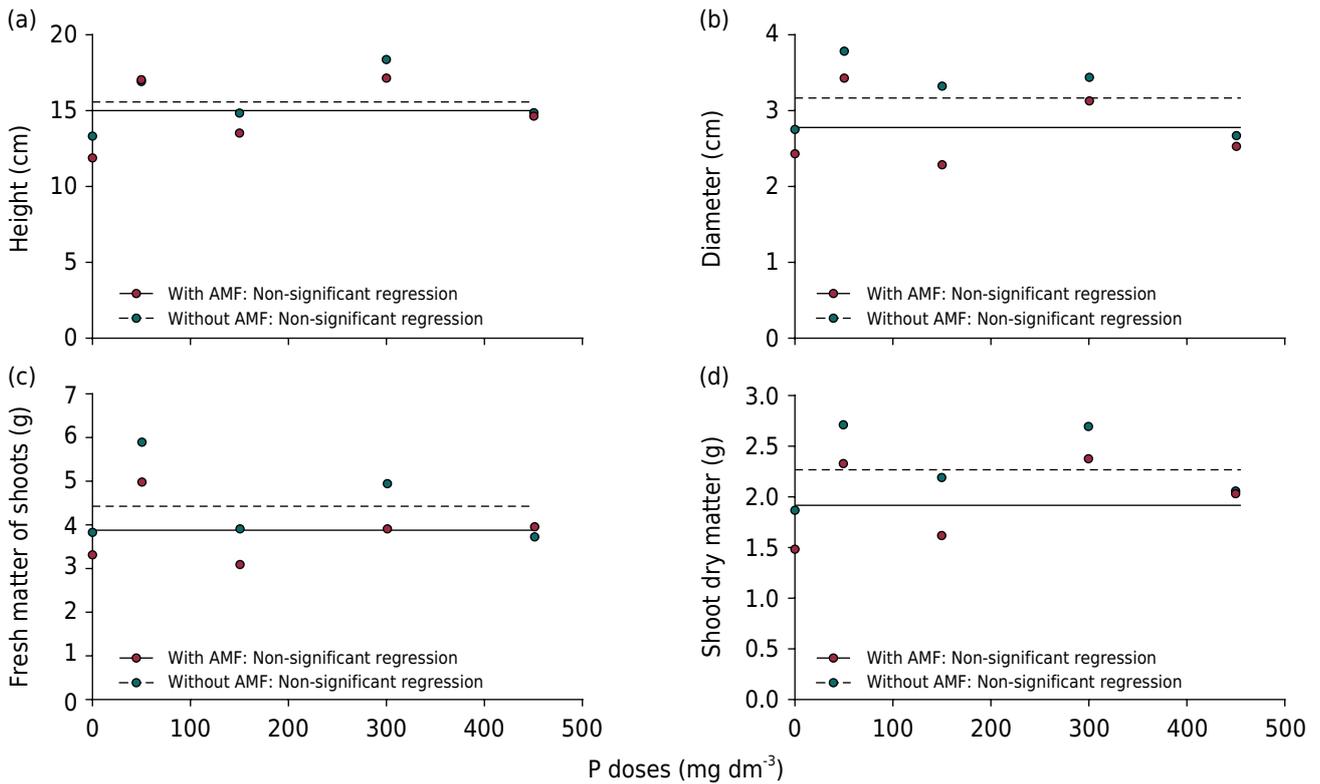


Figure 3. Response of *M. brauna* seedlings at doses of P (0, 50, 150, 300, and 450 mg dm⁻³ of soil), inoculated or uninoculated with a mixture of species of arbuscular mycorrhizal fungi (*Claroideoglomus etunicatum*, *Rhizophagus clarus*, *Gigaspora albida*), after 90 days of cultivation: (a) height; (b) diameter; (c) fresh, and (d) shoot dry matter.

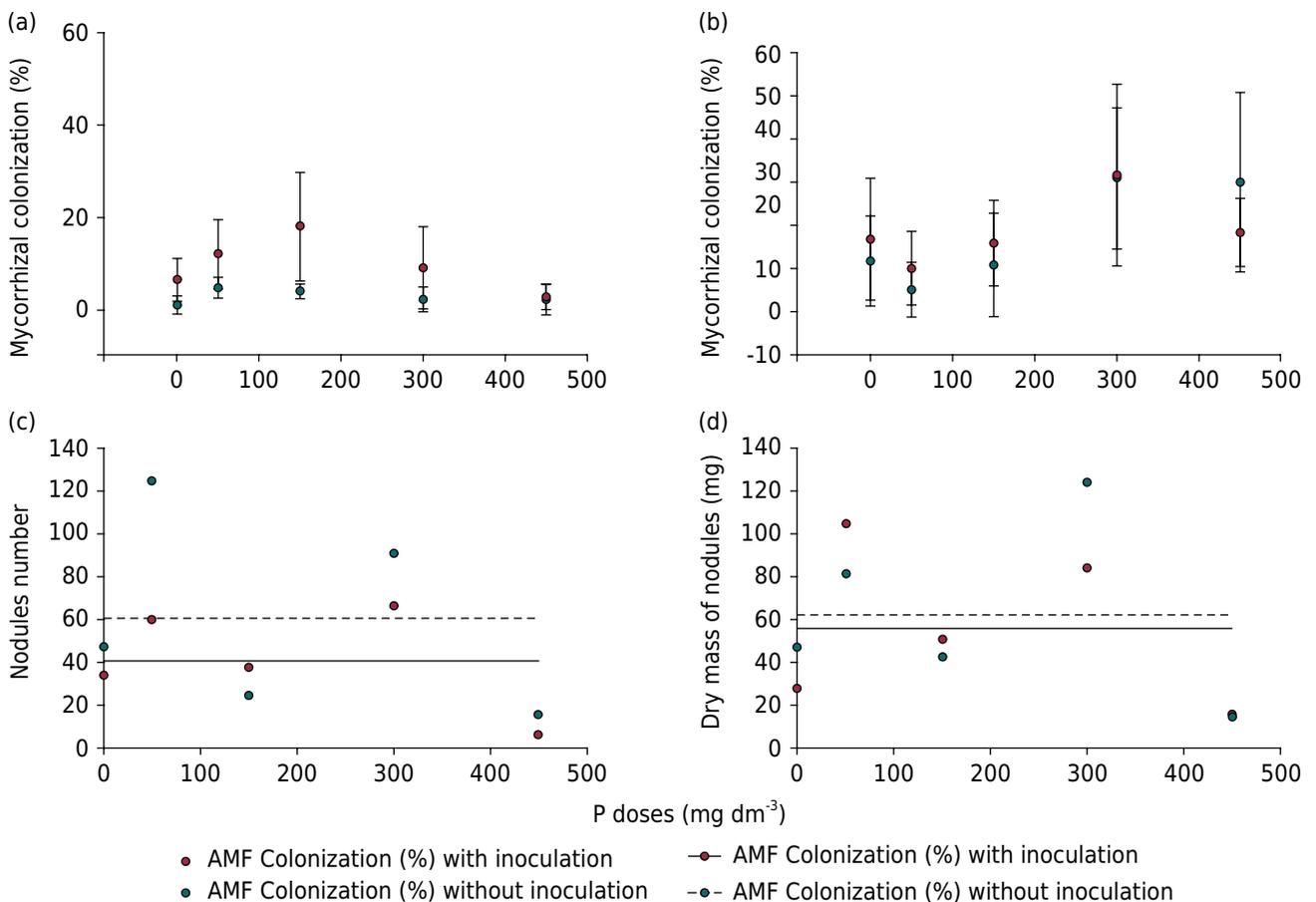


Figure 4. The percentage of mycorrhizal colonization of *P. reticulata* (a) and *M. brauna* (b) and number (c) and dry matter of nodules (d) in *M. brauna* after 90 days of cultivation, fertilized with P (0, 50, 150, 300, and 450 mg dm⁻³ of soil), inoculated or not with three species of arbuscular mycorrhizal fungi (*Claroideoglomus etunicatum*, *Rhizophagus clarus*, *Gigaspora albida*). Bar: standard deviation.

Table 2. Doses of P (mg dm⁻³) for mycorrhizal responsiveness of *P. reticulata* and *M. brauna* after 90 days of cultivation in the greenhouse

Phosphorus dose mg dm ⁻³	Mycorrhizal responsiveness
	<i>Plathymentia reticulata</i> Benth.
0	61.30
50	69.20
150	75.00
300	61.70
450	0.00

Response of *P. reticulata* to P fertilization when inoculated with AMF, showed a quadratic behavior, indicating that the highest plant growth occurs when the availability of P is below the recommended dose of 300 mg dm⁻³ (Figure 1), corroborating the recommended inoculated with rhizobium and AMF (Pagano et al., 2009), and fertilization for *P. reticulata* (Freitas et al., 2017). It can result in savings in phosphate fertilization and the production of seedlings with greater tolerance to water and salt stress, as well as better seedling nutrition, increasing the probability of success in the field (Goetten et al., 2016; Zhang et al., 2019; Anwar et al., 2020). *P. reticulata* showed high responsiveness to inoculation (Table 2), so the recommended doses of P are between 160 and 280 mg dm⁻³, which implies financial savings and production of good-quality seedlings. Doses of P higher than 300 mg dm⁻³ reduce the benefits of mycorrhizal association, as well as the percentage of colonization (Table 2).

Since when P is available to plants, AMF can act as a photoassimilate drain, without resulting in gains in plant growth, because the maintenance of mycorrhizal colonization becomes a loss for the plant, once the fungi receive the photoassimilates and is not reciprocate in terms of plant growth (Johnson et al., 1997; Andriano et al., 2020).

The benefits of AMF inoculation can go beyond increased growth, as it improves tolerance to biotic and abiotic stress (Johnson et al., 2010; He et al., 2019), vigor, and nutrient content in tissues (N, P, K, Ca, Mg, Zn, Mn, and Cu) (Goetten et al., 2016; Birhane et al., 2018; Karthikeyan and Arunprasad, 2021). The highest nutrient content in the tissues is indicative of healthy seedlings and may favor them when transplanted into the field. Inoculation is an important strategy for better utilization of nutrients in the cultivation substrates, especially the less mobile ones such as P, Zn, and Cu (Liu et al., 2000; Pasqualini et al., 2007; Nguyen et al., 2019) due to the capacity of the AMF to exploit higher volume (Smith and Read, 2008) and reduce the leaching of nutrients (Köhl and van der Heijden, 2016), making it possible to accumulate in the plant tissue.

Absence of a response from *M. brauna* to inoculation with AMF and fertilization with P (Figure 3) may be related to the source and form of P release, since controlled-release fertilizer (8000 mg dm⁻³) favor its growth (Gibson et al., 2019). Besides, due to the deforestation of the Atlantic and Cerrado forest, *M. brauna* are isolated in small forest fragments that decrease gene flow and duration of the experiment may have affected the result obtained with the experiment because this species is categorized as a slow-growing plant. Thus, the origin of seeds may represent genetically distinct populations and different responses to fertilization and inoculation with AMF strains. The species from tropical regions are exposed to environmental heterogeneity, including edaphoclimatic conditions that influence plant-soil feedback (van der Putten et al., 2013; Revillini et al., 2016; Prates Júnior, 2018) and can be influenced by different pathogenic and mutualistic microorganisms. There is evidence that species such as *M. brauna*, considered a late secondary species, are less responsive to inoculation and fertilization with P (Siqueira et al., 1998; Goetten et al., 2016). There is the possibility

that this plant species, during seedling phase, in natural conditions, may be fed by the mycorrhizal mycelium network formed with the roots of co-specific or heterospecific plants that occupy the canopy. Although the specificity of AMF with host plants is low, the source and potential of native inoculum can result in different plant responses (Pasqualini et al., 2007; Birhane et al., 2018). It is valid to evaluate the interaction with other sources and AMF species that may have a preferential association with *M. brauna*. The species may show high responsiveness to nodulation with N-fixing bacteria or with other beneficial fungi, such as Dark Septate Endophytes.

Our data showed that there was no difference in mycorrhizal colonization in *P. reticulata* (Figure 4a), contrasting with other forest species where the decrease in mycorrhizal colonization is followed by increasing P doses (Camenzind et al., 2014; Hailemariam et al., 2018). There was no difference in mycorrhizal colonization in *M. brauna*, indicating that AMF species in the soil from an adult plant can colonize the plant's roots (Figure 4b) because there are local adaptations among plants and AMF (van der Putten et al., 2013; Revillini et al., 2016). Thus, there are differences in mycorrhizal colonization depending on the successional group of host species, as well as the diameter and density of the root tissue (Zangaro et al., 2013; Asmelash et al., 2021), *P. reticulata* has fine roots, while *M. brauna* has thick roots, with few root hairs. The root architecture influences the hydraulic and nutritional performance of the plants and the responsiveness of AMF colonization, in *M. brauna* for example, that is poor in root hairs, it is suggested higher responsiveness on soil microbial symbionts, such as phosphate-solubilizing ones.

Absence of nodules in *P. reticulata* and the abundance of nodules in *M. brauna* indicates that the growth conditions, interaction with other microorganisms and plant species may or may not stimulate nodule formation and mycorrhizal association (Pagano et al., 2009; Lahrouni et al., 2012). The symbiotic relationship with rhizobia should improve by adapting the physico-chemical characteristics of the cultivation substrates (Castro Pires et al., 2017; Prates Júnior, 2018) and selecting efficient isolates in biological nitrogen fixation (Araújo et al., 2017). Although species of subfamily Mimosoideae (*P. reticulata*) seem to have more records of being associated with nodulating bacteria than members of Caesalpinioideae (*M. brauna*), genera *Melanoxylon* are a noduliferous group (Sprent, 2007; 2009). So, the responses of both species to inoculation with rhizobia, AMF, and fertilization may be evaluated together with liming, as suggested by Fonseca et al. (2010), to better understanding the adaptive responses of these plant species to symbiosis and phosphate fertilization.

Interestingly, *P. reticulata* may respond negatively to liming (Freitas et al., 2017), and there was no information regarding the effects of liming in *M. brauna*. The negative response of *P. reticulata* may be related not only to plant physiology, but also to groups of associated microorganisms that may respond negatively to pH increase (Prates Júnior, 2018). Under acidic conditions, common in soils where *P. reticulata* occurs naturally (Pagano et al., 2009), the phosphate ions are strongly bound to the clay fraction and, consequently, remain scantily available to the plants. Thus, for the best use of P present in the soil and/or substrate, it is essential to evaluate the response of the microbial community associated with *P. reticulata* and *M. brauna*. Thus, alternatives for improving the fertility of soils and/or substrates can be found without compromising the essential role of symbiotic microorganisms such as AMF and rhizobia.

CONCLUSIONS

Inoculation with Arbuscular Mycorrhizal Fungi (AMF) is an alternative to produce *P. reticulata* seedlings since the plants are well-nourished and have an association with AMF, helping plants to survive under nursery conditions. Besides, the positive effect of AMF, even with high doses of P (approximately 300 mg dm⁻³), indicates that the fertilization traditionally carried out for the implantation of this species in the field

does not interfere with mycorrhizal colonization due to the high responsiveness of *P. reticulata* with this association. However, we recommended the use of 150 mg dm⁻³ to guarantee the production of healthy seedlings of *P. reticulata*, resulting in economic and environmental gains. Despite of low colonization, *P. reticulata* receives beneficial effects from AMF and was more responsive than *M. brauna*. Inoculation with AMF and P fertilization does not promote the growth of *M. brauna* seedlings. On the other hand, it is paramount to evaluate the response of the microbial community associated with *M. brauna* to understand the role of the different symbionts related to the plant.

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