


Effects of Nutrient Deprivation on the Growth and Development of *Tabebuia rosea* Seedlings

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Abstract

Tabebuia rosea is a native tropical tree species with high potential of implementation in commercial reforestation and ecological restoration. We studied the effect of the suppression of essential nutrients on the growth of *T. rosea* in the nursery. The design was completely randomized with ten treatments: one that included full fertilization, another without fertilization, and the others with the suppression of N, P, K, Ca, Mg, S, B, and cationic microelements. Biometric variables (height, stem diameter, shoot dry weight, root dry weight) and functional traits (leaf area and relative growth rate) were evaluated. Phosphorus was the most limiting element. On the contrary, the suppression of Ca generated plants with growth and development similar to those of the FF treatment. Our results confirmed the importance of knowing the particular nutritional needs of individual species, which is to support nursery fertilization practices that produce highly vigorous and quality plants.

Keywords: seedling production, forestry nursery, morphological–functional traits.

1. INTRODUCTION AND OBJECTIVES

During 1990–2015, deforestation in the tropical zone decreased forest cover by 10% (196 million hectares), a scenario that unfortunately has an increasing projection until the year 2030 (D'annunzio et al., 2015; Keenan et al., 2015; Morales-Hidalgo et al., 2015). Ecological restoration and commercial reforestation with native tree species represent alternatives for the recovery of the structure, composition, and functionality of degraded ecosystems, as well as to reduce and mitigate the pressure on existing forest ecosystems (Hall et al., 2011; Cubillos et al., 2016).

The success of reforestation activities with native species mainly depends on the quality and nutritional status of the plant material used (Gregorio et al., 2015; Oliet et al., 2016; Wulandari et al., 2016). It is considered that plants produced under precise fertilization management will offer greater guarantees of survival and development in the

field, as a result of the improvement of plant performance (Ashiono et al., 2019; Mack et al., 2019).

Nevertheless, nursery fertilization practices of the species used in these projects are carried out in a generalized manner, without meeting the specific requirements of individual species (Camacho et al., 2014; Clark & Zheng, 2015). In addition, since each nutrient fulfills a specific function in the plant (Grossnickle, 2012), nutritional deficiencies can have a negative impact on their ability to adapt and grow (Grossnickle, 2012; Grossnickle & MacDonald, 2017). Hence, fertilization from the nursery must be consistent with the nutritional requirements of each native tree species, thus producing high quality and healthy plants with a higher probability of success at planting sites (Eser & Gülcü, 2019).

Colombia has a high potential for commercial reforestation (24.8 million hectares), however, forest plantations scarcely cover 450,000 hectares of the country (UPRA, 2014). On the other hand, given the high annual deforestation rates

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(220,000 hectares from 2016 to 2017: IDEAM, 2018), the need for ecological restoration is evident. Among the tree species with the greatest employment potential, *T. rosea* stands out because of the high quality and commercial value of its wood, its high rate of survival and growth in the field (Plath et al., 2011; Plath et al., 2012), and the recognition of tax benefits for its use in commercial reforestation (DNP, 2014). However, unlike other species, very little is known about its nutritional management in the nursery. This may affect the quality of the plant material produced, consequently limiting its success and decreasing the possibility of its use on a larger scale. The generation of information related to plant nutrient management is required, since, in the context of precision forestry, technical support of both *T. rosea* and other species is lacking, particularly at the early stage of the forest chain.

Usually, plant nutrient management studies evaluate the effects of nutrient treatments on tree seedlings by means of biometric variables (height, stem diameter, shoot dry weight, root dry weight) (Moretti et al., 2011; Sepúlveda et al., 2014; Corcioli et al., 2016). Nevertheless, other variables, such as, functional traits and plant quality indices offer additional valuable information of plant performance (Pérez-Harguindeguy et al., 2013).

In this study we considered both biometric variables and functional traits for: i. Identifying the key nutrients for growth and development of *T. rosea* seedlings in the nursery phase; and ii. Evaluating the response of this species to the deprivation of individual nutrients. Visual deficiency symptoms associated with each nutrient were also characterized.

2. MATERIALS AND METHODS

2.1. Study site

The study was carried out in a nursery located at Piedras Blancas Forest Station of Universidad Nacional de Colombia, Medellín (6°15'38" N y 75°30'23" W), at an altitude of 2,450 m. The mean annual precipitation is 1,815 mm, the mean temperature is 14.9°C, and the relative humidity is above 85%. This corresponds to an ecological zone of a lower montane humid forest (Holdridge, 1987).

2.2. Experimental establishment

The seeds of *T. rosea* were obtained from 15 mother trees, located in open areas in southwestern Antioquia, characterized

by high phenotypic and sanitary quality and by being at least 100 m apart from each other (Medina-Macedo et al., 2016). The collection of the seeds was carried out in August 2017 when the mature fruits acquired a light brown color and were brittle, that is, before they dehiscence. The fruits were collected from the middle and upper part of each tree. These fruits were air dried in order to facilitate their dehiscence and release the seeds (Gálvez-López et al., 2018). The seeds were sown in sterilized sand and manually irrigated daily in the morning. When the plants developed true leaves (average height = 2.83 cm; standard deviation = 0.23), we transplanted the seedlings to the growth substrate.

The substrate consisted of a volumetric mixture of soil and sand (3:2). The Andosol soil (Bw horizon, 30–50 cm) was sifted (4 mm mesh) and air dried. Subsequently, the substrate was disinfected with Basamid® [active ingredient: dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione)] at a dose of 200 gm⁻³. The substrate was covered with a plastic sheet for 10 days, and then aerated for 5 days.

2.3. Experimental design

The experiment consisted of 10 treatments, one that included full fertilization (FF), another without fertilization (Control), and the others resulting from the deprivation of macro and microelements from the FF treatment: without nitrogen (-N), without phosphorus (-P), without potassium (-K), without calcium (-Ca), without magnesium (-Mg), without sulfur (-S), without boron (-B), and without cationic microelements (-CM: Fe, Mn, Cu, Zn). Each treatment had 10 repetitions, and the experimental units were completely randomized. Nutrient sources and amounts applied to the substrate according to the corresponding fertilization treatment are presented in Table 1.

The nutrient supply in each of the deprivation treatments, was performed on a single occasion before filling the bags. Particularly, the nitrogen dose was fractionated and supplied at 15 and 30 days after transplanting. Similarly, the dose for the MC treatment was divided and supplied twice, at the beginning and 15 days after transplanting. The fractionation of the fertilization dose over time of these nutrients was carried out in order to reduce toxicity and/or nutritional imbalance risks (Raj et al., 2020). During the growth time of the seedlings in the nursery, no additional applications of nutrients were made to those mentioned above. A physical-chemical analysis was performed on the substrate before and after fertilization (Table 2).

Table 1. Nutrient sources and amounts applied to the substrate according to the corresponding fertilization treatment. FF: full fertilization (with B, Ca, P, Mg, N, K, S, Mn, Fe, Cu, Zn), -Ca: FF minus Ca, -Mg: FF minus Mg, -K: FF minus K, -S: FF minus S, -N: FF minus N, -P: FF minus P, -B: FF minus B, -CM: FF minus Mn, Fe, Cu and Zn.

Sources	FF	-Ca	-Mg	-K	-S	-N	-P	-B	-MC
Calcium chloride	CaCl ₂ ·2H ₂ O (g)	2.28	-	2.28	2.28	2.28	2.28	2.28	2.28
Magnesium chloride	MgCl ₂ ·6H ₂ O (g)	2.22	2.22	-	2.22	2.22	2.22	2.22	2.22
Potassium chloride	KCl (g)	0.42	0.42	0.42	-	0.42	0.42	0.42	0.42
Ammonium sulphate	(NH ₄) ₂ SO ₄ (g)	0.06	0.06	0.06	0.06	-	-	0.06	0.06
Magnesium sulphate	MgSO ₄ ·7H ₂ O (g)	-	-	-	-	-	7.34	-	-
Urea	CO(NH ₂) ₂ (g)	0.15	0.15	0.15	0.15	0.15	-	0.15	0.15
Phosphoric acid	H ₃ PO ₄ (mL)	4.30	4.30	4.30	4.30	4.30	4.30	-	4.30
Boric acid	H ₃ BO ₃ (g)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	-
Zinc sulfate	ZnSO ₄ ·H ₂ O (mg)	0.01	0.01	0.01	0.01	-	0.01	0.01	0.01
Iron sulphate	FeSO ₄ (mg)	0.01	0.01	0.01	0.01	-	0.01	0.01	0.01
Manganese sulphate	MnSO ₄ (mg)	0.01	0.01	0.01	0.01	-	0.01	0.01	0.01
Copper sulphate	CuSO ₄ (mg)	0.01	0.01	0.01	0.01	-	0.01	0.01	0.01
Zn chelate	EDTA-Zn-Na ₂ (mg)	-	-	-	-	0.03	-	-	-
Fe chelate	EDTA-Fe-Na (mg)	-	-	-	-	0.03	-	-	-
Mn chelate	EDTA-Mn-Na ₂ (mg)	-	-	-	-	0.03	-	-	-
Cu chelate	EDTA-Cu-Na ₂ (mg)	-	-	-	-	0.03	-	-	-

Table 2. Physical and chemical properties of the substrate before and after fertilization. The fertilized substrate corresponds to the fertilization formula of the full fertilization treatment (FF) in this study. CEC: Cation Exchange Capacity.

Property	Unfertilized substrate	Fertilized substrate
pH (1:1, water)	6	5.8
CEC (cmol _c kg ⁻¹)	2.8	3.8
Sand (%) (Bouyoucos)	96	96
Loam (%) (Bouyoucos)	2	2
Clay (%) (Bouyoucos)	2	2
Ca (cmol _c kg ⁻¹) (ammonium acetate 1N)	2.4	3
Mg (cmol _c kg ⁻¹) (ammonium acetate 1N)	0.3	1.1
K (cmol _c kg ⁻¹) (ammonium acetate 1N)	0.1	0.3
S (mg kg ⁻¹) (calcium phosphate, 0.008 M)	14	15
N (%) (Micro- Kjeldahl)	0.2	0.3
P (mg kg ⁻¹) (Bray II)	4.6	78.4
B (mg kg ⁻¹) (Hot water. Azometin)	0.2	1
Cu (mg kg ⁻¹) (Olsen-EDTA)	0.6	5.4
Fe (mg kg ⁻¹) (Olsen-EDTA)	41	41
Mn (mg kg ⁻¹) (Olsen-EDTA)	2	13.5
Zn (mg kg ⁻¹) (Olsen-EDTA)	1.1	6.2

Plastic bags (10 x 22 cm, 2 kg) were filled with this substrate corresponding to each treatment, and homogeneous plants were placed in the bags. The plants were kept in a humid greenhouse for seven months after transplanting, which preserved the moisture of the substrate in the bags (about 60% of the total pore volume).

2.4. Growth and visual symptoms monitoring

The growth variables monitored were: (i) the height (H), measured from the scar of the cotyledons to the highest bud; and (ii) the stem diameter (SD), measured with a digital caliper. Both were monitored monthly. In addition, the visual deficiency symptoms were recorded as they appeared and included a description of the symptom(s) and photographs of the representative leaves of the different treatments for comparison with the full fertilization treatment.

2.5. Plant harvest

After seven months of growth, the shoot and root portions of each plant were separated. For the fresh leaves, the leaf area (cm²) was obtained with a leaf area meter (LICOR LI-3000A). Subsequently, both shoot and root portions were oven dried (60°C, 72 h) until a constant mass was reached. The respective dry weights of each plant portions were obtained (SDW: shoot dry weight, g; RDW: root dry weight, g).

2.6. Leaf chemical analysis

Leaf samples were taken from five randomly selected plants corresponding to each treatment, and then combined for elementary chemical analysis. The combined leaf samples were ground and reduced to ashes (600°C). The chemical analyzes were performed differently according to the treatment. That is, for the FF and Control treatments, all the nutrients

considered in this experiment were analyzed (Ca, Mg, K, N, P, S, CM, and B). However, for each deprivation treatment (-Ca, -Mg, -K, -P, -N, -S, -CM, and -B), the nutrient not supplied was analyzed. Chemical analyzes followed the procedures described in Kalra (1998).

2.7. Data processing

Plant growth and quality indices were calculated (area under the curve and plant quality index) (Dickson et al., 1960; Shaner & Finney, 1997) as well as functional traits (leaf area and relative growth rate) (Pérez-Harguindeguy et al., 2013):

Area under the curve (AUC): height gain of plants in the growth period [1].

$$AUC = \sum_{i=2}^n (t_i - t_{i-1}) \left(\frac{h_{ti} + h_{ti-1}}{2} \right) [1]$$

Where n = number of periods evaluated, $t_i = i$ th day after transplanting, h_{ti} = height recorded on the i th day after transplanting.

Plant quality index (PQI): integrates the diameter and height of the plant with the ratio between the total dry weight of the plant (g) and the ratio of shoot portion to root portion [2] (Dickson et al., 1960).

$$PQI = \frac{TDW}{\frac{H}{SD} + \frac{SDW}{RDW}} [2]$$

Where H = height at harvest time (cm), SD = stem diameter at the time of harvest (mm), SDW = shoot dry weight (g), RDW = root dry weight (g), TDW = total dry weight (g).

Relative growth rate (RGR): increase in plant size over a given time interval [3].

$$RGR = \frac{(\ln TDW_2 - \ln TDW_1)}{t_2 - t_1} [3]$$

Where TDW_2 = total dry weight at the time of harvest (g), TDW_1 = initial total dry weight (g), t_2 = time elapsed from transplant to harvest (days), t_1 = initial time (0 days).

The nutrient content stored in the shoot portion of the plants was also determined. This was calculated, for each treatment, as the product between the nutrient leaf concentration and the SDW. In addition, in order to establish comparisons between the full fertilization treatment and the

nutrient deprivation treatments, relative performance (RP) was calculated for the variables H, SD, SDW, and RDW [4]:

$$RP = \frac{V_i(\text{Deprivation treatment})}{V_i(\text{FF})} [4]$$

Where V_i = the biometric variable (H, SD, SDW, RDW) and FF = the full fertilization treatment.

2.8. Data Analysis

One-way analysis of variance was performed to evaluate the effects of the treatments on the variables of interest. Next, multiple comparisons were made using the Tukey test. In all cases, a significance level of 5% was used. All analyzes were programmed using the R language (R Core Team, 2019).

3. RESULTS AND DISCUSSION

3.1. Biometric performance of the plants

During the seven months in the nursery, three response groups were identified with regard to the height (H) of the *T. rosea* seedlings, according to nutrient deprivation. The -Ca treatment group showed superior performance (similar to the FF treatment group) to that of the other nutrient-deprived treatment groups. The -P treatment group showed similar results to that of the Control treatment group with a 33.3% performance to the best performing group. The remaining nutrient-deprived treatment groups showed a 62.5% performance with respect to the best performing group (Figure 1).

At the end of the study, nutrient deprivation generated significant effects on the biometric variables ($P < 0.05$) (Table 3). The plants that performed better with respect to the Control treatment were those of the FF and -Ca treatment groups, with no statistically significant differences between them. The most limiting nutrient was P. Plants belonging to the -P treatment group showed similar results to those of the Control treatment group, and statistically significant differences in the biometric variables with respect to the other treatments were found. The remaining treatments exhibited intermediate values, and significant differences with respect to the FF treatment group were found.

The deprivation of nutrients caused changes in the relative performances of H, SD, SDW, and RDW with respect to the FF treatment group (Figure 2). Plants in the -Ca treatment group performed up to 45% better than those in the FF treatment group (Figure 2B). The -S, -P, and Control treatments showed performance values below 50% for each of these biometric variables compared to the FF treatment group, and -P had a performance value below 5% of the FF treatment group (Figure 2C and D).

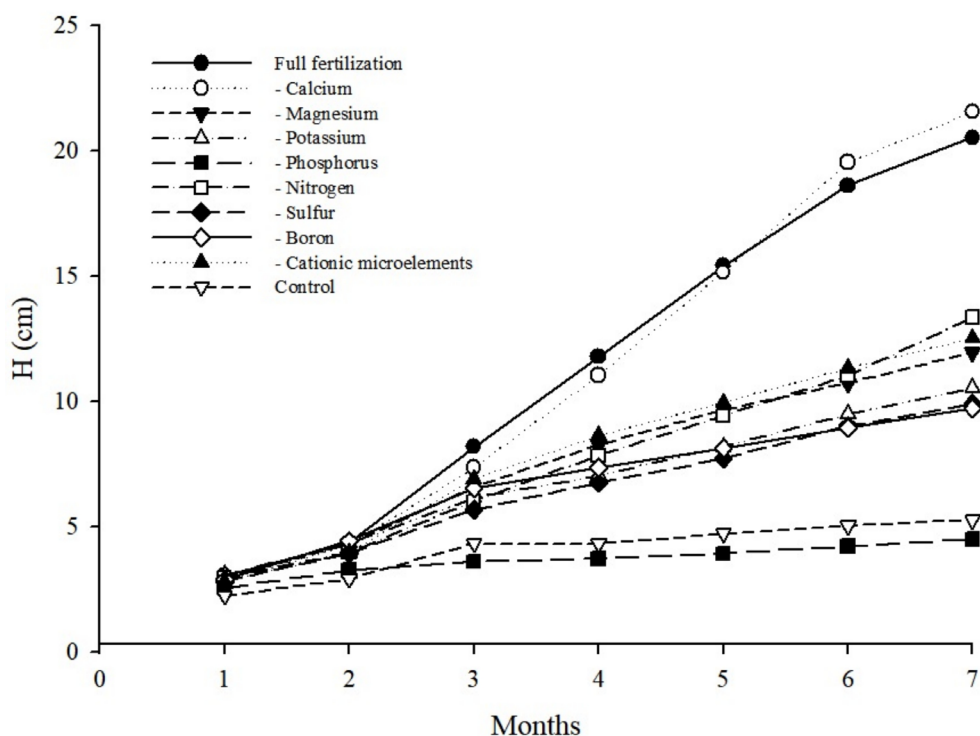


Figure 1. Monthly height of *T. rosea* under the different nutrient deprivation treatments.

Table 3. Effects of nutrient deprivation on the growth of *T. rosea* after seven months in the nursery. Different letters in the column represent significant differences between treatments for each variable (Tukey test, $P = 0.05$). Values in parentheses represent the standard deviation. H: height, SD: stem diameter, SDW: shoot dry weight, RDW: root dry weight, AUC: area under the curve. Control: without fertilization, FF: full fertilization (with B, Ca, P, Mg, N, K, S, Mn, Fe, Cu, Zn), -Ca: FF minus Ca, -Mg: FF minus Mg, -K: FF minus K, -S: FF minus S, -N: FF minus N, -P: FF minus P, -B: FF minus B, -CM: FF minus Mn, Fe, Cu and Zn.

Treatment	H (cm)	SD (mm)	SDW (g)	RDW (g)	AUC (cm ²)
FF	20.51 a (2.2)	7.13 b (1.0)	2.88 a (0.69)	2.5 a (0.38)	1991.55 a (315.87)
-Ca	21.53 a (2.5)	9.49 a (1.2)	3.64 a (0.63)	2.55 a (0.36)	1977.3 a (277.18)
-Mg	11.95 bcd (2.1)	4.81 cd (0.7)	0.86 b (0.43)	0.53 b (0.23)	1297.95 b (185.20)
-K	10.53 cd (2.0)	4.6 cde (0.9)	0.58 bc (0.45)	0.54 b (0.20)	1146 b (156.42)
-S	9.88 cd (2.2)	3.71 ef (0.7)	0.34 c (0.22)	0.27 c (0.10)	1080.60 b (248.31)
-N	13.34 b (2.0)	5.41 c (0.8)	0.91 b (0.44)	0.5 b (0.19)	1286.55 b (291.30)
-P	4.5 e (0.9)	2.61 g (0.4)	0.05 d (0.02)	0.05 e (0.01)	570.3 c (45.48)
-B	4.5 e (1.9)	3.76 def (0.5)	0.35 c (0.31)	0.48 bc (0.25)	1137.9 b (139.56)
-CM	12.49 bc (1.8)	4.7 cde (0.5)	0.81 b (0.29)	0.41 bc (0.41)	1349.7 b (162.66)
Control	5.3 e (0.9)	2.82 fg (0.5)	0.08 d (0.03)	0.13 d (0.13)	661.68 c (113.83)

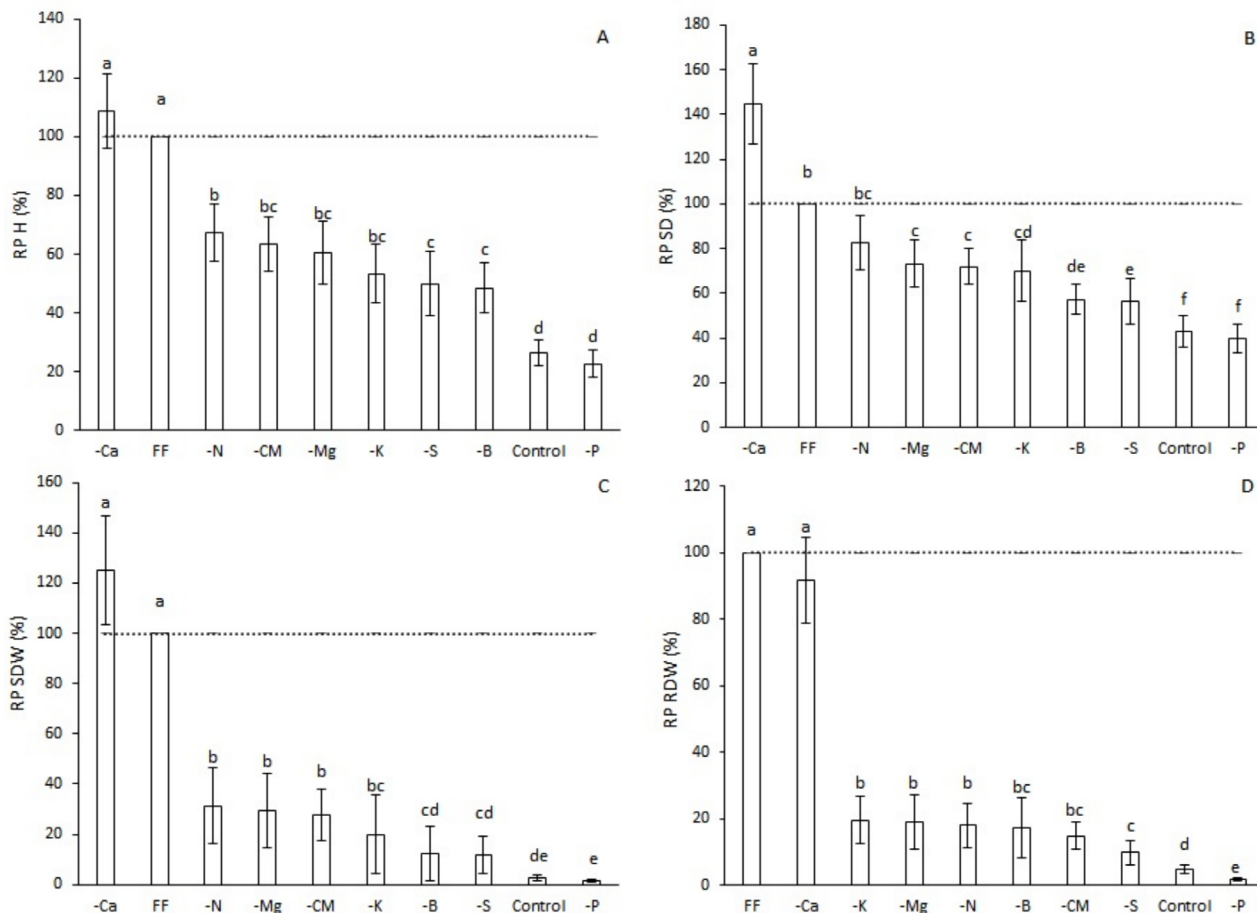


Figure 2. Relative performance (RP, %) of the biometric variables of *T. rosea* under different nutrient deprivation treatments with respect to the FF treatment, after seven months in the nursery. A. (height, H), B. (stem diameter, SD), C. (shoot dry weight, SDW), D. (root dry weight, RDW). Different letters on the bar represent significant differences between treatments for each variable (Tukey test, P = 0.05). Control: without fertilization, FF: full fertilization (with B, Ca, P, Mg, N, K, S, Mn, Fe, Cu, Zn), -Ca: FF minus Ca, -Mg: FF minus Mg, -K: FF minus K, -S: FF minus S, -N: FF minus N, -P: FF minus P, -B: FF minus B, -CM: FF minus Mn, Fe, Cu and Zn.

3.2. Functional traits and plant quality indices

The treatments generated significant differences among the functional traits and plant quality indices (P < 0.05) (Table 4). The FF and -Ca treatment groups showed higher PQI and RGR values than the other treatment groups. On the contrary, the Control and -P treatments had the

lowest values, reaching an average of 4.3% of the PQI and 7.8% of the RGR of the FF treatment group. An RGR of zero was obtained for the -P and Control treatment groups. The highest LA values were obtained for the -Ca, FC, -CM, and -N treatment groups, which were significantly different (P < 0.05) from those of the Control and -P treatment groups.

Table 4. Functional traits and plant quality indices of *T. rosea* under different nutrient deprivation treatments after seven months in the nursery. Different letters in each column represent significant differences between treatments for each variable (Tukey test, $P = 0.05$). Values in parentheses represent the standard deviation. PQI: plant quality index, LA: leaf area, RGR: relative growth rate. Control: without fertilization, FF: full fertilization (with B, Ca, P, Mg, N, K, S, Mn, Fe, Cu, Zn), -Ca: FF minus Ca, -Mg: FF minus Mg, -K: FF minus K, -S: FF minus S, -N: FF minus N, -P: FF minus P, -B: FF minus B, -CM: FF minus Mn, Fe, Cu and Zn.

Treatments	PQI	LA (cm ²)	RGR (g day ⁻¹)
FF	1.33 b (0.24)	306.02 b (26.20)	0.027 a (0.0007)
-Ca	1.67 a (0.21)	332.13 a (15.98)	0.027 a (0.0008)
-Mg	0.34 c (0.14)	36.31 e (15.66)	0.015 bcd (0.0030)
-K	0.34 c (0.16)	26.46 g (10.56)	0.016 bc (0.0033)
-S	0.15 cde (0.04)	36.94 f (10.43)	0.011 d (0.0016)
-N	0.31 c (0.11)	92.15 d (14.36)	0.0183 b (0.0017)
-P	0.04 e (0.01)	2.67 i (0.87)	0.000 f (0.0003)
-B	0.25 cd (0.12)	17.22 h (11.30)	0.014 cd (0.0031)
-CM	0.27 cd (0.08)	119.58 c (13.57)	0.016 bc (0.0028)
Control	0.09 de (0.03)	2.06 i (0.65)	0.004 e (0.0011)

3.3. Nutritional response

In general, the plants belonging to the FF treatment group presented higher amounts of stored nutrients (mg/plant) with respect to the Control treatment and the rest of the treatments (Table 5). The Ca content in the plants from the -Ca treatment group was high, i.e., 80% of the total Ca content in the FF treatment plants. On the other hand,

the content of this nutrient in the Control represented only 2% of that corresponding to the FF group. Very low P was stored in the plants from the -P and Control treatment groups, i.e., 1% and 2% of the P in the FF treatment group were observed in the -P and Control treatment groups, respectively. The foliar content of S in the -S treatment group and of B in the -B treatment group represented 10% and 4%, respectively, of the leaf content of S and B of the FF treatment group.

Table 5. Nutrients stored (mg/plant) in the shoot portions of the *T. rosea* plants under the nutrient deprivation treatments. Control: without fertilization, FF: full fertilization (with B, Ca, P, Mg, N, K, S, Mn, Fe, Cu, Zn), -B: FF minus B, -Ca: FF minus Ca, -P: FF minus P, -Mg: FF minus Mg, -N: FF minus N, -K: FF minus K, -S: FF minus S, -CM: FF minus Mn, Fe, Cu and Zn. *: The analysis of the microelements (CM) (Cu, Fe, Mn, Zn) and S for the Control treatment could not be performed due to a shortage of samples.

Nutrient	Ca mg/plant	Mg mg/plant	K	S	N	P	B	Cu	Fe	Mn	Zn
FF	50	20	45	3	55	3	0.11	0.02	0.39	0.54	0.04
-Ca	40										
-Mg		0.4									
-K		3									
-S		0.3									
-N		12									
-P		0.03									
-B		0.004									
-CM			0.005	0.11	0.14	0.01					
Control*	1	0.2	1	--	0.5	0.05	--	--	--	--	--

3.4. Visual symptoms of nutritional deficiency

The visual symptoms began to appear within two months of growth after transplanting. The differences between the treatments were evident in the heights of the plants, in the color of the leaves and their shape, texture, and size (Figures 3 and 4).

-Mg: Intervenal chlorosis followed by necrosis from the apex in the old leaves in addition to twisted edges.

-K: Generalized interveinal chlorosis and the presence of necrotic spots on the old leaves with curling of the affected parts.

-S: Visual symptoms not very evident but mild interveinal chlorosis from the apex to the base of the new leaves was observed.

-N: The leaves presented a yellowish color with necrotic spots. There was also twisting of the edges of the leaves.

-P: The plants showed a strong stunted growth, which was the most striking feature. Leaf necrosis and subsequent death and detachment were observed.

-B: Deficient leaves were more elongated and narrower. In addition, interveinal necrosis in the new leaves. Brown spots without a clear distribution were observed in the leaves.

-CM: Although there was a decrease in the growth of the plants compared to the FF treatment, no clear symptoms were observed.

Control: The plants showed strong changes with respect to the FF treatment. Characteristics were small leaf area, leaf necrosis, short internodes, and the permanent loss of leaves.



Figure 3. Appearance of *T. rosea* plants after seven months of growth in the nursery under different nutrient deprivation treatments. Control = without fertilization, FF: full fertilization (with B, Ca, P, Mg, N, K, S, Mn, Fe, Cu, Zn), -B: FF minus B, -Ca: FF minus Ca, -P: FF minus P, -Mg: FF minus Mg, -N: FF minus N, -K: FF minus K, -S: FF minus S, -CM: FF minus Mn, Fe, Cu and Zn.

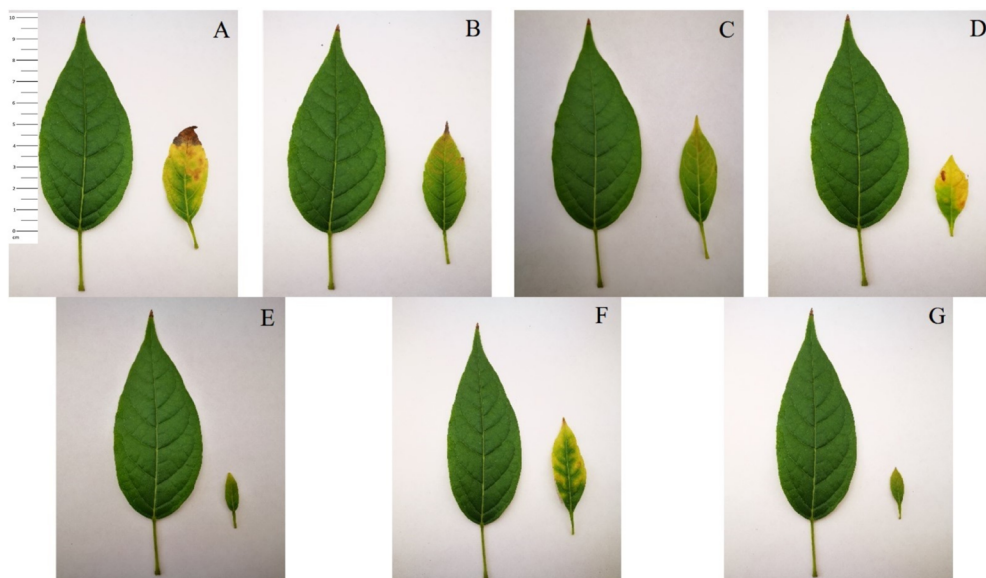


Figure 4. Comparison between *T. rosea* leaves from the full fertilization treatment (left) and nutrient deprivation treatments. A) -Mg: FF minus Mg; B) -K: FF minus K; C) -S: FF minus S; D) -N: FF minus N; E) -P: FF minus P; F) -B: FF minus B; Control: control treatment (without fertilization).

4. DISCUSSION

The deprivation of essential nutrients had significant differential effects on the growth and development of *T. rosea*. These results confirm the importance of knowing the particular nutrient requirements of individuals species to technically support nursery fertilization practices. This is a key factor for the production of high quality and vigorous plants to transplant to the field (Akpo et al., 2014).

In order to classify the plants according to their vigor and quality, four variables are relevant: height (H), plant quality index (PQI), relative growth rate (RGR), and leaf area (LA). Height (H) has been commonly used as an indicator for the appropriate time to transplant the plants to the field, given its close relationship with photosynthetic capacity and competitive vigor (Pérez-Harguindeguy et al., 2013). However, if this is the only criterion used to qualify plant attributes, key aspects could be lost compared to a more comprehensive evaluation. The proportionality of plant development with respect to the structural components (aerial and radical biomass) and functional traits that may determine better performance in terms of the ability to acquire resources (water, light) are examples of information that may be acquired given a comprehensive evaluation. To collect this information, it is necessary to measure PQI, RGR, and LA. The PQI index indicates balanced plants with respect to the aerial and radical dry mass and their robustness (Cruz et al., 2012). The RGR can be used to select plants

that acquire more biomass in less time (Kołodziejek, 2019). Finally, the LA can be used to identify plants with greater photosynthetic capacity (Juneau & Tarasoff, 2012).

Considering this set of variables, we were able to differentiate three response groups according to the degree of limitation generated by the nutrient deprivation treatments and, consequently, to classify the specific nutrient requirements for *T. rosea*: -P > -S, -B, -K, -Mg, -CM, -N > -Ca. Clearly, P was the most critical nutrient for the growth of *T. rosea*. Particularly, there were strong negative consequences derived from the deprivation of this nutrient, such as low H and LA values, an almost nil RGR, and a low PQI. Thus, the relative performance values of all the variables evaluated of the plants subjected to P deprivation were less than or equal to 20% of those in the FF treatment group, indicating severe nutritional deficiencies (Moretti et al., 2011). Similar negative effects were reported for other species, when compared to plants that received full fertilization formulas (FF), such as *Myracrodruon urundeuva* Allemão (Mendonça et al., 1999) and *Stryphnodendron adstringens* (Mart.) Coville (Carlos et al. 2013).

The adequate supply of P during the nursery stage is essential to obtain vigorous and high quality plants, as observed in this study. In fact, low soil P availability has been identified as one of the factors that restrict plant productivity the most, mainly as a result of its high fixation in the soil (Yang et al., 2016). It is more critical in the tropics and particularly from volcanic ash-derived soils (Brenner et al., 2019), as used here, which

exhibited a very low P content ($P = 4.6 \text{ mg kg}^{-1}$). In turn, this low P availability reflected in the low amount of the nutrient stored in the shoot portions of the plants that were not supplied with the nutrient, representing scarcely 1% of the P in the plants that were given the full fertilization formula.

In addition to P, N is widely recognized as a limiting nutrient for plant growth (Deng, 2018). Particularly, in this study, the deprivation of N generated slight limitations in the growth of *T. rosea*. Thus, the values obtained for the variables H, PQI, RGR, and LA, represented 65%, 23%, 67%, and 30%, respectively, of the values found in the FF treatment plants. The concentration of N in the substrate of the -N treatment group (0.3%) was lower than that suggested by Sepúlveda et al. (2014) (0.5%) for the growth of *Quercus humboldtii* Bonpl. species. The above study utilized Andosol substrates and the same experimental approach as the one presented here (nutrient deprivation). Despite the low concentration of N in the substrate used here, and taking into account that in volcanic ash-derived soils, N is limiting for plants given its low mineralization rate (Mayes et al., 2019), the deprivation of this nutrient did not affect *T. rosea* as drastically as the absence of other nutrients.

Greater limitations to those determined by the deprivation of N were found by the deprivation of other nutrients. Particularly, the greatest effect was observed in the plants from the -S and -B treatments. Plants that were not supplied with these two nutrients showed average PQI and LA values lower than 20% of those in the FF treatment plants. Similarly, the mean values obtained for RGR and H were less than 50% of those in the FF treatment plants. It should be noted that the strong effect of the deprivation of S on the variables occurred despite the fact that the content of S in the growth substrate was considered adequate for correct plant growth ($S = 14 \text{ mg kg}^{-1}$; Singh, 2017). These results allowed the identification of S and B as key nutrients for *T. rosea*. Similarly, Camacho et al. (2014) reported negative effects derived from the deprivation of S and B for *Bombacopsis glabra* (Pasq.) Robyns.

Figure 5 classifies the degree of limitation imposed by the nutrient deprivation treatments, considering the effects generated on the variables individually and together. Particularly, considering the average of the four variables, the degree of nutrient limitation for *T. rosea* follows this decreasing pattern: $P > S, B, K, Mg > CM, N > Ca$.

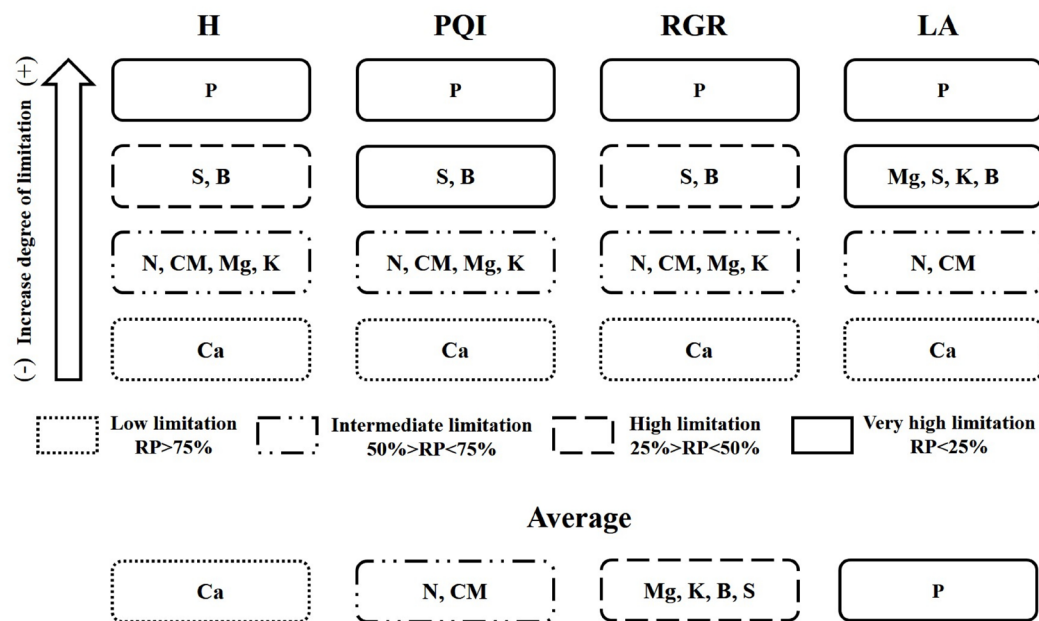


Figure 5. Degree of nutrient limitation on the growth and development of *T. rosea*. The degree of limitation was determined based on the relative performance (RP) values of the variables H, PQI, RGR, and LA of each deprivation treatment, with respect to the values of these variables found in the full fertilization treatment (FF) plants. The indicated nutrient groups correspond to the homogeneous groups established through the Tukey test ($P = 0.05$). In the upper part of the figure, the nutrients are grouped according to their degree of limitation on each of the variables, while in the lower part of the figure, the nutrients are grouped according to their degree of limitation on the average of the four variables. The four limitation groups considered were: low limitation ($RP > 75\%$), intermediate limitation ($50\% < RP < 75\%$), high limitation ($25\% < RP < 50\%$), and very high limitation ($RP < 25\%$).

The nutrient deprivation experimental approach allowed the identification of the most limiting nutrients for *T. rosea* growth and development and the determination of whether the levels of these nutrients in the substrate used were sufficient. Thus, despite being at adequate theoretical levels, the S content was not sufficient for the proper growth and development of this species. Therefore, it is necessary to supply greater quantities of S in the growth substrates for the future production of *T. rosea*. In contrast, the plants under Ca deprivation (-Ca) performed well, without limitations, even though the level of Ca in the growth substrate was relatively low ($2.4 \text{ cmol}_c \text{ kg}^{-1}$). Additionally, the substrate corresponding to the -Ca treatment had an adequate participation of Ca (63% of the CEC), as well as a balanced Ca: Mg ratio (2.2:1) (Osemwota et al., 2007). Consequently, it would not be necessary to supply additional Ca to substrates with similarly Ca levels.

In synthesis, the results of this study indicate the importance of establishing an adequate fertilization plan for the production of each tree species in nurseries designated for reforestation activities. Plants with adequate morphological and physiological characteristics (aerial/radical biomass balance, high photosynthetic and productive capacity, and high efficiency in water and nutrient absorption: Allen et al., 2017) will potentially offer competitive advantages in the field, compared to those whose fertilization practices have not been based on the particular requirements of the species. Consequently, the probability of success of reforestation projects may increase (Negiz et al., 2015), thus reducing the uncertainty of financial investments and favoring the use of high interest species, such as *T. rosea*, for reforestation.

5. CONCLUSIONS


T. rosea showed limited growth due to the deprivation of essential nutrients. Particularly, the deprivation of P followed by the deprivation of S and B generated drastic limitations on the growth and development of this species in the nursery phase. In particular, for both aerial and root biomass, the deprivation treatments for these three nutrients showed performances less than 20% of those corresponding to the FF treatment. Similarly, the large differences in relative performance of these biomass variables between Control and FF treatment were greater than 95%, which indicates the importance of nutrient supply for the proper growth and development of this species. On the other hand, the main morphological changes were observed at the leaf level, when Mg, K and N were not supplied. In contrast, the plants under Ca deprivation performed well, without limitations. The recognition of essential nutrients of individual species

contributes to precision forestry models in the early nursery stage, which generates vigorous and high quality plant material.

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