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Initial Silvicultural Performance of Pilocarpus microphyllus in Amazonian Agroforestry Systems under Different Pruning and Macronutrient Fertilization Regimes

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Initial Silvicultural Performance of *Pilocarpus microphyllus* in Amazonian Agroforestry Systems under Different Pruning and Macronutrient Fertilization Regimes

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Abstract

The Amazon region faces significant pressure from deforestation and cattle ranching, threatening the natural habitat of *Pilocarpus microphyllus*. This study evaluated the initial silvicultural performance of *P. microphyllus* under different treatments in agroforestry systems (AFS) in São Félix do Xingu, Pará, Brazil. The experiment followed a randomized complete block design in a 2 \times 6 factorial scheme with three replicates. Variables measured included collar diameter, seedling height, crown area, number of shoots, and mean branch length. Results indicated that the AFS presented distinct light regimes (17,445.5; 3,787.5; and 2,954.5 Lux). Significant differences were observed among systems, with AFS 1 (intermediate light) showing the best growth responses for all variables. A significant interaction between pruning and fertilization was found only for seedling height. In the absence of pruning, NPK doses of 175 g and 75 g produced the highest growth. Conversely, when pruning was applied, no significant differences were found among fertilization treatments, although the combination of 45 g NPK + 45 g MAP showed the best performance at 20 months. Pruning significantly enhanced the number of shoots and mean branch length from the 19 th month onward. Higher NPK doses were effective in increasing crown area. We recommend planting *P. microphyllus* in AFS with moderate solar radiation, combined with formative pruning and targeted fertilization (175 g NPK without pruning; 45 g NPK + 45 g MAP with pruning).
Keywords: pilocarpine; silvicultural treatments; jaborandi

Introduction

Pilocarpus microphyllus Stapf ex Wardle (jaborandi) has been economically exploited in the Brazilian Amazon since the 1990s, particularly in the Alto Xingu region, where local communities harvest its leaves for commercialization (Amaral et al., 2022). However, increasing market demand, together with deforestation, forest degradation, and the potential impacts of climate change on the distribution, productivity, and secondary metabolite production of medicinal plants, has raised concerns about the conservation of natural populations of P. microphyllus, which is currently classified as Vulnerable (VU) in Brazil, reinforcing the need for sustainable conservation and cultivation strategies. (Ministério do Meio Ambiente, 2022; Mykhailenko et al., 2025). Agroforestry systems (AFSs) represent a promising strategy for the sustainable cultivation of *P. microphyllus* because they combine biodiversity conservation with agricultural production; however, little is known about the effects of light availability and silvicultural management on the establishment and early growth of this species.

The species has considerable economic value because it is native to the Amazon and constitutes the only commercial natural source of pilocarpine, an alkaloid widely used in the pharmaceutical industry. Previous studies have demonstrated that *P. microphyllus* adapts well to cultivation within its native range and can provide an early economic return through sustainable leaf harvesting (Caldeira et al., 2017). Historically, the commercial supply of pilocarpine has depended almost exclusively on extractivism and, more recently, on monoculture plantations. However, declining natural stocks, increasing demand, and the limitations of extractive production have reinforced the need for sustainable cultivation systems in the Amazon (Homma, 2014). Although cultivation initiatives have been established in monocultures (Homma, 2012), information regarding the performance of *P. microphyllus* in diversified production systems remains scarce.

Beyond ensuring a sustainable supply of raw material, cultivation practices may also influence the quality of harvested leaves, particularly their pilocarpine concentration. Earlier studies reported higher pilocarpine concentrations in native populations than in monocultures (Gumier Costa, 2016), whereas recent evidence indicates that soil fertility and plant nutritional status are closely associated with pilocarpine

production, highlighting the importance of adequate nutrient management during cultivation (de Jesus et al., 2022). Despite these advances, little is known about how silvicultural practices under agroforestry systems influence the early silvicultural performance of *P. microphyllus*.

Among the environmental and management factors affecting the establishment of forest species, light availability and mineral nutrition play central roles. Nitrogen (N), phosphorus (P), and potassium (K) are essential macronutrients involved in photosynthesis, biomass accumulation, and seedling establishment under field conditions (Teixeira et al., 2019; Nieri et al., 2019). Because Amazonian soils generally exhibit low natural fertility, nutrient management becomes a key factor for the successful cultivation of native species and for improving plant productivity (Costa et al., 2023).

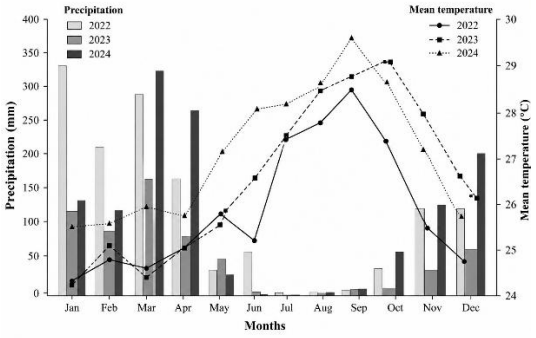
Agroforestry systems have become increasingly important in the Amazon by reconciling environmental conservation with diversified agricultural production and income generation for smallholder farmers (Silva et al., 2023). These systems also provide a favorable environment for cultivating species of high economic value, such as *P. microphyllus*, whose leaves remain the only commercial natural source of pilocarpine (Grandi, 2021; De Sá et al., 2021). In Pará State, approximately 50 thousand tonnes of leaves were produced in 2022, with commercialization supported by local cooperatives that organize harvesting, processing, and marketing (IBGE, 2022).

Although recent studies have improved the understanding of pilocarpine production and the cultivation of *P. microphyllus*, little information is available regarding the combined effects of light environment, pruning, and fertilization on the species' early silvicultural performance under Amazonian agroforestry systems. We hypothesized that the interaction between light availability, pruning, and macronutrient fertilization significantly affects the early silvicultural performance of *P. microphyllus*. Therefore, this study aimed to evaluate the initial silvicultural performance of *P. microphyllus* under different light environments, pruning regimes, and macronutrient fertilization in agroforestry systems established in Alto Xingu, Pará.

Material and Methods

The study was conducted on three smallholder farms located in the municipality of São Félix do Xingu, Pará State, Brazil. According to the Köppen climate classification, the region has a tropical savanna climate (Aw), characterized by a well-defined dry season and a rainy season, with mean annual precipitation of approximately 2,100 mm and a mean annual air temperature of 26.5 °C. The native vegetation is predominantly Dense Ombrophilous Forest, with smaller patches of Cerrado and other vegetation types. Monthly precipitation and mean air temperature data for the experimental period (2022–2024) were obtained from the ERA5-Land reanalysis dataset, available through the Copernicus Climate Change Service (C3S), which provides land surface climate variables at approximately 9-km spatial resolution (Muñoz Sabater et al., 2021). Monthly precipitation and mean air temperature during the experimental period are presented in Figure 1.

Figure 1. Monthly precipitation (blue bars) and mean air temperature (red line) for the experimental period (2022–2024). Precipitation is expressed in millimeters (mm) on the left y-axis and means air temperature in degrees Celsius (°C) on the right y-axis.



Source: Authors' elaboration based on ERA5-Land reanalysis data (Copernicus Climate Change Service, 2022).

The agroforestry systems (AFSs) were established on dystrophic red-yellow Argisols, one of the most abundant soil classes in Brazil, typically occurring on more rugged relief and characterized by low natural fertility. *P. microphyllus* was planted in three distinct AFS compositions; each AFS was treated as a replicate. Soil characterization for the 0–20 cm layer is summarized in Table 1.

Table 1 – Summary of soil analyses (0–20 cm depth) for agroforestry systems

AFS	depth (cm)	pH	OM	K	Fe	P	Ca	Mg	Al	H+Al	T	V	M
			%	mg dm^{-3}			cmol(c) dm^{-3}					%	
1	0 - 20	4,83	4,89	67,40	0,03	5,38	1,60	1,00	0,10	3,31	6,08	45,58	3,48
2	0 - 20	4,80	19,00	37,60	86,30	3,00	1,90	0,40	0,10	3,40	5,80	41,38	4,00
3	0 - 20	5,10	20,00	35,70	46,60	2,30	2,10	0,40	0,00	1,80	4,39	59,00	0,00

Em que: OM = organic matter; T = cation exchange capacity at pH 7.0; V = base saturation index; m = aluminum saturation index.

The three AFS compositions are multistrata silviagricultural systems that provide different light regimes for cultivated plants. P. microphyllus seedlings were planted in the rows of these systems. Each composition was considered a replicate (block), as the study focused on evaluating the initial silvicultural performance of P. microphyllus under the applied silvicultural treatments.

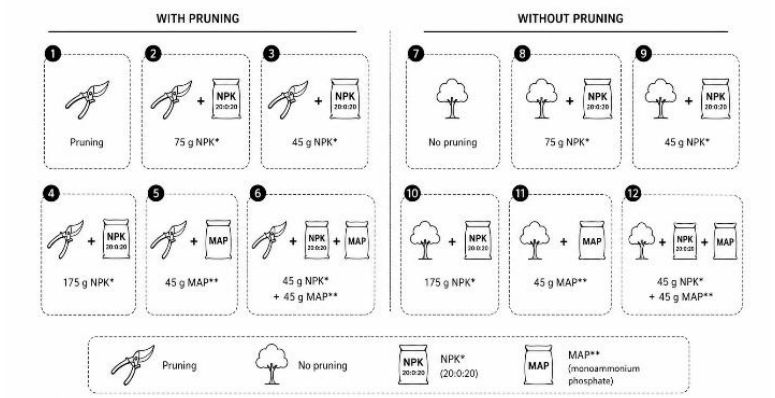
AFS 1: comprised *Musa* spp., *Ananas comosus*, *Manihot esculenta*, *Tithonia diversifolia*, *Theobroma cacao*, and *Handroanthus albus*. The tree species (*Theobroma cacao* and *Handroanthus albus*) were spaced 15 m apart, while the remaining species were cultivated in the spaces between them, at a spacing of 1 m between plants. The distance between rows was 3 m.

AFS 2: comprised *Malpighia emarginata*, *Plinia cauliflora*, *Anacardium occidentale*, *Citrus sinensis*, *Eugenia uniflora*, *Mangifera indica*, *Citrus limon*, *Spondias purpurea*, *Lafoensia glyptocarpa*, *Musa* spp., *Passiflora edulis*, *Theobroma cacao*, *Khaya ivorensis*, *Euterpe oleracea*, *Handroanthus albus*, *Spondias mombin*, *Inga edulis*, *Maximiliana maripa*, *Guazuma ulmifolia*, *Psidium guajava*, and *Anadenanthera colubrina*. The tree species were arranged at a spacing of 4 m between plants within rows and 4 m between rows.

AFS 3: characterized by greater shading, this system included *Mauritia flexuosa*, *Theobroma cacao*, *Euterpe oleracea*, *Theobroma grandiflorum*, *Artocarpus heterophyllus*, *Persea americana*, *Mangifera indica*, *Averrhoa carambola*, *Syzygium jambos*, *Maximiliana maripa*, *Genipa americana*, *Ipomoea carnea*, *Calycophyllum spruceanum*, *Inga edulis*, *Oenocarpus bacaba*, *Sclerolobium paniculatum*, and *Croton urucurana*. The system had no defined spacing between plants within rows; however, the distance between rows was 3 m.

The *P. microphyllus* seedlings were donated by the Centroflora nursery and originated from local seed sources. Planting was carried out in 2022 at a spacing of 50 cm × 50 cm, in the rows between the combinations of forest tree species established in the evaluated agroforestry systems (AFS). The experiment was conducted using a randomized complete block design (RCBD) in a 2 × 6 factorial arrangement (two pruning regimes × six fertilization combinations), with three blocks (replicates). Each block corresponded to an AFS characterized by a specific light environment for *P. microphyllus* seedlings, determined by the composition and spatial arrangement of the forest tree species within the system (Figure 2). Each experimental plot contained three plants, totaling 108 experimental units.

Figure 2. Treatments (pruning and fertilization combinations applied to *Pilocarpus microphyllus* in the different AFS compositions)



NPK*: the source used was the 20:0:20 formulation; MAP**: monoammonium phosphate with 61% P_2O_5 and 12% N.

Formative pruning was applied at experiment establishment at 30 cm height or when specimens reached an equivalent stem diameter of 4 mm. Fertilizers were applied once in lateral side holes, except for treatments 2 and 4 (higher fertilizer rates), which received two split applications: one at establishment and a second dose 30 days later to reduce nutrient losses.

Pruning was performed at experiment establishment at a height of 30 cm or when specimens reached a stem diameter of 4 mm. Fertilization was applied once in lateral planting pits for all treatments except treatments 2 and 4, which received higher fertilizer rates and were therefore applied in two split doses: the first at experiment establishment and the second 30 days after the first application to reduce nutrient losses.

Results were obtained by measuring root collar diameter, total seedling height, crown area, number of shoots > 30 cm, and mean shoot length. Measurements were taken every 30 days after experiment installation.

To better characterize treatment responses, the light regime in the three AFS compositions was recorded using a thermo-hygro-anemometer to assess whether illumination significantly affected the analyzed variables. For subsequent analyses, the mean of the three plants per plot was calculated for root collar diameter, total seedling height, crown area, number of shoots > 30 cm, and mean shoot length.
Crown area (CA) was calculated using the following equation:

$$CA = \frac{(CWI)x(CWR)x\,\pi}{4} \qquad (1)$$

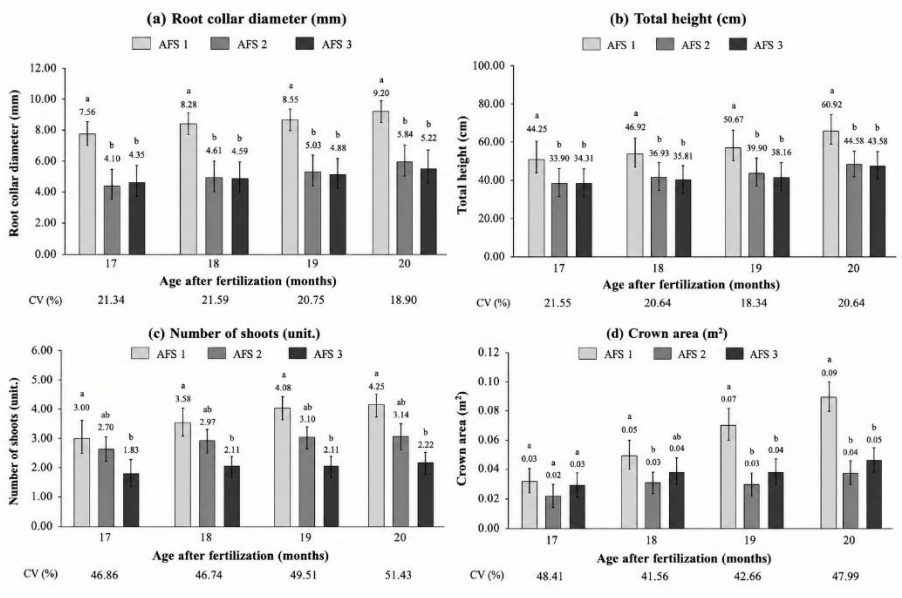
Where,
CA: is crown area per plant (m²)
CWI: is crown width in the interrow direction (m);
CWR: is crown width in the row direction (m);
π: constant (3.141592654).

Data were tabulated and subjected to analysis of variance (ANOVA). When effects were significant, mean comparisons were performed using Tukey's test and/or the Scott-Knott test at a 5% significance level, implemented in R statistical software.

5 Results

The measurement of light intensity showed that AFS 2 had the highest mean irradiance (17,445.5 lux), followed by AFS 1 (3,787.5 lux) and AFS 3 (2,954.5 lux). Comparison among the systems revealed a significant effect on root collar diameter (DC), total height (HT), number of shoots (NS), and crown area (CA). For DC and HT, AFS 1 exhibited significantly higher means than the other systems (Figure 3).

Figure 3 – Comparison among agroforestry systems (AFS) for root collar diameter (DC), total height (HT), number of shoots (NS), and crown area (CA) at 17, 18, 19, and 20 months after planting.

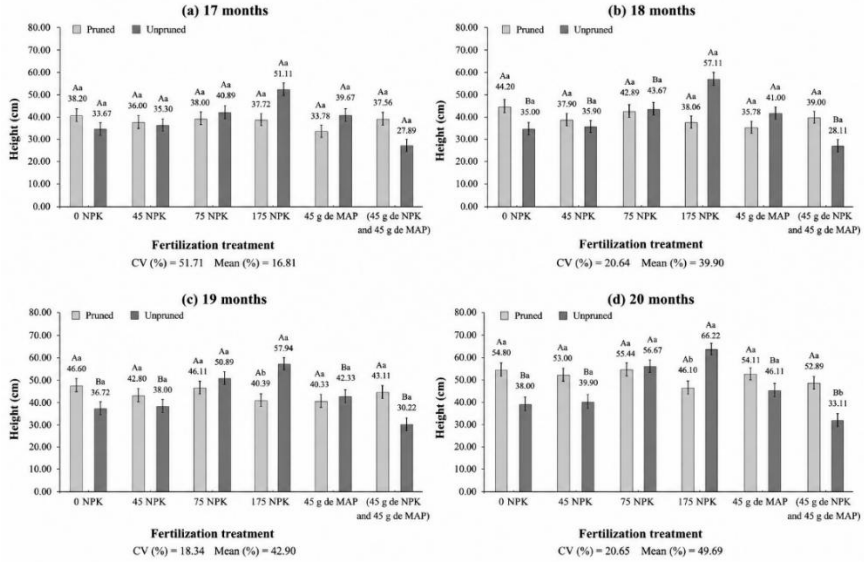


* Means followed by at least one identical letter within a column do not differ significantly by Tukey's test at the 5% probability level.

Over the monitoring period, AFS 1 consistently exhibited higher means for root collar diameter (DC) and total height (HT) at all evaluated ages, while AFS 2 and AFS 3 showed statistically lower means. For number of shoots (NS) and crown area (CA), AFS 1 demonstrated an advantage beginning at the 19th month. AFS 3 presented the poorest overall performance, which was associated with greater shading, whereas AFS 2, despite having the highest measured irradiance, did not show superior growth, indicating that factors other than light availability influenced seedling performance.

Analysis of the effect of silvicultural treatments on the agroforestry systems revealed a significant interaction between pruning (P vs. NP) and fertilization for the variable total height (HT). When plants were pruned, no statistically significant differences were observed among the fertilization doses tested. In contrast, under no pruning (NP) conditions, the higher NPK doses (175 g NPK and 75 g NPK) produced the most pronounced height growth, particularly from the 19th month onward (Figure 4).

Figure 4 – Evaluation of the effect of pruning in combination with fertilization on the variable Total Height (HT) in cm at 17, 18, 19, and 20 months after planting.



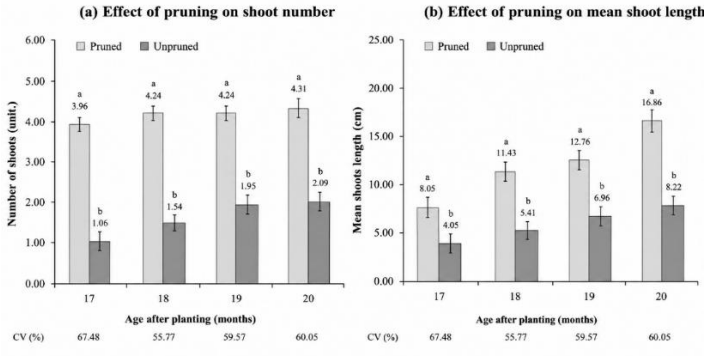
Uppercase letters indicate differences among fertilization treatments within each pruning condition according to the Scott-Knott test ($p \leq 0.05$), whereas lowercase letters indicate differences between pruning

treatments within each fertilization treatment according to Tukey's test ($p \leq 0.05$). Bars followed by at least one identical letter do not differ significantly at the 5% probability level.

Multiple comparison tests indicated differences both among rows and among columns (Figure 4). In the absence of pruning, the largest NPK doses (175 g and 75 g) promoted the greatest increases in height, while under pruning the combined fertilization treatment 45 g NPK + 45 g MAP stood out at the 20th month.

These results suggest that pruning modifies the seedlings' response to fertilization, reducing the relative effect of fertilizer dose on height growth, whereas in unpruned plants fertilizer dose is a key driver of vertical growth. There was no interaction between the factors for number of shoots (NS) and mean shoot length (MSL); however, a significant isolated effect of the pruning factor was observed. Pruning resulted in higher mean values for both NS and MSL in all evaluated months (17, 18, 19, and 20 months after planting) (see Figure 5).

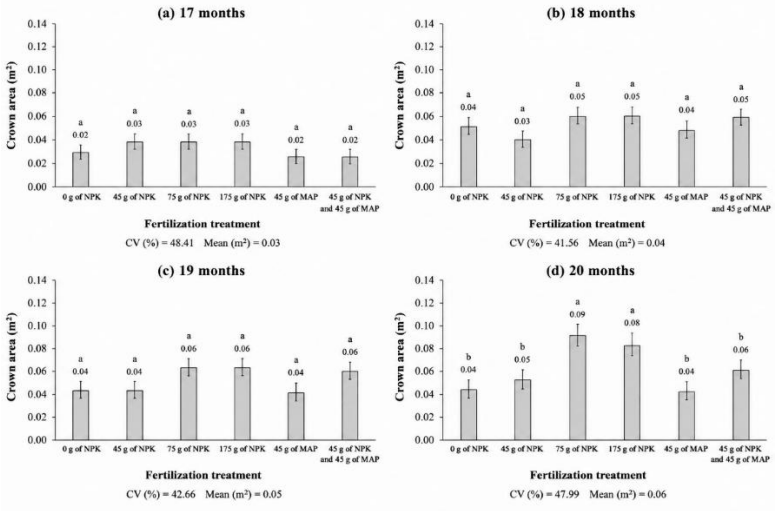
Figure 5 – Evaluation of the effect of pruning on Number of shoots (NS) and Mean shoot length (MSL) at 17, 18, 19, and 20 months after planting



* Means followed by at least one identical letter in the column do not differ significantly according to the Scott-Knott test at the 5% probability level.

At 20 months (Figure 6), crown area (CA) showed a significant effect of fertilization, with the 75 g NPK and 175 g NPK doses standing out relative to the others. The Scott-Knott analysis at the 5% probability level indicated that these two doses formed the group of superior means at the end of the evaluation period.

Figure 6 – Effect of fertilization on Crown Area (CA) at 17, 18, 19 and 20 months after planting



* Means followed by at least one identical letter in the column do not differ significantly according to the Scott-Knott test at the 5% probability level.

Discussion

The results indicate that, under the evaluated conditions, intermediate lighting (AFS 1, ~3,787.5 Lux) favored the initial vegetative development of *Pilocarpus microphyllus*, as reflected in greater root collar diameters, total heights, number of shoots, and crown area. This pattern suggests that the species, during the seedling and establishment phase, may exhibit better performance under moderate light intensity rather than under extreme full sun or intense shading.

The observed discrepancy between the higher irradiance in AFS 2 and its inferior performance can be explained by complementary mechanisms: stress due to excessive radiation (possible photoinhibition or increased water demand) and edaphic limitations associated with the pasture history (reduced fertility, soil compaction, and low organic matter content), which compromise the conversion of available light into growth. Conversely, the reduced performance in AFS 3 is consistent with the classic effects of shading on photosynthesis and the accumulation of photoassimilates, resulting in a lower resource allocation to the stem and crown (Fonseca, 2002; Engel et al., 1990; Viana et al., 2022).

Compared to the literature, some studies report greater sprouting and leaf number under higher radiation (Assis et al., 2016; Ribeiro et al., 2011; Cardoso et al., 2012), whereas other works on analogous species indicate optimal responses under half-shade conditions (Mazuchowski et al., 2007). Thus, the findings of this study do not contradict the literature but highlight that the response of *P. microphyllus* is dependent on the environmental context and soil management; the optimal light intensity may vary according to water availability, nutrients, and land-use history (Siebeneichler et al., 2008; Rayol, 2018).

For practical applications, the data suggest that management practices promoting intermediate lighting (controlled partial shading or intercropping that reduces excessive direct radiation) can optimize the initial growth of jaborandi seedlings in Agroforestry Systems (AFS). In areas recovered from pasture, soil evaluation and correction prior to planting are recommended to minimize edaphic limitations that could negate the benefits of higher light availability (Mizobata et al., 2017; Pinho et al., 2021).

Upon evaluating the application of silvicultural treatments, the findings indicate that the height response of *P. microphyllus* depends on the interaction between pruning management and fertilization. The superiority of the 175 g and 75 g NPK doses in the absence of pruning suggests that when the plant maintains a larger leaf area, the higher availability of nutrients (especially nitrogen) is effectively utilized for vertical growth, corroborating previous observations regarding the importance of N for the initial development of jaborandi (Marques and Costa, 1994; Brasil and Viégas, 1999).

The observation that there were no differences among fertilization treatments under pruning indicates that the removal of part of the aboveground biomass alters resource demand and allocation, reducing the immediate response to the different doses tested. In this context, the better performance of the 45 g NPK + 45 g MAP combination in the presence of pruning at the 20th month reinforces the need for balanced formulations that supply nitrogen and phosphorus for recovery and sprouting after pruning management, aligning with the recommendations for split fertilization in jaborandi (Marques and Costa, 1994).

The results observed in *P. microphyllus* also converge with evidence from other forest species, in which fertilization with N, P, and K promotes height increments (Vieira et al., 2017; Souza et al., 2013). Studies such as those by Vieira et al. (2017), Souza et al. (2013), Nieri et al. (2019), and Silva et al. (2022) demonstrate that adequate doses of nitrogen and phosphorus increase height growth, which supports the interpretation that nitrogen was a determining factor in the responses observed in this experiment.

From a physiological standpoint, nitrogen is fundamental in the synthesis of chlorophyll, proteins, and enzymes, favoring photosynthesis and growth, while phosphorus participates in energy transfer and essential metabolic processes; the deficiency of these nutrients at the beginning of the cycle can irreversibly compromise development (Souza et al., 2013). Thus, higher NPK doses can accelerate initial growth when the plant has sufficient leaf area to capture light and convert nutrients into biomass, which explains the greater height response in the absence of pruning.

For practical management, the data suggest that in plantations without intensive pruning, it is advantageous to apply higher doses of NPK (up to 175 g) to promote height growth, whereas in systems with scheduled pruning, the NPK + MAP combination at moderate doses can favor recovery and sprouting. These recommendations resonate with historical guidelines for jaborandi (Marques and Costa, 1994) and with evidence that nitrogen is one of the main limiting factors for the species' initial growth (Brasil and Viégas, 1999).

The findings indicate that fertilization with higher NPK doses (75 g and 175 g) favored the development of the *P. microphyllus* crown area up to the 20th month, suggesting a positive effect of fertilization on leaf mass expansion. This result can be attributed, in particular, to the nitrogen and potassium

present in the 20-0-20 formulation used: nitrogen is an essential component of the protoplasm and chlorophyll, influencing cell division and photosynthetic capacity; potassium regulates the osmotic potential, cell turgor, and enzymatic processes that affect transpiration and CO2 diffusion, contributing to a higher photosynthetic rate and, consequently, greater crown growth (Santos et al., 2010).

The positive influence of nitrogen fertilization on shoot production is corroborated by Cruz et al. (2006), who observed greater aboveground dry matter gains in *Samanea inopinata* seedlings with higher applications of ammonium sulfate. Similarly, the results of this study reinforce the role of N in promoting vegetative growth in forest species and commercial seedlings.

It is noted, however, that the significance of the differences emerged mainly in the final months of evaluation, indicating that responses to fertilization may require time to fully manifest in terms of crown area. This pattern reinforces the need for medium-term monitoring, as effects on morphological attributes and foliar biomass accumulation tend to consolidate with plant development.

From a management perspective, the data suggest that NPK applications at higher doses can be recommended to promote greater crown development during the initial stages of establishment, which can accelerate canopy closure and foliar biomass production. However, it is recommended that such guidelines be accompanied by soil analyses and fertilization programs tailored to the actual nutrient availability and the phenological needs of the species.

Finally, considering that pilocarpine levels and other phytochemical attributes of jaborandi typically stabilize only at more advanced ages (approximately four years in field experiments), it is imperative to extend the experimental monitoring to evaluate whether the fertilization regimes that maximize crown area also positively influence phytochemical quality and long-term productivity. Future studies should include foliar analyses, physiological measurements, and monitoring of N and K dynamics in the soil to elucidate mechanisms and optimize fertilization recommendations for *P. microphyllus* (Santos et al., 2010; Cruz et al., 2006).

Conclusion

The different agroforestry systems demonstrated that jaborandi develops better under intermediate light incidence. In the presence of pruning, fertilization did not affect height growth. Pruning positively influenced both the number of shoots and their length.

Fertilization with 175 g and 75 g NPK promoted increases in crown area and seedling height, with pronounced effects observed only by the twentieth month, indicating the need for prolonged monitoring to better characterize responses. When pruning is performed, to maximize height growth the recommended fertilization is 45 g NPK combined with 45 g MAP.

Silvicultural treatments benefited the development of *P. microphyllus* seedlings; planting the species in agroforestry systems that provide moderate solar radiation is recommended. Among silvicultural practices, formative pruning is advised to stimulate new shoot development and crown expansion, thereby enhancing leaf production.

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