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ARTICLE

Interaction of *Acaena elongata* L. with Arbuscular Mycorrhizal Fungi under Phosphorus Limitation Conditions in a Temperate Forest

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ABSTRACT

The aim of this study was to analyze the performance of *Acaena elongata* colonized by arbuscular mycorrhizal fungi (AMF) to different phosphorus (P) concentrations, as a measure of AMF dependency. *A. elongata*, is a species from soils where P availability is limited, such as temperate forests. Our research questions were: 1) How do different P concentrations affect the AMF association in *Acaena elongata*, and 2) How does the AMF association influence *A. elongata*'s growth under different P concentrations? *A. elongata*'s growth, P content in plant tissue, AMF colonization and dependency were measured under four P concentrations: control (0 g P kg^{-1}), low (0.05 g P kg^{-1}), intermediate (0.2 g P kg^{-1}) and high (2 g P kg^{-1}) in different harvests. A complete randomized block design was applied. *A. elongata*'s growth was higher under −AMF in intermediate and high P concentrations, and the lowest growth corresponded to +AMF in the low and intermediate P concentration. We observed a negative effect on the root biomass under +AMF in intermediate P concentration, while the P concentration had a positive effect on the leaf area ratio. The AMF colonization in *A. elongata* decreased in the highest P concentration and it was favored under intermediate P concentration; while the low and the high concentrations generated a cost-benefit imbalance. Our results suggest that the performance of some plant species in soils with low P availability may not be favored by their association with AMF, but a synergy between AMF and intermediate P concentrations might drive *A. elongata*'s growth.

KEYWORDS

Abies religiosa forest; arbuscular mycorrhizae; plant growth; soil fertilization

1 Introduction

The availability of essential nutrients for the plants, as nitrogen (N) and phosphorus (P), is limited in soil of temperate forests, severely affecting the growth and reproduction of plant species in these forests [1,2]. Phosphorus availability is determined by the interaction of biotic and abiotic factors, such as the parental material type, weathering, pedogenesis, and organic matter decomposition [3]. The soils of some temperate forests are Andosols, which show slightly acid pH values (pH = 5.4 to 6.5) as well as a high content of organic matter (OM = 13% to 34%), which favor the retention of phosphates (PO_4^{3-}) [4,5]. In these soils with andic properties, the limited P availability is attributed to their high capability of phosphates retention (PR) ≥ 85%, caused by their amorphous clay fraction (e.g., allophane, imogolite and ferrihydrite) and the formation of complex aluminum (Al^{3+}) and ferric (Fe^{2+}) organo-minerals. These compounds show a high specific surface and many reactive sites for the retention of phosphates, which are increased in soils with acid pH [6,7]. It is estimated that P availability in soils vary from 2 ppm to 30 ppm [8]. This high capability of phosphates retention in forest acid soils affect the growth and nutrition of plants, since phosphorus is critical for several physiological, biochemical and structural processes of plants [9–11].

In temperate forests, anthropogenic disturbances can induce P limitation affecting the supply of other nutrients, most often N [12]. The increase in P cycling observed in N-fertilized soils typically is insufficient to balance the increased rate of N inputs, and P often becomes limiting [13]. Also, it has been shown that livestock and habitat deterioration promote a reduction in available P and an increase in soil pH [14]. This could be explained due to cation release during the mineralization of cattle manure, which increases soil pH and favors P precipitation in unavailable forms for plants [15]. On the other hand, these anthropogenic disturbances also modify the structure and composition of plant communities, favoring the establishment of species that show adaptive strategies to colonize disturbed sites [16]. An anthropogenic disturbance indicator plant species is either a native or introduced species that increases its abundance in sites that have already been changed by humans [16,17]. Such is the case of *Acaena elongata* L. (Rosaceae), which geographic distribution is restricted to temperate forests and mountain areas; mainly in the understory of *Abies religiosa* (Kunth) Schltdl. & Cham., (Pinaceae) forests [18,19]. This species is a successful colonizer of disturbed sites because of its life history traits; for instance, it has a high growth rate, an exozoochorous dispersal, a high tolerance to a great variety of environmental conditions and a constant flower and fruit production throughout the year [5,20,21]. The establishment of this type of plants has important ecological implications, due to the fact that they can contribute to the maintenance of microenvironmental conditions of the soil; thus, favoring the community microbial activity and functionality in the soil, as is the case of the arbuscular mycorrhizal fungi (AMF) [22,23].

The plants that are associated with AMF increase their P absorption in soils with low availability of this nutrient [24]. It is known that AMF improve the growth, survival and reproductive success of their hosts as a response to an increase in the uptake of nutrients and water, through extraradical hyphae [25–27]. The functionality of AMF, as well as the plant's growth, are strongly regulated by the P availability in the soil [28]. A low P availability has been linked to a higher AMF colonization in plant roots and to a positive effect of the AMF association on host growth (mutualism); while an increase in the availability of this nutrient can negatively affect or even inhibit the interaction between AMF and roots but a positive effect on host growth (antagonism), with species-specific thresholds [29,30]. This variation in the response of the AMF association and the host growth have ecological implications, which are important in the ecosystem function because it may favor the dominance of certain plant species [31,32]. Thus, the high dominance of a species may have strong affectations in the ecosystem functions, because it can cause a homogenization in the AMF composition; for instance, especially if dominant plants are specialists and dependent on AMF colonization [31]. Given the importance that soil P availability has for the functionality of AMF association and in the growth and development of plants, research on those species that have a high capability to colonize and develop in disturbed sites is needed, this is the case of *A. elongata*, which has a high competitive capability, for what has been proposed as a anthropogenic disturbance indicator plant species with the possibility of impacting the structure and composition of communities, and the ecosystem functions as well [5,33].

It has been suggested that disturbance indicator plant species with highly competitive capability do not show a strong dependency on their interaction with AMF [24,34]. However, there is evidence that the response of this type of plants to AMF colonization is variable and can favor their growth and survival [35]. The relation between anthropogenic disturbance indicator species that become dominant in the vegetation and the AMF, in terms of the plant's performance in an ecosystem, where P availability is low has been scarcely studied. Therefore, the aim of this study was to analyze the performance of *A. elongata*, colonized by AMF to different P levels, as a measure of AMF dependency. Therefore, our research questions were: 1) How do different P concentrations affect the AMF association in *Acaena elongata*, and 2) How does the AMF association influence *A. elongata*'s growth under different P concentrations? It is known that a low and intermediate P availability in soil may favor the AMF association, while a high availability of this nutrient can have negative effects on this association [30]. Thus, we expect that the AMF colonization and *A. elongata*'s growth will be increase under low and intermediate P concentrations; while under the highest P concentration, the AMF colonization will decrease but not plant growth.

2 Materials and Methods

2.1 Study Site

At the Magdalena river basin (MRB), the *Abies religiosa* forest is part of the temperate forest that constitutes the remaining vegetation at Mexico City (CDMX) [36]. This forest has approximately 1,130 ha of extension and represents 37.8% of the total surface, which makes it the most extensive vegetation type within the MRB [37]. The climate is temperate sub-humid, where mean annual temperature is 18°C, mean annual precipitation is 1, 200 mm and thermal oscillation is lower than 5°C. The rainy season extends from May to October, while the dry season occurs from November to April. Landscape slopes vary from 2° to 50°. The two dominant soil groups are Leptozols and humic Andosols [4]. In these soils, the interval of available P concentration varies between 2 ppm and 20 ppm, which is similar to the reported for other temperate forests [38,39]. Tab. 1 shows some soil parameters of the study site.

Table 1: Mean values ± SD of soil parameters in the *Abies religiosa* forest in the Magdalena river basin during the rainy and the dry seasons in 2017

Season	pH	EC 1:5 H_2O mmhos/c dSm^{-1}	MO (%) Walkley-Black	PO_4-Olsen (ppm)	Nt (%) Kjeldahl
Rainy	5.94 ± 0.4	0.08 ± 0.1	25.01 ± 5.7	7.0 ± 4.4	0.72 ± 0.2
Dry	5.98 ± 0.2	0.08 ± 0.02	19.93 ± 4.79	5.87 ± 3.5	0.60 ± 0.6

EC = electric conductivity, MO = organic matter, P = phosphorus and Nt = total nitrogen

2.2 Soil Sampling and Processing

In the *A. religiosa* forest, the reported AMF spore density is 170/50 g of dry soil [40]. Twenty-nine morphospecies of AMF have been registered. The most abundant genera are *Acaulospora* with 78% of the total spores, *Ambispora* with 9%, *Claroideoglomus* with 5%, *Funneliformis* with 4%, *Archaeospora*, *Diversispora*, *Glomu*s, *Rhizophagus*, *Sclerocystis* and *Scutellospora*, had all together 4% [5,40].

Therefore, we sampled 560 kg of soil in different spots within the *A. religiosa* forest of the MRB; these samples corresponded to the first 20 cm deep, previously removing the litter. The soil was homogenized and divided in two parts. The first one was used with the native AMF inoculum for the "with AMF = +AMF" treatment. The second part was pasteurized (100°C; 1.4 kg cm^{-2}; 1 h, this procedure was repeated for three consecutive days) and used for the "without AMF = −AMF".

2.3 Seeds Collect and Germination

Approximately 1,250 seeds (achenes, fruits) of *A. elongata* were collected in October and November 2016; since these months have been registered as the highest for the presence of mature fruits [5]. Seeds were dried at room temperature for a month. All seeds were disinfected with 10% sodium hypochlorite for 10 minutes, and were set for germination in pots with sterile vermiculite and watered daily, and after 60 days 91% of germination was reached.

2.4 Determination of Soil P Concentrations for Experimental Treatments

Phosphorus concentrations were selected based on 180 data of soil available P (ppm, PO_4^{3-} Olsen) registered in previous sampling from 2012 to 2017, in this study site. In this way, we established four levels of P concentration (Tab. 2). The fertilizer used was triple superphosphate [Ca $(H_2PO_4)_2.H_2O$], also known as monocalcium phosphate.

Table 2: Phosphorus concentration in the soil of the *Abies religiosa* forest at the Magdalena river basin, at Mexico City and P concentration under experimental conditions in the greenhouse

Treatments	P in forest soil (g PO_4^{3-} kg^{-1})	P added (g PO_4^{3-} kg^{-1})
Control		0
Low	0.002	0.05
Intermediate	0.01	0.2
High	0.036	2

2.5 Experimental Design

We set a greenhouse (3 m × 6 m) in the *A. religiosa* forest of the MRB, where we placed eight tables (blocks, 2 m × 1 m each). We use black plastic bags (with 1 kg of soil), perforated in the base to allow water drainage.

We designed the experiment considering the AMF factor (+AMF, –AMF), P factor with four concentrations (0 g P kg^{-1}, 0.05 g P kg^{-1}, 0.2 g P kg^{-1} and 2 g P kg^{-1}) and six times of measurement (harvests). A complete randomized block design was used in order to reduce noise factors, as well as to reduce and control the variance of the experimental error, as it has been implemented in other studies by [41,42]. Blocks were incorporated as a random factor. In total we had 480 seedlings (240 +AMF and 240 −AMF).

Acaena elongata plants were transplanted once they reached 20.5–35.1 mm height. Fifteen days after the transplant, we added the fertilizer in the mentioned concentrations. Plants were watered with distilled water twice per week throughout a year.

We also installed a HOBO data logger (Lascar, EasyLog EL-USB2, Massachusetts, USA); in the greenhouse to measure mean monthly temperature values. During the experiment a mean annual temperature of 9.6°C, a minimum of −2.5°C and a maximum of 27°C were registered.

2.6 Growth Parameters

For each treatment we selected 10 plants. The number of leaves was counted, and height, cover and stem diameter were measured in each plant every two months, through a year. The cover of each individual was obtained using the perpendicular diameters registered with a digital vernier (Mitutoyo, 500-196-20/30 0–150 mm/0-6" Japan); we assumed that, for each individual, the cover presents a circular shape, it was calculated following the equation:

$$C = \pi\left(\frac{D1 + D2}{4}\right)^2 \quad (1)$$

where: C = cover, D1 = horizontal diameter, D2 = vertical diameter.

On the other hand, with the rest of the plants, six harvests were carried out with five individuals randomly selected, per treatment, in six different times. We obtained indirect measurements of growth through a destructive method. We registered the leaf area of each plant with field portable leaf area meter device (OPTI-SCIENCES, AM300, USA); the chlorophyll content with a chlorometer (OPTI-SCIENCES, CCM-300, USA); and the total fresh weight (TFW) afterwards the plants were dried in a Riossa oven at 72°C, during 24 hours until reaching a constant weight, the total dry weight was measured (TDW), as well as the root and shoot weight, using an analytical balance (OHAUS, AX1502, USA). Finally, the dried plant material was grounded and analyzed for P concentration, following the modified semi-Kjeldahl method and using ammonium molybdate-ascorbic acid colorimetric method for autoanalyzers [43].

For the plant growth measurement, indirect parameters were determined through the following equations proposed by [44]:

i) Relative growth rate (RGR):

$$RGR\ DW = \frac{ln\ TDW_{t+1} - ln\ DW_t}{(t+1) - t} \quad (2)$$

where: RGR DW = Relative Growth Rate of the dried weight, $\ln TDW_t$ = natural logarithm of the total dried weight at time t, $\ln TDW_{t+1}$ = Natural logarithm of the total dried weight at time t + 1.

ii) Dried matter (DM): Shows the percentage that the dried weight represents in relation to fresh weight:

$$DM = \frac{DW}{FW} * 100 \quad (3)$$

where: DM = Dried matter in percentage, DW = Weight of the sample after being dried, FW = Weight of the sample before being dried.

iii) Relation root/shoot (R/S):

$$R/S = \frac{DWr}{DWs} \quad (4)$$

where: DWr = Root dried weight, DWs = Shoot dried weight.

iv) Root mass fraction (RMF):

$$RMF = \frac{DWr}{TDW} \quad (5)$$

where: DWr = Dried weight of the root, TDW = Total dried weight of the plant.

v) Leaf area ratio (LAR):

$$LAR = \frac{LA}{TDW} \quad (6)$$

where: LA = Leaf area, TDW = Total dried weight of the plant.

2.7 AMF Colonization of Roots

AMF colonization was carried out in five individuals randomly selected from each treatment. The finest roots were extracted. Roots were processed according to the method of Koske et al. [45], with KOH 10%, HCl 10% and stained with trypan blue 0.05%. Afterwards, 20 to 25 root fragments, of approximately 2 cm long, were fixed on permanent slides. The number of fragments depended on the age of the plant and P concentration, and they were placed in a parallel way on the slide. Finally, we quantified the AMF colonization through the method of McGonigle [46] using an optic microscope (NYKON, eclipse E-100LEDMV, Japan), with 20X and 40X objectives, where the minimum number of observed fields was 80.

The colonized fields were observed by the presence of aseptated hyphae, arbuscules and vesicles. Thus, we determined the colonization percentage through the following equation:

$$\text{AMF colonization (\%)} = \frac{\text{Number of colonized fields}}{\text{Total number of observed fields}} (100) \tag{7}$$

2.8 Relative Field Mycorrhizal Dependency

Relative field mycorrhizal dependency (RFMD) is an index that estimates the effect of AMF on the DW of plants with and without mycorrhiza. The value intervals of this index are between $-\infty$ and 100% [47].

$$\text{RFMD} = \left(\frac{\text{TDWm (g)} - \text{TDW nm(g)}}{\text{TDWm}}\right) (100) \tag{8}$$

where: TDWm = total dried weight of the mycorrhized plant and DWnm = total dried weight of the non-mycorrhized plant.

3 Data Analysis

To know the effect that each factor and time had on the height, cover, stem diameter, number of leaves, chlorophyll content, total dried weight, dried matter, root/shoot ratio, radical proportion, leaf area ratio and AMF colonization percentage, we carried out mixed effects linear models (LME library) [48], with the R software, version 3.3.1 [49]. We used the residuals to check normal distribution and homogenous variances. The coefficients of linear prediction were estimated using the method of maximum restricted likelihood (REML). Most of the data were transformed, except for LAR, in order to accomplish with the model assumptions. Height, cover, stem diameter, leaf number, dried matter and the root/shoot ratio were processed through the Rank function (average method); the TDW value and chlorophyll content were calculated through the log function. AMF colonization percentage was transformed with the arcsin (x)/100 function.

We also carried out a generalized linear model (lme4 library, GLM), McCullagh et al. [50] to know the effect of the interaction of the AMF factor with P concentration and time on the relative growth rate (RGR) and on the P content in the plant tissue. The RGR was analyzed as repeated measures. We worked with a Gamma probabilistic model and an "identity" binding function. The control treatment under −AMF in 0 g P kg^{-1}, was used as a reference level.

The RFMD index was analyzed with a one-way analysis of variance (ANoVA). In this case, we applied a multiple comparison of means through a Tukey test [51], to know the differences between treatments.

We calculated Spearman's correlation coefficients (library corrplot) for the variables of height, cover, stem diameter, leaves number, DW, DM, R/S, RMF, LAR, AMF colonization and P concentration in the plant. In all cases, $p \leq 0.05$ was taken as significant.

4 Results

4.1 Growth of **Acaena elongata**

We observed that AMF factor with P concentration and time had a significant effect ($p < 0.001$) on the plant's height, cover, stem diameter, number of leaves, DW, root/shoot ratio, root mass fraction (RMF), leaf area ratio and chlorophyll content; while the interaction between these factors did not have a significant effect on these growth variables (Tabs. 3 and 4). The growth of *A. elongata* increased significantly under −AMF in low and intermediate P concentrations, while it was not favored under +AMF in intermediate P concentration.

Table 3: Summary of the statistical results of the mixed effects linear model (LME) for height, cover, stem diameter and number of leaves of *Acaena elongata* grown in greenhouse conditions. Where: AMF factor x [P] is the interaction in AMF factor (+AMF and −AMF) with P concentrations (control, low, intermediate and high)

Source of variation		Height		Cover		Stem diameter		Leaves	
	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
AMF x [P]	7, 565	910.9	**<0.001**	9.5	**<0.001**	3.2	**<0.01**	4.9	**<0.001**
Time	7, 565	10.8	**<0.001**	129.9	**<0.001**	102.8	**<0.001**	129.9	**<0.001**
AMF x [P]: Time	49,565	0.7	0.843	0.7	0.921	0.8	0.800	0.8	0.76

Note: Significant differences are shown in bolds ($p < 0.05$).

Table 4: Summary of the statistical results of the mixed effects linear model (LME) for TDW = Total dried weight, R/S = root/shoot ratio, RMF = root mass fraction, LAR = leaf area ratio and Chlor = Chlorophyll content of *Acaena elongata* L. grown in greenhouse conditions. Where: AMF factor x [P] is the interaction in AMF factor (+AMF and −AMF) with P concentrations (control, low, intermediate and high)

Source of variation		TDW		R/S		RMF		LAR		Chlor	
	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
AMF x [P]	7, 192	8.2	**<0.01**	3.5	**<0.01**	3.1	**<0.01**	3.3	**<0.01**	7.75	**<0.01**
Time	5,192	164.5	**<0.01**	32.1	**<0.01**	35.1	**<0.01**	34.7	**<0.01**	31.21	**<0.01**
AMF x [P]: Time	35, 192	1.8	0.1	1.5	0.052	1.4	0.050	1.7	**0.013**	4.17	**<0.01**

Note: Significant differences are shown in bolds ($p < 0.05$).

Plant's height ($\bar{X}$ = 89.2 S.D. = ±7.4 mm), cover ($\bar{X}$ = 0.030 ± 0.0027) m^2, and TDW ($\bar{X}$ = 2.29 ± 0.41 g) were the highest under −AMF in 0.2 g P kg^{-1}; while the lowest means of height ($\bar{X}$ = 47.32 ± 3.02 mm), cover ($\bar{X}$ = 0.011 ± 0.0013 m^2), number of leaves ($\bar{X}$ = 72 ± 8.66 leaves) and TDW ($\bar{X}$ = 1.08 ± 0.36 g) occurred under +AMF in 0.2 g P kg^{-1}. Stem diameter was higher under +AMF in 0.05 g P kg^{-1} and 2 g P kg^{-1} ($\bar{X}$ = 2.22 ± 0.14 mm, $\bar{X}$ = 2.64 ± 0.19 mm, respectively); while, the lowest value was observed under +AMF in 0.2 g P kg^{-1} ($\bar{X}$ = 1.42 ± 0.09 mm).

The root/shoot ratio ($\bar{X}$ = 2.56 ± 0.57) was the highest under +AMF in 0.2 g P kg^{-1}, and the lowest was registered at +AMF in 0.05 g P kg^{-1} ($\bar{X}$ = 0.54 ± 0.085). The highest chlorophyll content ocurred under −AMF fungi in P control ($\bar{X}$ = 389.74 ± 18.9 (mg/m^2) and the lowest in the treatment under +AMF in 2 g P kg^{-1} ($\bar{X}$ = 342.15 ± 25.5 mg/m^2). The RMF resulted the highest under +AMF and −AMF in P control ($\bar{X}$ = 0.46 ± 0.036, $\bar{X}$ = 0.41 ± 0.025) but the lowest was observed under +AMF in 0.05 g P kg^{-1} ($\bar{X}$ = 0.32 ± 0.028). LAR was higher under +AMF in 0.05 g P kg^{-1}, 0.2 g P kg^{-1} and 2 g P kg^{-1}.

According to the generalized linear model (GLM), AMF factor with P concentration and time had a significant effect on RGR; while the interaction between these factors did not have a significant effect. A significant decrease was observed in the RGR under +AMF and −AMF in 0.05 g P kg^{-1} and 0.2 g P kg^{-1} with +AMF (Tab. 5).

4.2 AM Fungal Colonization of A. elongata Roots

The P concentration had a significant effect ($P<0.05$) on the AMF colonization percentage according to the LME. Under 0 g P kg^{-1} ($\bar{X}$ = 31.6 ± 12.1%), 0.05 g P kg^{-1} ($\bar{X}$ = 33.5 ± 14.1%) and 0.2 g P kg^{-1} ($\bar{X}$ = 28.49 ± 11.5%), highest values of AMF colonization were observed, in contrast under 2 g P kg^{-1} we observed the lowest AMF colonization percentage $\bar{X}$ = 20.7 ± 14.7% (Fig. 1).

Table 5: Summary of the statistical results of the general linear model (GLM) for RGR = relative growth rate of *Acaena elongata* L. under greenhouse conditions. Where: P_0 = 0 g P kg^{-1}, P_0.05 = 0.05 g P kg^{-1}, P_0.2 = 0.2 g P kg^{-1}, P_2 = 2 g P kg^{-1}. Time2 = 140 days, Time3 = 196 days, Time4 = 252 days, Time5 = 308 days, Time6 = 364 days

Source of variation		RGR ($g*g*d^{-1}$)		
	df	Coeficient	t	[P(>|t|)]
Intercep	180	0.057	5.427	**<0.0001**
P_0 A+MF	180	−0.003	−0.759	0.454
P_0.05 -AMF	180	−0.008	−2.401	**0.023**
P_0.05 +MF	180	−0.006	−1.841	**0.076**
P_0.2 -AMF	180	−0.004	−1.088	0.286
P_0.2 +AMF	180	−0.007	−2.002	**0.055**
P_2 -AMF	180	−0.004	−1.221	0.232
P_2 AMF	180	0.008	1.280	0.211
Time2	180	−0.044	−4.378	**<0.0001**
Time3	180	−0.042	−4.133	**<0.0001**
Time4	180	−0.041	−3.970	**<0.0001**
Time5	180	−0.048	−4.755	**<0.0001**
Time6	180	−0.043	−3.990	**<0.0001**

Note: Significant differences are shown in bolds.

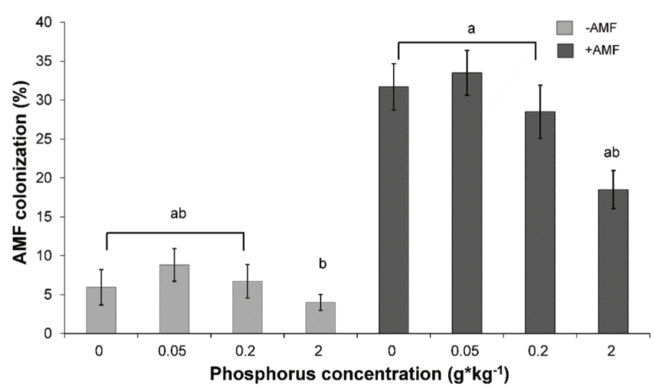

Figure 1: Arbuscular mycorrhizal fungal colonization percentage in the roots of *Acaena elongata* in different P concentrations. Grey bars correspond to AMF factor: –AMF, +AMF. Means ± S.D. Different letters indicate significant differences at $p < 0.05$

The Relative field mycorrhizal dependency (RFMD) showed significant differences among P concentrations, according to ANoVa ($F_{(3,24)} = 2.85$, $p = 0.049$). This index was positive only for control, 0 g P kg^{-1} ($\bar{X} = 24.1 \pm 26.1$, Fig. 2).

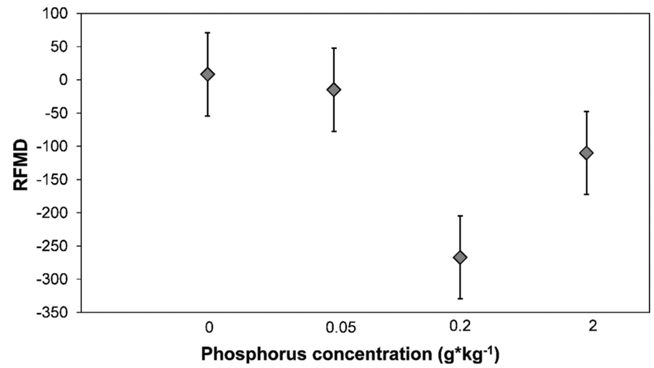

Figure 2: Relative field mycorrhizal dependency (RFMD) for P concentrations. Means ± SD

4.3 Spearman's Correlations

Positive and significant correlations were found ($p < 0.05$) in height, cover, stem diameter, number of leaves and chlorophyll content for all P concentrations. Indirect variables of growth such as: TDW, DM, R.S, RMF and LAR, showed a heterogeneous behavior according to P concentration. The AMF colonization showed a positive correlation with LAR and P concentration at treatment 0 g P kg^{-1}. This last correlation was maintained for treatment 0.05 g P kg^{-1}; while for treatment 0.2 0 g P kg^{-1} the correlation with P was negative (Fig. 3).

5 Discussion

The association between anthropogenic disturbance indicator plant species and AMF could have positive effects on the plants growth through helping them to endure the low P availability in the temperate forest soils [52,53]. It is known that under low or intermediate P concentrations in the soil, AMF colonization can promote the absorption of P by the host plant, giving as a result a benefit in its growth [30,54,55]. In this study, we observed that AMF did not show a significant positive effect on most of the measured growth parameters of *A. elongata*, since the highest values of height, cover, leaves, chlorophyll, TDW, DM and RMF corresponded to the plants under –AMF in low (0.05 g P kg^{-1}), intermediate (0.2 g P kg^{-1}) and high (2 g P kg^{-1}) P concentrations. However, AMF seem to have a positive effect on some growth parameters of their host under +AMF in the control (0 g P kg^{-1}) and the low (0.05 g P kg^{-1}) P concentrations, for example in the plant's tissue (%) and leaf area ratio. This result could be supported by the Spearman's correlation values, where the percentages of AMF colonization showed a positive correlation with LAR and the P content in the plant`s tissue. This can presumably be associated to the fact that AMF affect the leaf area by total dry weight unit ($m^2 \times kg^{-1}$); this is why they probably and indirectly promote an increase of fixed carbon (C) by photosynthesis, from which it is known that between 20 and 50% is translocated to the maintenance of the functionality of the AMF association [56]. It is already known that the C flux from the plant to the AMF is proportional to the amount of P that these fungi give back to their host [57].

Phosphorus availability in soil is a driver for the plant's growth and for the AMF function [24,28,30]. We observed an increase in the AMF colonization percentage under low and intermediate concentrations, with respect to the control treatment; while in a high concentration the colonization percentage significantly decreased. It is important to mention that LAR values were significantly higher under +AMF in the highest P concentrations; this is why, in the case of *A. elongata* growth (in terms of the LAR), this parameter was presumably favored by the increase of P concentration in the soil, more than by the AMF association. Under high nutrient concentrations in the soil, the growth of -AMF plants surpasses the growth of +AMF plants, this is due to the fact that a higher P availability promotes the increase of dried weight and root growth in the plants, which is related to higher growth rates of central and lateral roots, as well as a higher elongation and production of root hairs, suggesting that the plant is, thus, more efficient in nutrient acquisition; therefore, showing an possibly antagonistic interaction associated to the fact that the costs of the association exceed the benefits for the plant [30,58]. In Fig. 4, we can clearly observe how the AMF colonization is higher in low (0 and 0.05 g P kg^{-1}) P concentration; on the opposite way, when soil P availability is higher, the colonization percentage is reduced significantly.

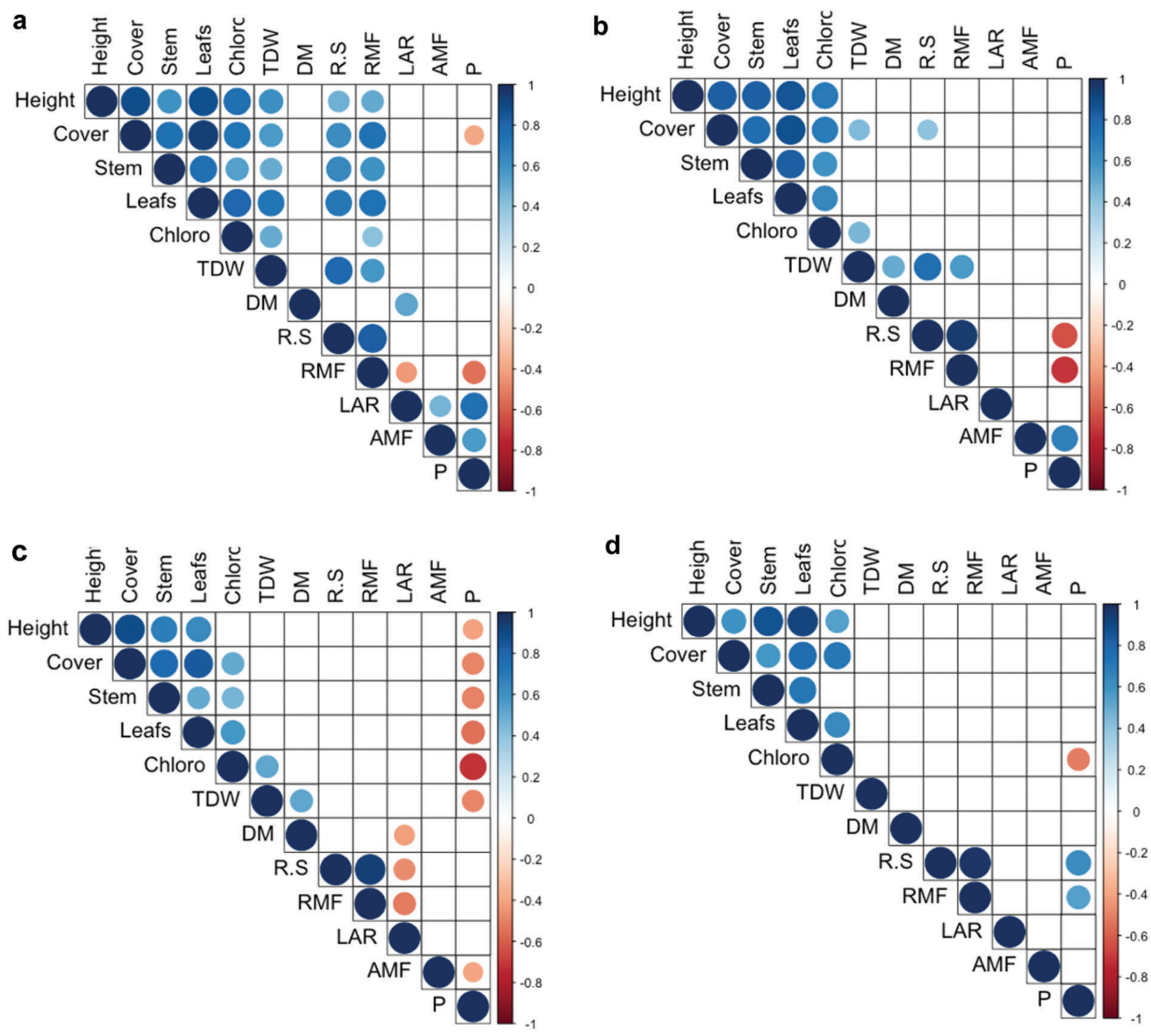

Figure 3: Significant Spearman's correlation values ($p < 0.05$) are shown, positive correlations in blue, and negative correlations in red, where 0 g P kg^{-1}, b = 0.05 g P kg^{-1}, c = 0.2 g P kg^{-1}, and d = 2 g P kg^{-1}. The intensity of color determines the degree of correlation

It is important to consider that the life history traits of *A. elongata* are part of some evolutionary factors that can also shape its association with AMF, and that possibly could contribute to explain our results. *A. elongata* is an anthropogenic disturbance indicator species and it has biological attributes that allow it to be successful in soils with low P availability. These features involve a fast increase in the plants size, in order to acquire more resources (light, water and nutrients) and an efficient root system to explore more soil volume [16]. For instance, growth rates also have a genetic component, which would explain the absence of significant differences in the RGR among the examined treatments. Nevertheless, under conditions of nutrients limitations, the AMF can work as facilitators in soil buffering stress conditions that the host plant has to face in disturbed sites, and therefore, having a positive impact on some processes of the plant's life cycle. This could have been reflected in the control treatment, which was the only one that showed a positive response to mycorrhiza (RFDM), which suggests that *A. elongata* showed a favorable response in terms of the TDW to AMF colonization; while in the treatments with higher P concentrations, plants showed negative RFDM. According to [59], in infertile soils the response of plants to mycorrhizal inoculation would be positive, because fungal cost in terms of C is equivalent to the P absorption by the mycorrhiza, even though this absorption can also decrease due to any other limiting nutrient [24,60].

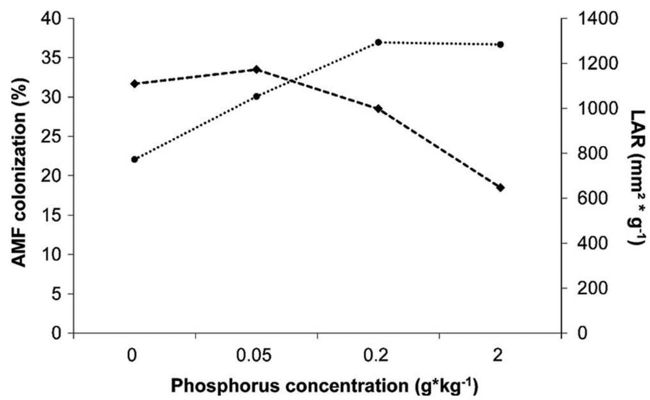

Figure 4: Change in the AMF colonization percentage (fragmented line with diamond marker) and in LAR = Leaf area ratio is the photosynthetic surface area per unit dry weight of a plant (dotted line with circle marker) according to added P concentration

It has been demonstrated that the association between anthropogenic disturbance indicator plant species and AMF can have positive impacts on their establishment, survival, growth or reproductive success, in this way increasing their competitive capability [31,26,61,62]. Hence, it is necessary to focus future research on other aspects of the role of these fungi on the germination, reproductive phenological phases, establishment and survival at long term of *A. elongata*, as an anthropogenic disturbance indicator species critical for the dynamics of the *A. religiosa* forest here studied.

The success of *A. elongata* in disturbed sites within the *A. religiosa* forest, at the MRB, is linked to its fast growth and mycorrhizal dependency positive under P limitation conditions. Thus, AMF can play an important role in the establishment of this anthropogenic disturbance indicator species, since they confer tolerance to environmental stress in disturbed sites, as an adaptive biotic mechanism to the low P availability in the soil.

Besides, our results indicate that available P in the soil regulates the growth and the response to mycorrhiza of *A. elongata*, and that an increase in soil P availability, may have critical ecological implications for both symbionts, on one side the fast growth of *A. elongata* may be favored, and on the other, the amount of P in the soil can also decrease or inhibit the AMF colonization, since the plant will have a negative response to AMF association, which could possibly negatively affect the AMF community and favoring the dominance of this disturbance indicator plant species.

6 Conclusion

Our study is the first one that provides an experimental data that explains the relationship between *A. elongata* and AMF under variability conditions of P added, which contributes to understanding the role of AMF on the performance of anthropogenic disturbance indicator plants inhabiting temperate forests. Ours results indicate that AMF not necessarily benefit *Acaena elongata*'s growth in soils with low P availability, and that there might be a synergy between AMF and intermediate P concentrations in the soil.

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