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Native Microbial Consortia: A Sustainable Strategy for Improving the Quality of Forest Seedlings in the Peruvian Amazon

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Abstract: Forest plantations represent an alternative to reduce timber extraction pressure in the Amazonian forests. In order to tolerate the hostile field conditions of deforested areas, high-quality seedlings are required. This study aimed to find the optimal dose of a native microbial consortium (NMC), which enhances seedling quality indicators, in three forest species at nursery phase. A completely randomized design (3 × 5) was used. Factor 1: Bolaina blanca (*Guazuma crinita* Mart.), Capirona (*Calycophyllum spruceanum* Benth. Hook. f.), and Marupa (*Simarouba amara* Aubl.). Factor 2: Incremental doses of 0, 160, 320, 480, and 640 mL NMC per plant. The nursery survival (%), robustness index, root height/length ratio, shoot–root index, Dickson Quality Index (DQI), Nitrogen (%), Phosphorus (%), and Potassium (%) content in tissues were analyzed. Statistical analyses consisted of two-way ANOVA per variable and correlation analysis. The results indicated that increasing doses of NMC did not improve nursery survival for any species; did not decrease the robustness index, plant height/root length ratio, or the shoot–root index for any species; and did not increase the DQI, P%, or K% for any species; however, they did increase the N% for all species. In conclusion, the incremental dose of 160 mL was chosen for increasing the N% without affecting nursery survival.

Keywords: shoot–root index; robustness index; Dickson quality index; survival; *Guazuma crinita*; *Calycophyllum spruceanum*; *Simarouba amara*

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1. Introduction

In Peru, demand for roundwood and sawn wood has increased in the last three years. According to the National Forestry and Wildlife Service of Peru (SERFOR—the Spanish acronym) [1], transportation records for roundwood increased from 1,284,688.02 m^3 in 2022 to 1,309,152.48 m^3 in 2023. Additionally, the volume of non-coniferous wood destined for sawmills increased from 626,760 m^3 in 2020 to 1,455,180 m^3 in 2022 [2]. Most of the national wood production comes from the Amazonian region of Ucayali, which accounted for 51.97% of the national roundwood production and 38.42% of the total sawn wood in 2023 [1]. Despite these high production volumes, this region only has 7.47% of the registered area of forest plantations, totaling just 8,739.71 ha [3]. This suggests that most of the timber comes from natural areas, which implies a significant disruption in the ecosystems by extractive activities. Even under conditions of selective logging, mechanized operations decrease the capacity of the forest to provide ecosystem services such as carbon fixation and water infiltration [4]. In addition, the opening of roads for logging facilitates the initiation of shifting agriculture, which ultimately leads to increases in soil degradation [5]. Given this situation, the establishment of forest plantations emerge as a more sustainable alternative to meet the growing demand for timber.

Since forest plantations are often established on degraded soils, seedlings must cope with several stress conditions, such as dry seasons, nutrient-poor soils, grazing, and fires [6]. Selecting species which adapt to these conditions is critical to the success of plantations. In this study, we worked with three native species to the Peruvian Amazon: Bolaina blanca (*Guazuma crinita* Mart.), Capirona (*Calycophyllum spruceanum* Benth. Hook. f.), and Marupa (*Simarouba amara* Aubl.). They have demonstrated good adaptability to harsh conditions, rapid growth, and good wood workability [7,8]. In order to ensure the success of these plantations high quality seedlings capable of establishing in adverse conditions are needed. [9–11]. The seedling quality depends on both genetic and environmental factors. The genetic factor is related to the selection of vigorous parents adapted to conditions where the plantations will take place [12]. The environmental factor includes the appropriate choice of substrates [13,14] and containers [15], irrigation management [13], the control of light intensity [13,16], temperature, and relative humidity [13]. Many of these subfactors and their effects on seedling quality are yet to be investigated for the three species of our study. In this work, we will focus on nutritional management.

Available reports on these three species have focused on the physical and chemical benefits of adding organic amendments to the growing media. In that sense, the authors tested chicken manure + decomposed saw dust [17]; cow manure + 'Guano de Isla' (Island manure) [18]; and vermicompost [19]. In all of them, consistently better results were found in treatments that received any organic input. Despite this, the analysis was founded on physical and chemical explanations. Biological interactions with soil microbiota were just superficially examined. For that reason, a native microbial consortium was tested to observe the possible benefits of inoculating endemic soil microorganisms during the nursery phase.

As reviewed by Calvo et al. [20], microbial inoculants promote plant growth by either facilitating plant nutrition (biostimulants) or antagonizing with plant pathogens (biocontrollers). Some positive consequences involved larger nutrient availability and root growth enhancement. The analyzed mechanisms were asymbiotic nitrogen fixation, solubilization of nutrients like Phosphorus, sequestering of Iron by siderophores, and the production of volatile compounds. More recently, Liu et al. [21] highlighted that single-species inoculants could be affected by competition with native microbial communities. After reviewing 51 reports, they found that under pot/greenhouse conditions, bacterial consortia had an average of 51.7% increase in plant growth compared to single-species inoculum, which only achieved 30.4% increase. This was explained on the basis that multiple species cover a wide array of mechanisms which not only enhances synergy with roots but also complementarity relationships among different microbial populations. In the Peruvian Amazon, Nottingham et al. [22] studied the diversity of soil bacteria and fungi at different altitudes. They described that between 194 to 210 m.a.s.l., in the organic horizon of soils, there were predominantly Sordariomycetes, Agaricomycetes, Acidobacteria, Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria. The above-mentioned classes bring together very well-studied individual genres that have been tested as biostimulants [23,24]. That is the case of the antagonist fungi *Trichoderma* spp. or the mycorrhizal *Laccaria bicolor.* In the case of bacteria, well-known genres like *Streptomyces* sp., *Rhizobium* spp., *Azospirillum* spp., or *Pseudomonas* spp. also belong to the bacteria classes reported by Nottingham et al. [22]. Given that such beneficial microorganisms are naturally present in the Amazonian soils, it would be fair to think that multiplying local strains and using them as biostimulants would synergistically interact with native plant species. If that is the case, in nurseries, plantlets that survive the propagation process would show better quality indicators like an appropriate stem elongation in relation with their diameter, a well-balanced growth of the root and aboveground biomass, or better chances of field survival. That is why analyzing quality indicators like the nursery survival percentage, the robustness index [25], the height:root length ratio [25], shoot:root index [26], and Dickson's quality index [27] can give us an idea of the resilience of trees in harsh field conditions. According to Rueda-Sanchez et al. [28], high-quality tree plantlets show the following values for the above mentioned indices: robustness index <6.0; plant height:root length ratio ≤2.0; shoot:root index of 1.5–2.0; and Dickson's quality index ≥0.5. In the context of Peruvian Amazon forest nursery management, the optimal dose of a native microbial consortium (NMC) that favors Bolaina blanca, Capirona, and Marupa is still unknown, so the objective of this study was to determine the optimal dose of NMC that allows for achieving the best plantlet quality indicators for each of the three tropical forest species.

For the species Bolaina blanca, Marupa, and Capirona, we hypothesized that the nursery survival percentage will quadratically increase with higher doses of native microbial consortium (NMC) as a result of better plant nutrition. This happens in a differentiated manner for each species. The Nitrogen, Phosphorus, and Potassium contents in leaves are indicators of this. Regarding plantlet quality indicators, they also respond differently for each species. Our hypothesis was complimented with the following: The robustness index linearly decreases with higher doses of NMC as a result of larger shoot diameters (mm) in relation to height (cm), not exceeding the value of 6; the height:root length ratio linearly decreases with higher doses of NMC as a result of larger root length (cm) in relation to plant height (cm), not exceeding the value of 2; the shoot:root index linearly decreases with higher doses of NMC as a result of larger root biomass (g) in relation to the aboveground biomass (g), not exceeding the value of 2; and the Dickson quality index linearly increases with higher doses of NMC as a result of larger shoot diameters (mm) and root biomass (g), being above the value of 0.5. The capacity of the NMC to improve the quality of the seedlings was evaluated by analyzing differences in the morphological and nutritional parameters based on increasing doses of the native microbial consortium.

2. Materials and Methods

2.1. Location of the Experiment

The experiment was carried out in the agroforestry nursery of the Pucallpa Agrarian Experimental Station of the National Institute of Agrarian Innovation (INIA) located in the district of Calleria, province of Coronel Portillo, Ucayali region, Peru, between May 2022 and August 2022. The geographical coordinates of the place are 8°23′14.7″ S and 74°33′34.8″ W at an altitude of 156 m.a.s.l. The weather conditions during the test were the following: daily average temperature of 27.1 °C, daily mean relative humidity of 82.4%, and accumulated precipitation of 1279.7 mm [29].

2.2. Experimental Design

A completely randomized design was used in a factorial arrangement (3 × 5). Factor 1 consisted of three native species: Bolaina blanca (*Guazuma crinita* Mart.), Capirona (*Calycophyllum spruceanum* Benth. Hook. f.), and Marupa (*Simarouba amara* Aubl.). Factor 2 consisted of five incremental dose levels of native microbial consortium (NMC) applied to seedlings of the three forest species. Doses were 0, 160, 320, 480, and 640 mL per plantlet seedling. That is the total volume of stock solution of NMC applied to each seedling over a period of 105 days. Applications took place every 15 days, making a total of 8 applications. The inoculation process took place in the following way: stock solution of NMC was measured using a plastic container previously labeled with the following marks: 20, 40, 60, and 80 mL (corresponding to the treatments 160, 320, 480, 640, respectively). For instance, the treatment of 160 mL NMC consisted of 8 applications of 20 mL stock solution of NMC. The same procedure was done for the other doses. In every application, corresponding measured stock solution of NMC was mixed with irrigation water until reaching a volume of 80 mL. In the case of 640 mL treatment, no dilution was needed. Every plantlet within each experimental unit was drenched individually. The experimental unit (EU) was defined as a set of 25 plantlets (4 months old). Each plantlet was placed in an individual nursery plastic bag and grouped together with the same members of their respective experimental unit. Each EU was randomly placed within the experimental nursery area. Four replicates per treatment combination were used, making a total of 60 experimental units. Final evaluation took place on day 120 in order to allow 15 extra days to see the effect of the last application.

2.3. Production Process of the Native Microbial Consortium (NMC)

The NMC production process was carried out in two phases according to Kalema and Chacon [30]. Collection and Microorganism reproduction phase: An amount of 50 kilos of forest mulch was collected in November 2021 and sifted though a 0.5 cm metal mesh. The forest mulch is made up of a layer of organic material (leaves, branches, roots, wood, fruits, and seeds in a state of decomposition) that covers the soil in forest ecosystems and was the main source of NMC microorganisms. It was collected from a residual forest located at the Alexander Von Humboldt Experimental Facility (8°49′54.19″ S; 75°3′22.94″ W) managed by the National Institute of Agrarian Innovation (INIA—Spanish acronym). The sieved forest mulch was subsequently mixed with 50 kg of rice dust, 2 gallons of cane molasses, and 4 L of non-chlorinated water. To reproduce the NMC microorganisms in an anaerobic process, 2/3 of the previous mixture was placed in a hermetically sealed drum for 30 days and located in a cool and shaded place. For aerobic reproduction, 1/3 of the mixture was taken into a bucket, covered with a cloth to protect it from environmental factors, and constantly stirred for 8 days in order to reduce excess heat and prevent microorganism populations from falling drastically. Activation phase: After 30 days, a homogeneous mixture of 6.5 kg of anaerobic NMC and 3.5 kg of aerobic NMC was transferred to a 200 L container (previously filtered with a cloth). Then, 2 gallons of molasses and non-chlorinated water were added until the mixture thickened to the expected texture. After 10 days, the microorganisms were activated and the NMC was ready for use. A sample was sent to perform a microbiological and chemical analysis at the Microbial Ecology and Biotechnology Laboratory "Marino Tabusso", Biology Department, Faculty of Sciences, Universidad Nacional Agraria La Molina (National Agrarian University La Molina). The results are shown in Table 1.

Table 1. Results of the microbiological and chemical analyses of the stock solution of native microbial consortium.

Parameter [a]	Unit	Value
lactic acid bacteria	CFU [b] mL^{-1}	5.1×10^7
viable mesophilic aerobes	CFU mL^{-1}	6.4×10^7
molds and yeasts	CFU mL^{-1}	1.0×10^3
aerobic bacteria (actinomycetes)	CFU mL^{-1}	b. d. l. [c]
pH	------	3.63
electrical conductivity	dS m^{-1}	7.71
organic matter in solution	%	2.54
Nitrogen	%	0.13
Phosphorus	%	0.013
Potassium	%	0.436
Calcium	%	0.105
Magnesium	%	0.044
Iron	%	0.002
Manganese	%	0.001

[a] Analyzes were conducted at the "Marino Tabusso" Laboratory of Microbial Ecology and Biotechnology, Department of Biology, Faculty of Sciences, Universidad Nacional Agraria La Molina (Av. La Molina s/n, La Molina, Lima 15024, Peru—http://www.lamolina.edu.pe/lmt/, accessed on 9 February 2025). [b] Colony formation unit. [c] Below detection limit.

2.4. Cultural Practices

Plant propagation was performed according to Flores [31]. Seeds were collected from seed stands managed for more than 50 years at the INIA Alexander Von Humboldt Experimental Facility (8°49′54.19″ S; 75°3′22.94″ W). In order to harvest them, telescoping pruning shears and a tree climber were used, cutting branches with abundant fruit and selecting the best seeds. Seed germination was carried out in plastic trays with a substrate of disinfected fine sand. Irrigation was carried out manually with medium-drop sprinkler nozzles in an environment with a permanent roof. The seeds of Bolaina blanca and Capirona germinated in 8 and 18 days, respectively, without the need for pre-germination treatments. Due to the scarce availability and low germination percentage of Marupa seeds, already germinated seedlings from the forest were used. They were transplanted bare root.

At 45 days after germination, Bolaina blanca and Capirona seedlings with 4 or more true leaves were transplanted. Marupa seedlings were transplanted directly into bags. The substrate used consisted of a mixture of alluvial soil material and fine sand in a 1:1 ratio. The container features were the following: polyethylene bags 12.7 cm in diameter and 20.2 cm long (2.6 dm^3) and the NMC treatments applied using polypropylene dispensers graduated to the milliliter. The seedlings were randomly grouped under homogeneous 85% shade provided by a cloth made of polyethylene Raschel fabric.

2.5. Data Collection

Assessment of all measured variables was carried out at 120 days after transplant (DAT). For each experimental unit, the 25 plants were measured, and the average was calculated for data analysis. Evaluated variables and their measurement procedures are detailed in Table 2.

Table 2. Evaluation methodology of measured and calculated variables.

Variable	Unit	Measuring Instrument	Procedure [a]
survival (S) [b]	%	manual counting	Number of survivors per EU was determined. The applied formula was $$\%S = \frac{\#\ \text{Survivors}}{25\ \text{plants}} \times 100$$
height (H) [b]	cm	millimeter ruler	Plants were measured from the base to the last node.
diameter (D) [b]	mm	digital vernier—precision 0.01 mm (CALDI-6MP, Truper, China)	The measurement was taken at the base of the seedling.
root length (RL) [b]	cm	millimeter ruler	Plants were carefully removed from the containers. They were immersed in water up to the shoot–root junction and scrubbed manually until all the substrate was removed. Plants were divided at the shoot–root junction. The main root was stretched, and the measurement was taken.
aboveground dry biomass (ABD) [b]	g	analytical balance precision 0.0001 g (JA303P, Eurolab, Germany)	canopy biomass was oven dried (U30, Memmert, Germany) at 70 °C for 72 h [32]. The weight was recorded.
root dry biomass (RDB) [b]	g	analytical balance precision 0.0001 g (JA303P, Eurolab, Germany)	The root biomass was dried in an oven (U30, Memmert, Germany) at 70 °C for 72 h [32]. The weight was recorded.
robustness index (RI) [b]	cm mm^{-1}	same as height and diameter	Plant height (cm) was used and divided by shoot diameter (mm) [25]. $$\text{RI} = \frac{\text{height (cm)}}{\text{diameter (mm)}}$$
root height to length ratio (r H/RL) [b]	cm cm^{-1}	root height and length	Plant height (cm) was used and divided by root length (cm) [25]. $$\text{r H/RL} = \frac{\text{height (cm)}}{\text{root length (cm)}}$$
shoot-root index (rABD/RDB) [b]	g g^{-1}	dry aboveground and root biomass	Weight of dry aboveground biomass (g) was used and divided by the dry root biomass [26]. $$\text{r ABD/RDB} = \frac{\text{dry aboveground biomass (g)}}{\text{dry root biomass (g)}}$$
Dickson quality Index (DQI) [b]	g(cm mm^{-1} + g g^{-1})$^{-1}$	biomass, height and diameter	Total biomass weight (g), plant height (cm), shoot diameter (mm), aboveground biomass (g), and root biomass (g) were used in the following formula [27]: $$\text{DQI} = \frac{\text{total dry biomass (g)}}{\frac{\text{height (cm)}}{\text{diameter (mm)}} + \frac{\text{aboveground dry biomass (g)}}{\text{root dry biomass (g)}}}$$
Nitrogen [c]	%	---	Modified Kjeldahl method [33] [d]
Phosphorus [c]	%	---	Phospho-vanadomolybdate colorimetry [34] [d]
Potassium [c]	%	---	Atomic absorption spectrophotometry [34] [d]

[a] All variables were assessed at 120 DAT. [b] The data were evaluated for the 25 plants in each experimental unit, and then the average was used for statistical analysis. [c] Leaves of 10 plants per experimental unit were collected and taken fresh to the laboratory. They were dried at 70 °C for 72 h. Subsequently, they were sieved through 1 mm sieve. [d] Analysis was carried out at the "Laboratory of Soil, Water and Plant tissue analysis laboratory INIA—LABSAF" (Campo Verde, Ucayali, Peru—https://app.inia.gob.pe/labsaf/publico/dashboard; accessed on 9 February 2025).

2.6. Statistical Analysis

Statistical analyses were carried out using R software version 4.2.2, through RStudio sversion 2023.06.0. For each variable, two linear models were tested: one with quadratic effect and one with linear effect only using the function "lm" from the "stats" package [35]. The relevance of the quadratic effect was determined using the "anova" function from the "stats" package [35], with a significance level of $\alpha = 0.05$. Subsequently, the most appropriate model was chosen. Then, the normality of residuals assumption was verified with the "shapiro.test" function, "stats" package [35], and the homoscedasticity assumption with the "bptest" function, "lmtest" package [36]. For the variables that met these assumptions, an Analysis of Variance (ANOVA type I) was performed using the "anova" function of the "stats" package [35], considering "species" and "doses" as factors in that order. In the cases where the interaction was significant, a simple effects analysis was performed with the "emmeans" and "contrast" functions, "emmeans" package. In case of significance at the main effects level, a Tukey test was performed using the "HSD.test" function, "agricolae" package [37], only on the dose factor. For variables that did not meet the assumptions, specific mathematical transformations were used, such as natural logarithm for %P and inverse for %N. Then, the above-mentioned tests were repeated. In cases where the transformations were not successful, the Robust Standard Errors method was used: "coeftest" function, "lmtest" package [36], and "vcovHC" function, "sandwich" package [38,39], followed by a type III ANOVA, "Anova" function, "car" package [40]. Finally, in order to identify relevant correlations between indices and morphological and physiological variables, a correlation analysis was performed using the "cor" function from the "stat" package [35]. Variables were segregated by species and NMC dose before calculating the Pearson coefficient. A value greater than $|0.89|$ was considered significant, since it is equivalent to explaining at least 80% of the variability. The details of the codes used can be found in Supplementary Material S1. The results of the statistical analyses can be found in Supplementary Material S2. Data matrix can be found in Supplementary Material S3. The coding process in RStudio was carried out with the support of ChatGPT [41]. The prompts can be found in Supplementary Material S4.

3. Results

3.1. Survival (%)

The interaction between forest species and the quadratic component of NMC dose (ml) was found to be significant ($p = 0.0019$) for the variable survival (%). The application of NMC doses significantly influenced the survival percentage in a differentiated manner according to the species type (Figure 1). Bolaina blanca survival for all doses was statistically similar to the control (Supplementary Material S2). In the case of Capirona, only the 640 mL dose proved to be statistically different from the control. This indicates that lower doses showed similar survival to the control. Survival in Marupa without the application of NMC had an average of 60% and showed a decreasing quadratic tendency when adding increasing doses of NMC. All doses above 160 mL of NMC showed a significantly lower survival than the control (Supplementary Material S2).

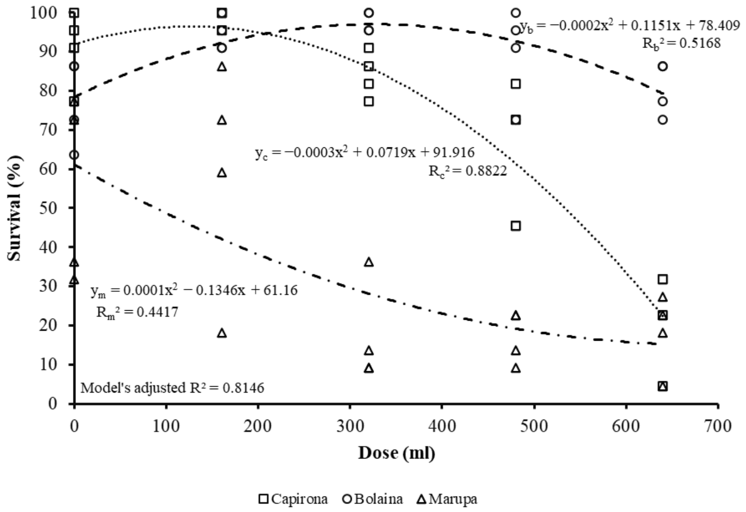

Figure 1. Effect of different native microbial consortium (NMC) doses (mL) on the survival (%) of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-·-△-·-) grown in nursery conditions. Using a type III ANOVA (α = 0.05), with robust standard errors, interaction between tree species and the quadratic component of NMC dose (mL) was found to be significant (p = 0.0019). Regression lines and equations represent the respective quadratic trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, coefficient analyses, and simple effects analysis can be found in Supplementary Material S2. Model's adjusted R^2: 0.8146.

3.2. Plant Height (H)

Although the interaction between tree species and the linear component of NMC dose was not significant (p = 0.2575), for the variable plant height (cm), the main effects of species type ($p < 0.05$) and NMC dose (p = 0.0026) were significant (Supplementary Material S2). This suggests that, at least one of the plant height averages (cm) for a single species (across all doses), was statistically different from the other two. However, it was not an objective to check which species was the tallest or the shortest. On the other hand, significance on the main effect of doses means that at least one of the plant height averages, for a single dose (across all species), was significantly different than the others. In this case, only the averaged 640 mL dose was statistically different from the control. (Supplementary Material S2). It also implies that the change in plant height (cm), for increasing doses of NMC, was similar for the three species. That is the explanation for the similar slopes for each of the species' regression lines (Figure 2).

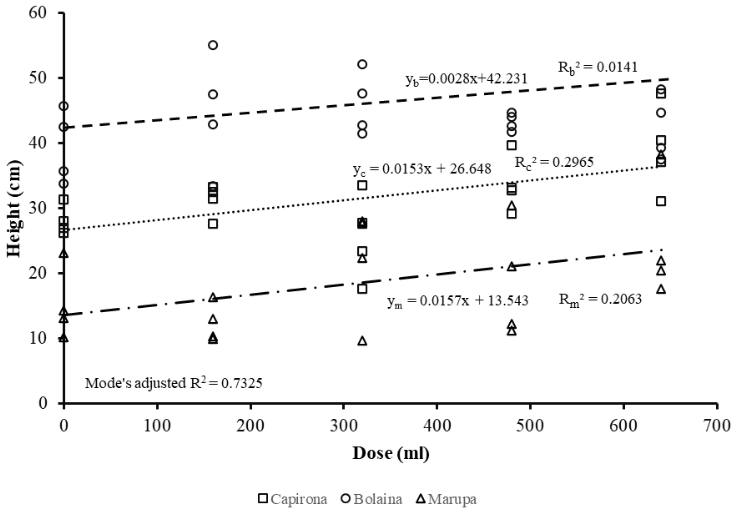

Figure 2. Effect of different native microbial consortium (NMC) doses (mL), in the height (cm) of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-··△-··) grown in nursery conditions. Using a type I ANOVA (α = 0.05). Interaction proved to be non-significant (p = 0.2575). Main effects of species type were found to be significant (p < 0.05). Main effect of NMC dose (mL) was found to be significant (p = 0.0026). Regression lines and equations represent the respective linear trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, coefficient analyses, and Tukey test can be found in Supplementary Material S2. Model's adjusted R^2: 0.7325.

3.3. Diameter at Shoot–Root Junction (D)

The interaction between species and the linear component of NMC dose (mL) was found to be non-significant (p = 0.5446) for the variable diameter (mm) at shoot–root junction. Significant main effects were found for species type (p < 0.05). The main effect of NMC dose (mL) was not significant (p = 0.2729) (Figure 3). This suggests that at least one of the diameter averages (mm) for a single species (across all doses) was statistically different from the other two. However, it was not an objective to check which species had the thickest or the thinnest at the shoot–root junction. Conversely, the lack of significance in the dose main effect indicates that the average of each dose (across all species) behaved similarly to the control treatment. That explains the very small value of the slopes in Figure 3. Details of the statistical tests can be found in Supplementary Material S2.

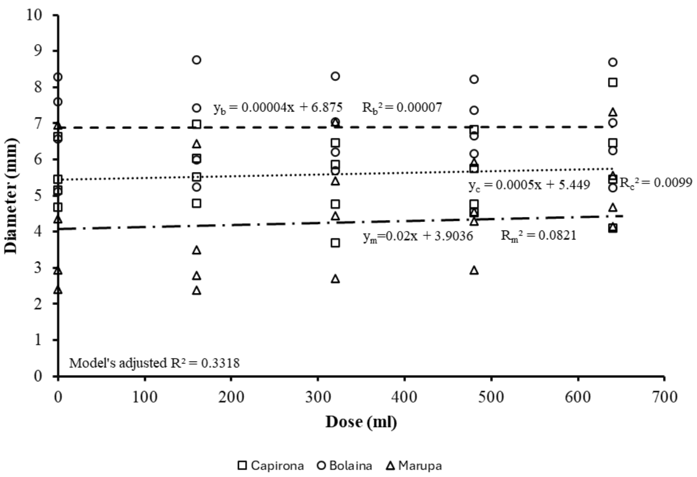

Figure 3. Effect of different native microbial consortium (NMC) doses (mL), in the diameter of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-··△-··) grown in nursery conditions.

Using a type I ANOVA (α = 0.05), interaction between tree species and NMC dose (mL) was found to be non-significant (p = 0.5446). Main effects of species type were found to be significant ($p < 0.05$). Main effect of NMC dose (ml) was found to be not significant (p = 0.2729). Regression lines and equations represent the respective linear trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, and coefficient analyses can be found in Supplementary Material S2. Model's adjusted R^2: 0.3318.

3.4. Robustness Index (RI)

The robustness index (RI) was calculated from the height (cm) and diameter (mm) data. The interaction between species and the linear component of NMC dose was not significant (p = 0.2433). The main effects were significant for both "species" ($p < 0.05$) and "dose" (p = 0.0045). for this variable (Supplementary Material S2). The main effects were significant for both "species" ($p < 0.05$) and "dose" (p = 0.0045). This suggests that at least one of the robustness index averages, for a single species (across all doses), was statistically different from the other two. However, it was not an objective to check which species had the smallest robustness index. On the other hand, significance on the main effect of doses means that at least one of the robustness index averages, for a single dose (across all species), was significantly different than the others. In this case, only the averaged 640 mL dose was statistically bigger than the control (Supplementary Material S2). It also implies that the change in the robustness index, for increasing doses of NMC, was similar for the three species. That is the explanation for the similar slopes for each of the species' regression lines (Figure 4). Apart from that, it was necessary to check whether or not this index value remained within the ranges given by Rueda-Sanchez et al. [27]. In the case of Bolaina blanca, the average remained above 6 for all NMC doses, including the control. This indicates not high quality, but medium quality instead. Regarding Capirona, the average values remained between 5 and 6. This indicates high quality for all NMC doses. Species Marupa showed average values between 3.5 to 4.5, which indicates high quality for all NMC doses. A positive slope in all three tree species (Figure 4) is a negative indicator in terms of the robustness index. Nonetheless, only the 640 mL dose was different from the control.

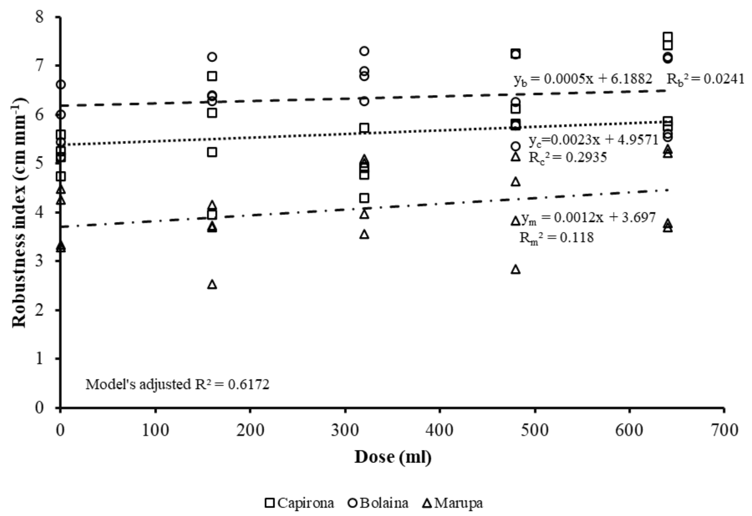

Figure 4. Effect of different native microbial consortium (NMC) doses (mL) in the robustness index of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-·-△-·-) grown in nursery conditions. Using a type I ANOVA (α = 0.05), interaction between tree species and NMC dose (mL) was found to be non-significant (p = 0.2433). Main effects of species type were found to be significant (p < 0.05). Main effect of NMC dose (ml) was found to be significant (p = 0.0045). Regression lines and

equations represent the respective linear trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, coefficient analyses, and Tukey test can be found in Supplementary Material S2. Model's adjusted R^2: 0.6172.

3.5. Root Length (RL)

In the case of root length (cm), the interaction between species and the linear component of NMC dose was not significant (p = 0.5585) (Supplementary Material S2). Main effects were significant for "species" (p < 0.05); however, they were not for "dose" (p = 0.3391). This suggests that at least one of the root length averages (cm) for a single species (across all doses) was statistically different from the other two. However, it was not an objective to check which species had the longest or the shortest root. Conversely, the lack of significance in the dose main effect indicates that the average of each dose (across all species) behaved similarly to the control treatment. That explains the very small value of the slopes in Figure 5. Details of the statistical analyses can be found in Supplementary Material S2.

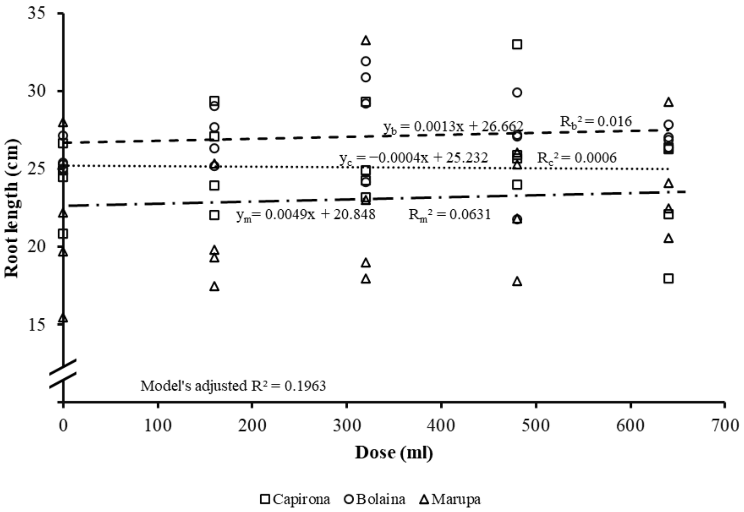

Figure 5. Effect of different native microbial consortium (NMC) doses (mL) on the root length of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-·-△-·-) grown in nursery conditions. Using a type I ANOVA (α = 0.05), interaction between tree species and NMC dose (mL) was found to be non-significant (p = 0.5585). Main effects of species type were found to be significant (p < 0.05). Main effect of NMC dose (ml) was found to be not significant (p = 0.3391). Regression lines and equations represent the respective linear trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, and coefficient analyses can be found in Supplementary Material S2. Model's adjusted R^2: 0.1963.

3.6. Plant Height/Root Length Ratio (r H/RL)

Regarding the ratio between plant height and root length, the interaction between species and the linear component of NMC dose was not significant (p = 0.1737) (Supplementary Material S2). The main effects were significant for both "species" (p < 0.05) and "dose" (p = 0.0063). This suggests that at least one of this ratio's averages, for a single species (across all doses), was statistically different from the other two. However, it was not an objective to check which species had the biggest or smallest height:root length ratio. Conversely, the significance on the main effect of doses means that at least one of this ratio's averages, for a single dose (across all species), was significantly different than the others. In this case, only the averaged 640 mL dose was statistically different from the control. (Supplementary Material S2). This also implies that the change in plant height:root length ratio, for increasing doses of NMC, was similar for the three species. That is the explanation for the similar slopes for each of the species' regression lines (Figure 6). Furthermore, it was necessary to check whether or not this index value remained within the ranges given by Rueda-Sanchez et al. [27][]. In the case of Bolaina blanca, the average remained between 1.5 and 2 for all NMC doses, including the control. This indicates high-quality for all tested NMC doses. Regarding Capirona, the average values remained between 1 and 1.5. This indicates high quality for all NMC doses. Species Marupa showed average values between 0.5 and 1.0, which indicates high quality for all NMC doses. A positive slope in all three tree species (Figure 6) is a negative indicator in terms of the plant height:root length ratio. Nonetheless, only the 640 mL dose was different from the control (Supplementary Material S2).

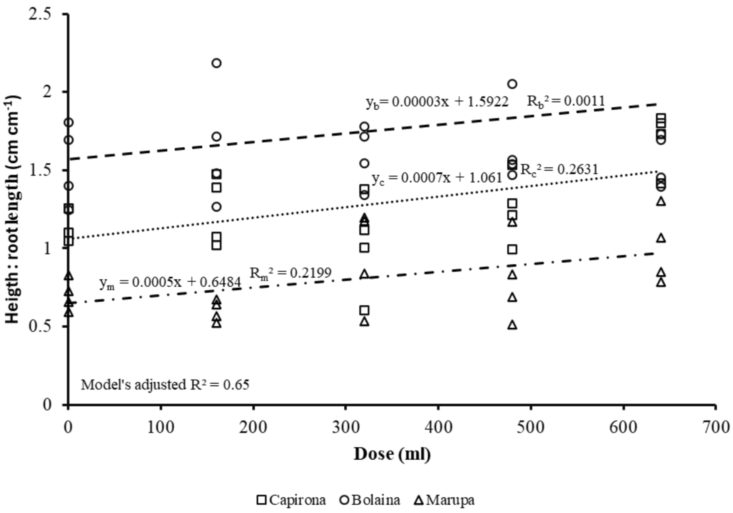

Figure 6. Effect of different native microbial consortium (NMC) doses (mL) in the robustness index of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-·-△-·-) grown in nursery conditions. Using a type I ANOVA ($\alpha = 0.05$), interaction between tree species and NMC dose (mL) was found to be non-significant ($p = 0.1737$). Main effects of species type were found to be significant ($p < 0.05$). Main effect of NMC dose (ml) was found to be significant ($p = 0.0063$). Regression lines and equations represent the respective linear trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, coefficient analyses, and Tukey test can be found in Supplementary Material S2. Model's adjusted R^2: 0.65.

3.7. Aboveground Dry Biomass (ABD) and Root Dry Biomass (RDB)

Regarding dry aboveground biomass, the interaction between species and the linear component of the NMC dose was not significant ($p = 0.8989$). The main effects of "dose" were also not significant ($p = 0.4498$). Only the main effects of species showed significance ($p < 0.05$). This suggests that at least one of the aboveground biomass averages (g) for a single species (across all doses) was statistically different from the other two. However, it was not an objective to check which species produced the largest or poorest aboveground biomass. Conversely, the lack of significance in the dose main effect indicates that the average of each dose (across all species) behaved similarly to the control treatment. That explains the very small value of the slopes in Figure 7. The results of the statistical analyses can be found in Supplementary Material S2.

Regarding dry root biomass, the interaction between species and the quadratic component of NMC dose was significant ($p = 0.0184$). This indicates that the responses to increases in NMC doses varied following a quadratic trend according to the three species (Figure 8). Bolaina blanca showed the highest values for this variable, showing an increase in dry root biomass up to a dose of 320 mL that was followed by a progressive reduction at higher doses. The simple effects analysis indicates that the differences between all treatments were not significant (p = [0.0525; 0.0537]) (Supplementary Material S2). Marupa, whose root mass was significantly lower than that of Bolaina blanca and similar to that of Capirona, showed that its root biomass was statistically similar to the control (p = [0.6394; 0.6397]) (Supplementary Material S2). In the case of Capirona, it was found that, like Marupa, the root biomass did not differ from the control at increasing doses of the NMC (p = [0.6982; 0.7008]) (Supplementary Material S2).

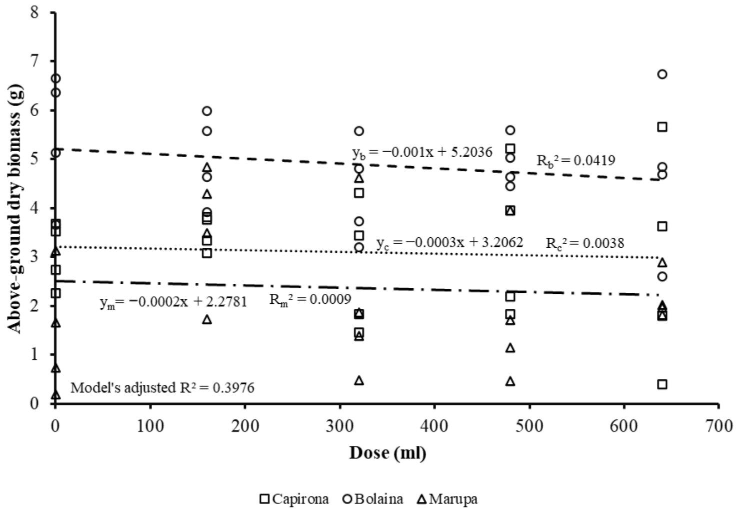

Figure 7. Effect of different native microbial consortium (NMC) doses (ml) in the above-ground dry matter of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-··△··-) grown in nursery conditions. Using a type III ANOVA ($\alpha = 0.05$) with robust standard errors, interaction between tree species and NMC dose (ml) was found to be non-significant ($p = 0.8989$). Main effects of species type were found to be significant ($p < 0.05$). Main effect of NMC dose (ml) was found to be not significant ($p = 0.4498$). Regression lines and equations represent the respective linear trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, and coefficient analyses can be found in Supplementary Material S2. Model's adjusted R^2: 0.3976.

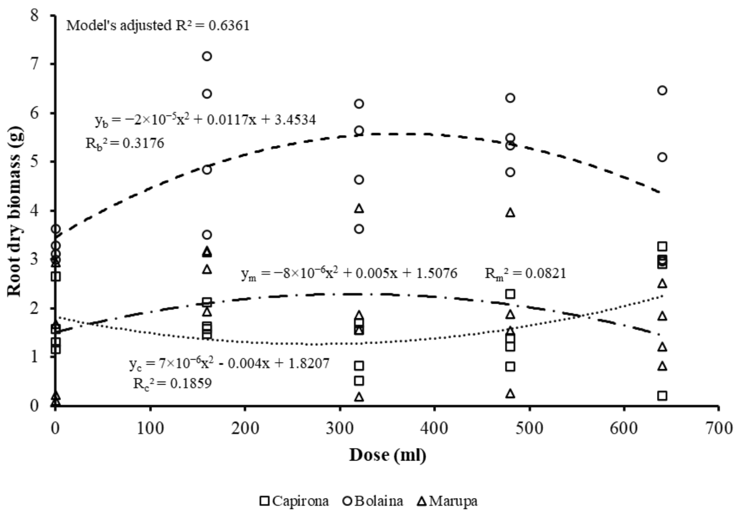

Figure 8. Effect of different native microbial consortium (NMC) doses (ml) on the root dry matter of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-··△··-) grown in nursery conditions. Using a type I ANOVA ($\alpha = 0.05$), interaction between tree species and the quadratic component of NMC dose (ml) was found to be significant ($p = 0.0184$). Regression lines and equations

represent the respective quadratic trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, and simple effects analyses can be found in Supplementary Material S2. Model's adjusted R^2: 0.6361.

3.8. Shoot–Root Index (SRI)

The shoot–root index is the ratio between the dry aboveground biomass (g) and the dry root biomass (g). The interaction between species and the quadratic component of the NMC dose was significant ($p = 0.0049$) in the quadratic component for this variable. This indicates that the responses to the increases in NMC doses varied following a quadratic trend according to the species type (Figure 9). Although the ANOVA indicates that there were statistically different results at the interaction level, when observing the results of the simple effects analysis, we see that, within each species, all treatments behaved statistically similar to the control ($p > 0.05$; for all cases) (Supplementary Material S2). Moreover, it was necessary to check whether or not this index value remained within the ranges given by Rueda-Sanchez et al. [27]. In the case of Bolaina blanca, the average remained between 0.7 and 1.8 for all NMC doses, including the control. This indicates high quality for all tested NMC doses. Regarding Capirona, the average values remained between 1.5 and 2.5. This does not indicate high quality, but medium quality instead for all NMC doses, including the control. Species Marupa showed average values between 1.0 and 1.8, which indicates high quality for all NMC doses. A negative slope in all three tree species (Figure 6), is a positive indicator in terms of the plant shoot–root ratio. Nonetheless, all doses were statistically similar to their control experimental units (Supplementary Material S2).

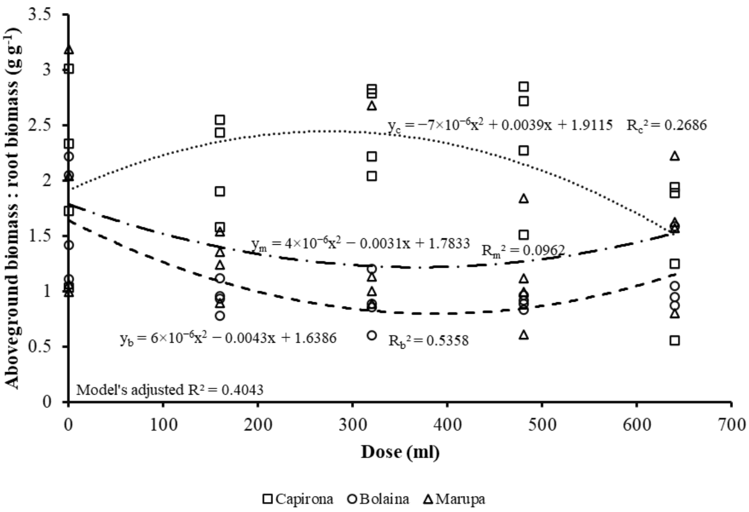

Figure 9. Effect of different native microbial consortium (NMC) doses (mL) in the shoot–root index of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-·-△-·-) grown in nursery conditions. Using a type I ANOVA ($\alpha = 0.05$), interaction between tree species and the quadratic component of NMC dose (mL) was found to be significant ($p = 0.0049$). Results of the Shapiro–Wilk test, Breusch–Pagan test, and simple effects analyses can be found in Supplementary Material S2. Regression lines and equations represent the respective quadratic trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Model's adjusted R^2: 0.4043.

3.9. Dickson Quality Index (DQI)

Regarding the Dickson quality index, the interaction between species and the linear component of NMC dose was not significant ($p = 0.6792$). As for the main effects, "dose" was also not significant ($p = 0.6525$). Only the main effects of species were statistically different ($p = 0.0338$) (Supplementary Material S2). This suggests that at least one of the

DQI's averages for a single species (across all doses) was statistically different from the other two. However, it was not an objective to check which species had the largest or smallest DQI. On the other hand, the lack of significance in the dose main effect indicates that the DQI average of each dose (across all species) behaved similarly to the control treatment. That explains the very small value of the slopes in Figure 10. Furthermore, it was necessary to check whether or not the DQI's averages remained within the ranges given by Rueda-Sanchez et al.][27]. In the case of Bolaina blanca, the average remained between 1.0 and 1.5 for all NMC doses, including the control. This indicates high quality for all tested NMC doses. Regarding Capirona, the average values remained between 0.5 and 1.0. This indicates high quality for all NMC doses, including the control. Species Marupa showed average values between 0.7 and 1.2, which indicates high quality for all NMC doses. A positive slope in all three tree species is a positive indicator in terms of the DQI. Nonetheless, all doses were statistically similar to their control experimental units (Supplementary Material S2), which is confirmed with non-significant slopes.

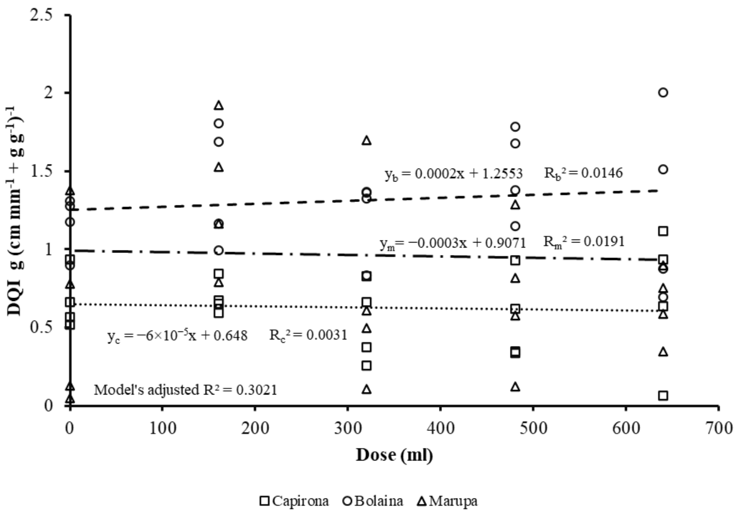

Figure 10. Effect of different native microbial consortium (NMC) doses (mL) in the Dickson quality index (DQI) of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-·-△-·-) grown in nursery conditions. Using a type III ANOVA (α = 0.05) with robust standard errors, the interaction between tree species and NMC dose (mL) was found to be non-significant (p = 0.6792). Main effects of species type were found to be significant (p = 0.0338). Main effect of NMC dose (ml) was found to be not significant (p = 0.6525). The results of the Shapiro–Wilk test, Breusch–Pagan test, and coefficient analyses can be found in Supplementary Material S2. Regression lines and equations represent the respective linear trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Model's adjusted R^2: 0.3021.

3.10. Nutritional Changes in Tissues (%K, %N, %P)

Regarding Potassium, the interaction between species and the linear component of NMC dose was not significant (p = 0.4354). Regarding the main effects, "dose" was also not significant (p = 0.7352). Only the main effects of species showed significance ($p < 0.05$). This suggests that at least one of the K% averages for a single species (across all doses) was statistically different from the other two. However, it was not an objective to check which species had a better Potassium absorption. Conversely, the lack of significance in the dose main effect indicates that the K% average of each dose (across all species) behaved similarly to the control treatment. That explains the very small value of the slopes in Figure 11. The results of the statistical analyses can be found in Supplementary Material S2.

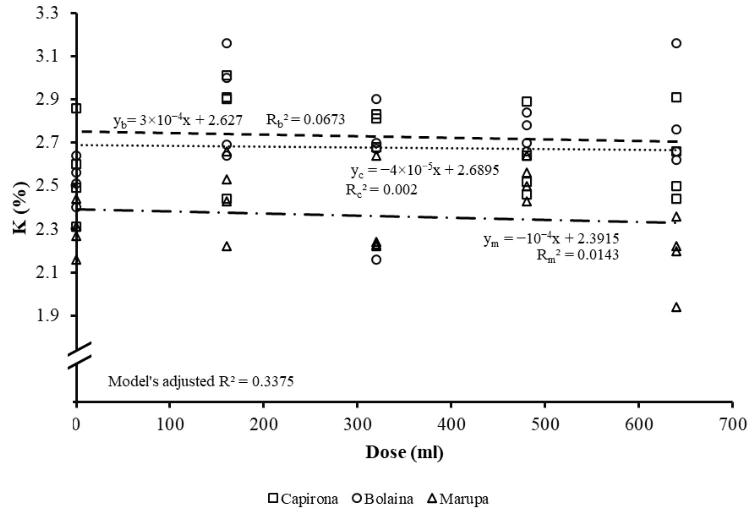

Figure 11. Effect of different native microbial consortium (NMC) doses (mL) in the K (%) of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-·-△-·-) grown in nursery conditions. Using a type I ANOVA ($\alpha = 0.05$), interaction between tree species and NMC dose (mL) was found to be non-significant ($p = 0.4354$). Main effects of species type were found to be significant ($p < 0.05$). Main effect of NMC dose (ml) was found to be non-significant ($p = 0.7352$). Results of the Shapiro–Wilk test, Breusch–Pagan test, and coefficient analyses can be found in Supplementary Material S2. Regression lines and equations represent the respective linear trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Model's adjusted R^2: 0.3375.

Regarding Nitrogen (N), after applying an inverse transformation, it was found that the interaction between species and the quadratic component of NMC dose was not significant ($p = 0.3762$). The main effects were significant for both "species" ($p < 0.05$) and "dose" ($p < 0.05$). This suggests that at least one of the $(N\%)^{-1}$ averages for a single species (across all doses) was statistically different from the other two. However, it was not an objective to check which species had the highest or the lowest Nitrogen absorption. On the other hand, significance on the main effect of doses means that at least one of the $(N\%)^{-1}$ averages, for a single dose (across all species), was significantly different than the others. In this case, all doses above 160 mL absorbed significantly higher amounts of Nitrogen than the control. Nevertheless, all doses above 160 mL were statistically similar among them (Supplementary Material S2). It also implies that the change in Nitrogen absorption, for increasing doses of NMC, was similar for the three species. That is the explanation for the statistically similar slopes for each of the species' regression lines (Figure 12) (Supplementary Material S2).

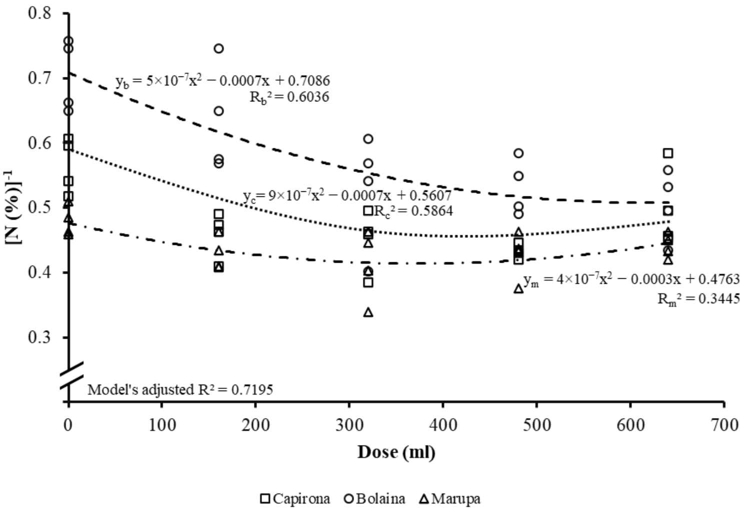

Figure 12. Effect of different native microbial consortium (NMC) doses (mL) in the inverse of N% of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-··△-··) grown in nursery conditions. Using a type I ANOVA (α = 0.05), interaction between tree species and the quadratic component of NMC dose (mL) was found to be non-significant (p = 0.3762). Main effects of species proved to be significant ($p < 0.05$), as well as main effects of quadratic effect of dose ($p < 0.05$). Regression lines and equations represent the respective quadratic trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, coefficient analyses, and Tukey test can be found in Supplementary Material S2. Model's adjusted R^2: 0.7195.

Regarding Phosphorus, after performing a transformation using the natural logarithm of %P, it was found that the interaction between species and the quadratic component of NMC dose was significant (p = 0.0014). This indicates that the responses to the increases in CMD doses varied following a quadratic trend according to the forest species (Figure 13). The simple effects analyses revealed that for the Bolaina blanca and Marupa species, all doses behaved statistically similar to the control. In the case of the Capirona species, P absorption was reduced with NMC doses greater than 160 mL (Supplementary Material S2).

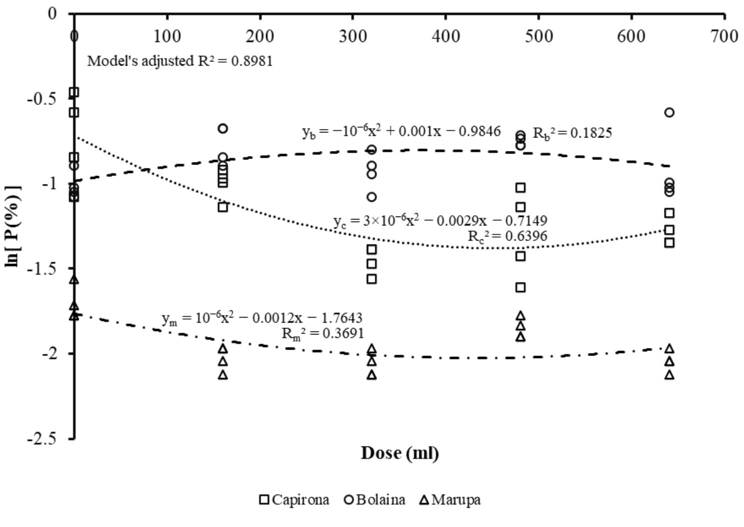

Figure 13. Effect of different native microbial consortium (NMC) doses (mL) in the natural logarithm of the P% of tree species Capirona (··□··), Bolaina blanca (--○--), and Marupa (-··△-··) grown in nursery conditions. Using a type I ANOVA (α = 0.05), interaction between tree species and the

quadratic component of NMC dose (mL) was found to be significant (p = 0.0014). Regression lines and equations represent the respective quadratic trend for each tree species (y_c, Capirona; y_b, Bolaina blanca; y_m, Marupa). Results of the Shapiro–Wilk test, Breusch–Pagan test, coefficient analyses, and simple effect analyses can be found in Supplementary Material S2. Model's adjusted R^2: 0.8981.

3.11. Correlations Between N, P Content, Survival, and Quality Indices

In the case of Bolaina blanca, the robustness index (height/diameter) was found to be directly related to the Phosphorus content for the control and the 480 mL dose of native microbial consortium (NMC) (Table 3). The root height/length ratio did not present any significant correlation with the Nitrogen or Phosphorus nutritional status. The shoot–root index only showed a positive correlation with the N content for the 640 mL dose of NMC. The Dickson quality index showed a negative correlation with the Phosphorus content for the control and for the 480 mL dose of NMC. The survival percentage was only inversely correlated with the Phosphorus content for 480 mL of NMC. In the case of Capirona, the robustness index was inversely related to the Phosphorus content for the 320 mL dose of NMC. The root height/length ratio only presented a significant correlation with the Nitrogen content in the control treatment. The shoot–root index was only found to be positively correlated with the Phosphorus content at the 540 mL dose of NMC. The Dickson quality index was only found to be correlated with the Nitrogen content in the control treatment. In the case of survival (%), it was inversely correlated with the Nitrogen content for the 160 and 320 mL doses of NMC. A direct correlation was also found with the Phosphorus content at 160 mL and an inverse correlation with the 480 mL dose. In the case of Marupa, the robustness index showed a directly proportional correlation with the Nitrogen and Phosphorus content corresponding to the 160 mL dose. In addition, a directly proportional correlation was observed with the Phosphorus content for the 320 mL dose. The height–root length ratio did not present any type of significant correlation. The shoot–root index only presented a significant positive correlation with the Nitrogen content at the 320 mL dose of NMC. The Dickson quality index only showed an inverse correlation between the Phosphorus and Nitrogen content at a dose of 160 mL of NMC. Finally, survival did not show any significant correlation (Table 3).

Table 3. Pearson's correlation coefficients between quality indices of each species and Nitrogen/Phosphorus content according to the tested doses (ml) of native microbial consortium (NMC).

Doses (mL)	Macro	Bolaina Blanca					Capirona					Marupa				
		RI	rH/RL	ITR	DQI	Surv	RI	rH/RL	ITR	DQI	Surv	RI	rH/RL	ITR	DQI	Surv
0	N	0.20	−0.42	−0.20	−0.45	0.11	−0.30	0.93 *	−0.66	0.90 *	0.00	−0.47	0.32	−0.69	0.00	0.12
	P	0.90 *	−0.56	−0.59	−0.98*	0.39	−0.58	−0.21	0.12	0.20	0.87	0.57	−0.72	0.79	−0.46	0.71
160	N	−0.80	0.40	0.40	0.00	−0.61	0.59	0.49	−0.58	0.00	−0.96 *	0.92 *	0.00	−0.60	−0.94 *	−0.82
	P	−0.33	0.73	0.73	−0.31	−0.17	−0.85	−0.73	0.73	0.50	0.93 *	0.91 *	0.00	−0.61	−0.95 *	−0.81
320	N	−0.26	−0.15	0.00	0.55	0.71	−0.25	−0.28	−0.25	−0.47	−0.91 *	−0.50	−0.64	0.90 *	−0.75	−0.42
	P	−0.34	−0.82	−0.52	0.80	0.00	−0.93 *	−0.74	−0.74	−0.14	−0.40	0.90 *	0.84	−0.57	−0.24	−0.64
480	N	0.71	0.75	0.41	−0.36	−0.48	0.00	−0.50	0.27	−0.45	0.48	−0.64	0.00	−0.31	0.42	0.55
	P	0.94 *	0.79	0.25	−0.99 *	−0.91 *	0.31	0.22	−0.71	0.41	−0.89 *	−0.70	−0.25	−0.30	0.00	0.87
640	N	0.23	0.81	0.98 *	0.00	0.35	−0.80	−0.58	0.7	0.43	−0.15	−0.38	−0.17	−0.57	0.26	−0.38
	P	0.64	−0.64	−0.43	−0.67	−0.33	0.29	0.00	−1.00*	0.00	0.20	−0.70	−0.50	−0.42	0.41	0.00

RI—robustness index (height and diameter relation); rH/RL—plant's height and root length ratio; ITR—shoot–root index (shoot and root dry biomass ratio); DQI—Dickson quality index; "Surv"—survival percentage. * Correlation value above |0.89| represents an explanatory power of more than 80%. More details can be found in Supplementary Material S2.

3.12. Correlations Between N, P, and Morphological Variables

In the case of Bolaina blanca, plant height (cm) and aboveground biomass (g) did not show any significant correlation with either Phosphorus or Nitrogen (Table 4). Plant diameter (mm) showed a negative correlation with Phosphorus content for the NMC dose of 480 mL. Root length (cm) showed only a direct correlation with Phosphorus content for the control. Root dry weight (g) had a positive correlation with Phosphorus at a NMC dose of 320 mL. In the case of Capirona, the parameters height (cm), root length (cm), and aboveground dry biomass (g) did not show any statistically significant correlation. Plant diameter (mm) only showed a direct correlation with Phosphorus content for the NMC dose of 160 mL. Root dry biomass (g) was positively correlated with Nitrogen content in the control only. In the case of Marupa, the parameters height (cm), diameter (mm), aboveground dry biomass (g), and root dry biomass (g) did not show any significant correlation with Phosphorus or Nitrogen. Only root length showed inversely proportional correlations with both Nitrogen and Phosphorus (Table 4).

Table 4. Pearson's correlation coefficients between morphological parameters of each species and Nitrogen/Phosphorus content according to the tested doses (mL) of native microbial consortium (NMC).

Doses (mL)	Macro	Bolaina Blanca					Capirona					Marupa				
		H	D	RL	RDB	ABD	H	D	RL	RDB	ABD	H	D	RL	RDB	ABD
0	N	−0.38	−0.28	0.54	−0.36	−0.37	0.55	0.49	−0.42	0.89 *	0.00	0.24	0.00	−0.55	0.00	0.00
	P	−0.46	−0.75	0.94 *	0.00	−0.75	0.42	0.48	0.47	0.13	0.00	−0.20	−0.41	0.00	−0.47	−0.35
160	N	−0.30	0.00	−0.43	−0.13	0.00	0.23	−0.64	−0.51	0.88	0.00	−0.69	−0.83	−0.93 *	−0.70	−0.67
	P	−0.62	−0.49	0.12	−0.46	−0.16	−0.60	0.89 *	0.62	−0.88	0.36	−0.68	−0.83	−0.93 *	−0.72	−0.68
320	N	0.41	0.33	0.49	0.45	0.49	−0.45	−0.34	−0.36	−0.54	−0.52	−0.86	−0.83	−0.63	−0.83	−0.73
	P	0.10	0.16	0.96 *	0.98 *	0.21	−0.71	−0.25	0.59	−0.47	0.11	0.48	0.11	−0.24	0.1	−0.22
480	N	0.19	−0.70	−0.83	0.00	0.16	−0.55	−0.43	0.11	−0.55	−0.33	−0.21	0.23	−0.68	−0.12	0.19
	P	0.19	−0.92 *	−0.85	−0.84	−0.81	0.69	0.34	0.43	0.64	0.25	−0.42	0.00	−0.67	−0.17	−0.20
640	N	0.67	0.18	−0.37	−0.16	0.41	0.12	0.52	0.53	0.00	0.60	0.10	0.31	0.39	0.31	−0.19
	P	−0.63	−0.73	−0.19	−0.58	−0.83	0.00	−0.17	0.00	0.56	−0.24	−0.19	0.15	0.21	0.21	0.12

H—plant height; D—plant diameter; RL—root length; RDB—root dry biomass; ABD—aboveground dry biomass. * Correlation value above |0.89| represents an explanatory power of more than 80%. More details can be found in Supplementary Material S2.

4. Discussion

The objective of this research was to determine the optimal dose of native microbial consortium (NMC) to achieve the best quality indicators in plantlets of three different tropical forest species: Bolaina blanca (*Guazuma crinita* Mart.), Capirona (*Calycophyllum spruceanum* Benth. Hook. f.), and Marupa (*Simarouba amara* Aubl.). We hypothesized that the nursery survival percentage was going to show a quadratic increase with higher doses of native microbial consortium (NMC) as a result of better plant nutrition. Differentiated responses for each species were expected. Moreover, higher Nitrogen, Phosphorus, and Potassium content in leaves was the confirmation indicator. This part of our hypothesis was partially rejected. Regarding nursery survival (%), the results indicate that the response of this variable was indeed different according to the tree species (Figure 1); however, with increasing doses of NMC, species Marupa and Capirona showed a tendency to reduce their survival percentage, while Bolaina blanca remained statistically similar to the control. Marupa, whose control treatment showed a survival of 60%, was the most affected species by the increasing doses of NMC (Figure 1). This might be a negative consequence of the aggressive "bare root" propagation method applied for this species (reasons behind this are explained in the methodology). Extracting seedlings from the ground and exposing the root system causes root tissue damage. This is an entrance gate for opportunistic fungi and bacteria that cause root rot symptoms. The NMC analysis indicates the presence of molds and yeasts (1.0×10^3 CFU ml^{-1}) (Table 1). Additionally, Nottingham et al. [22] reported the presence of Sordariomycetes as a predominant fungi species in the Peruvian Amazon soils. A common taxonomic genre which belongs to this class is *Trichoderma* spp., which is known to be a beneficial microorganism. Nonetheless, *Fusarium spp*. also belongs to this class. Unfortunately, it was not possible to identify the causal agent of plantlet rot, but it is highly probable that, if not Fusarium spp., a fungus present in the growing medium [42] with similar behavior could have caused the decreasing survival percentage. It is recommended that future research can identify microorganism genres present in the NMC produced in this geographical area. Apart from that, it should be said that possible increases in growing media salinity could have affected this species' survival, which do not tolerate very well high electrical conductivities [43]. A high ion concentration can generate an unfavorable environment for the roots, hindering their growth and development [44]. As explained in the methodology, each NMC dose treatment was split into eight applications. For example, in the 640 mL treatment, every plant received 80 mL of pure NMC. According to the analysis (Table 1), while the stock solution of NMC had an electrical conductivity of 7.71 dS m^{-1}. This is closely equivalent to the 50 mM tested by Das et al. [43], which showed salinity stress symptoms. Both the inoculum and the high osmotic potential conditions could have worsened the conditions for already damaged roots of Marupa. For this reason, in follow-up experiments, direct seed propagation is encouraged instead of bare root transplant from already germinated seeds. In the case of Capirona, despite its tendency to decrease its survival (%) with increasing doses of NMC (Figure 1), only the 640 mL dose showed significantly lower survival compared to the control (Supplementary Material S2). This can be associated with higher salt concentrations, which might have reached levels above the tolerance of this species. Unfortunately, there is no previous research on this topic, so it is recommended to keep track of changes in electrical conductivity for future experiments in this species. In the case of Bolaina blanca, all doses were statistically similar to the control (Figure 1), suggesting that even the highest dose of NMC does not negatively affect the survival of this species. There were some tendencies to improve survival with doses between 160 mL to 480 mL of NMC. However, they did not differ from the control (Supplementary Material S2). This could be due to an intrinsic tolerance of this species which has not been studied yet or a tolerance induced by the addition of native microorganisms [42]. In addition, the benefits of mycorrhizal symbiosis have been recognized as a factor that increases plant tolerance to abiotic stress [45]. Furthermore, no strong correlations among survival (%) and nutrient content in leaves were found (Table 3); therefore, that part of our hypothesis was also rejected. This implies that, no matter how high the concentration of N or P present in tissues, this will probably not improve the survival of plants. That is why the two factors that seem to influence survival are salinity tolerance and the level of root damage.

Regarding plantlet quality indicators, we also hypothesized that the robustness index linearly decreases with higher doses of NMC as a result of larger shoot diameters (mm) in relation to height (cm), not exceeding the value of 6. This part of the hypothesis is partially rejected too. It was found that the regression lines showed a positive significant linear trend, with similar effect for the three species (Figure 4) (Supplementary Material S2). This can be explained because the increasing doses of NMC did not show significant increases in the shoot diameters (mm) for any of the species (Figure 3), while they did show increases in plant height (cm) (Figure 2). This index is the quotient between the plant height (cm) and the shoot diameter (mm). In that sense, if the plant height grows more than the shoot diameter, the robustness index will grow bigger. That is what happened in this case, and it is an unwanted result because it means that the plant is growing tall but very unstable, as the shoot diameter is not growing accordingly. Under hostile environmental conditions in plantations, it is preferable for this index to have the lowest possible value [25]. A high value (above 6) suggests that the plant shoot is excessively elongated, making it susceptible to breaking by factors such as wind [46]. Notwithstanding the unfavorable trend for this variable, it was found that mean values for Capirona and Marupa remained below 6 (Figure 4). Only the Bolaina blanca averages showed values above 6, including the control. This indicates that this species' elongation is not a consequence of the increasing doses of NMC. The question now is, why did the plants grow taller, but not thicker? By looking at the correlations between plant height (cm), shoot diameter (mm), and nutritional values of N and P absorption (Table 4), it is possible to see that only for Bolaina blanca and Capirona, evidence was found of an inverse correlation between P% and shoot diameter (mm). There was only one significant coefficient out of five each, which implies a lack of consistency. When looking at the P% leaf tissue (Figure 13), Bolaina blanca showed no significant increases in P% with increasing doses of NMC, while Capirona showed reductions in P% in leaf tissue with increasing doses. This analysis concluded that variations of P% in leaf tissue did not explain changes in shoot diameter (mm). Interestingly, N% in leaf tissue did not show correlations with shoot diameter (mm) nor plant height (cm). This indicates that better nutrient availability as a consequence of the NMC incorporation had no influence in this index. Also, we can infer that either the number of replicates were too few to find a significant correlation, or there are other factors that might be more influential. Hartmann et al. [13] suggests that stem elongation can be a consequence of insufficient light. In this study, 85% shade was used to protect the plantlets. Perhaps it would be necessary to remove the shade for future experiments.

It was also hypothesized that the height: root length ratio linearly decreases with higher doses of NMC as a result of larger root length (cm) in relation to plant height (cm), not exceeding the value of 2. This part of the hypothesis is also rejected. It was found that the regression lines showed a positive significant linear trend, with similar effect for the three species (Figure 6) (Supplementary Material S2). This can be explained because the increasing doses of NMC did not show significant increases in the root length (cm) for any of the species (Figure 5), while they did show increases in plant height (cm) (Figure 2). This index is the quotient between the plant height (cm) and the root length (cm). In that sense, if the plant height grows more than the root length, the plant height:root length ratio will grow bigger. That is what happened in this case, and it is an unwanted result because it means that the plant is growing tall but the root is not growing accordingly, which can cause an imbalanced supply of water and nutrients to the stems. Under hostile environmental conditions in plantations, it is preferable for this index to be kept below a value of 2 [25]. Higher values might indicate that the stem is growing too much in comparison to the roots. In spite of the unfavorable trend for this variable, it was found that mean values for three species remained below the value of 2, even at the highest dose (640 mL NMC). The question now is, why did the plants grow taller, but the root did not grow longer? By looking at the correlations between plant height (cm), root length (cm), and nutritional values of N and P absorption (Table 4), it is possible to see that Bolaina blanca showed two out of five significant positive correlations with Phosphorus. Marupa showed one significant negative correlation with Phosphorus and one with Nitrogen. Capirona showed no evidence. When looking at the P% leaf tissue (Figure 13), Bolaina blanca and Marupa showed no significant increases of P% with increasing doses of NMC. This analysis concluded that variations in P% and N% in leaf tissue did not explain changes in root length (cm). This indicates that better nutrient availability as a consequence of the NMC incorporation has no influence in this index. Also, we can infer that either the number of replicates were too few to find a significant correlation or there are other factors that might be more influential. Hartmann et al. [13] suggests that problems with root growth can be a consequence of unsuitable growing media or inappropriate choice of containers. In this study, a mixture of alluvial material and sand in a 1:1 ratio was used. Probably a less denser substrate, like coconut fiber or compost, can help the roots to grow better. Also, a container of diameter 12.7 cm and height 20 cm was used per plant. Maybe a container with more than 30 cm height can be suitable for favoring root growth.

Apart from that, it was also hypothesized that the shoot:root index linearly decreases with higher doses of NMC as a result of larger root biomass (g) in relation to the aboveground biomass (g), not exceeding the value of 2. This part of the hypothesis was partially rejected. It was found that the regression lines showed a negative significant quadratic trend, with different effects for each of the three species (Figure 9) (Supplementary Material S2). However, the simple effect analysis proved that each NMC dose was similar to the control for each species. This can be explained because the increasing doses of NMC showed no significant changes in the root dry biomass (g) (Figure 8) nor the aboveground dry biomass (g) (Figure 7) for each species. This index is the quotient between the aboveground dry biomass (g) and the root dry biomass (g). In that sense, if the aboveground biomass grows more than the root biomass, the plant shoot–root index will grow bigger. Nevertheless, that was not case here, because both parts of the plant reacted similarly with the increasing doses of NMC. Under hostile environmental conditions in plantations, it is preferable for this index to be kept below a value of 2 [47]. Maintaining a balanced ratio between aerial and root biomass helps plants to be more resilient to drought stress or nutrient shortage [48]. It was found that mean values for Marupa and Bolaina blanca species remained below the value of 2, even at the highest dose (640 mL NMC). However, Capirona average values remained above 2, even without any application of NMC (Figure 9). This indicates that this species' unbalanced growth is not a consequence of the increasing doses of NMC. We were expecting more root biomass in comparison with aerial biomass. What happened then? By looking at the correlations between root dry biomass (g) and nutritional values of N and P absorption (Table 4), it is possible to see that Bolaina blanca showed one out of five significant positive correlations with Phosphorus. Capirona showed one significant negative correlation with Phosphorus, and Marupa showed no significant correlation at all. This analysis concluded that variations in P% and N% in leaf tissue did not explain changes in root dry biomass (g). This indicates that better nutrient availability as a consequence of the NMC incorporation has no influence in this index. Also, we can infer that either the number of replicates were too few to find a significant correlation, or there are other factors that might be more influential. As explained in the previous paragraph, Hartmann et al. [13] suggests that problems with root growth can be a consequence of unsuitable growing media or inappropriate choice of containers.

Moreover, we also hypothesized that the Dickson quality index (DQI) linearly increases with higher doses of NMC as a result of larger shoot diameters (mm) and root biomass (g) above the value of 0.5. The Dickson Quality Index (DQI) was designed to predict seedling survival in the field by integrating biomass, height, and diameter data [27]. The DQI is a useful comprehensive index to predict seedling quality. However, it may not be sufficiently sensitive to smaller increases in nutrients if changes in height, diameter, and biomass are not pronounced [49]. This part of the hypothesis was rejected. It was found that the regression lines showed a positive significant linear trend, with a lack of effect for the three species (Figure 10) (Supplementary Material S2). This can be explained because the increasing doses of NMC did not show significant increases in the shoot diameters (mm) (Figure 3) nor the root biomass (g) (Figure 8) for any of the species. This index is the quotient between the total dry biomass (g) and an arbitrary sum of the two previously mentioned indexes (Table 2). In that sense, if the sum of these two indices grows less than the total dry biomass, the DQI will grow bigger. A high DQI value was expected to indicate better plant performance, but it must be taken into account that this index was designed to correlate morphological parameters with empirical quality data in two conifer species,: white spruce (*Picea glauca*) and white pine (*Pinus glauca* Strobus.). Consequently, the DQI has a more robust application in conifers due to the historical correlation with quality data in these species [50]. Although our data did not show an increase in DQI with increasing doses of NMC (Figure 10), no decreases were observed either, which suggests that the seedlings of the three species studied would maintain a similar quality to that of their controls. Significant increases in Nitrogen contents were observed when applying doses of NMC of 160 mL or higher (Figure 12). In the case of Phosphorus, the Bolaina and Marupa species showed concentrations similar to the controls, while Capirona experienced a reduction in Phosphorus levels at doses higher than 160 mL (Figure 13). This phenomenon goes along with the behavior observed in some species where Phosphorus levels do not always increase with high doses of fertilizer, since absorption may be limited by competition between nutrients or by saturation of Phosphorus absorption [51]. As for Potassium, its levels remained constant during the trial; therefore, its role in nutrition during this study period was ruled out (Figure 11). The data suggest that plant tissues had higher Nitrogen concentrations when NMC doses of 160 mL or more were applied. This increase in Nitrogen content may be an indicator of better field performance, since high levels of N are associated with greater vigor and capacity of plants to cope with stress under adverse conditions [49]. However, this relationship depends on factors such as the species and the establishment environment, and long-term monitoring would be necessary to verify whether these Nitrogen levels are correlated with a greater probability of survival under adverse conditions. Regarding correlations, the DQI and Phosphorus content showed a negative correlation for the control and 480 mL NMC doses (Table 3), which suggests that an increase in Phosphorus could negatively affect DQI performance. For Capirona, a significant and positive correlation was only observed with Nitrogen in the control (Table 3), indicating that higher concentrations of N could be beneficial. Nonetheless, this relationship may be mediated by factors such as storage capacity and Nitrogen use efficiency [52]. In the case of Marupa, both Nitrogen and Phosphorus showed a negative correlation with the DQI at the 160 mL dose, suggesting that increases in N and P could harm this quality index. It should be noted that the correlations in Tables 3 and 4 were calculated from only four values, which means that the results are referential and would require a greater number of repetitions per dose to increase the reliability of the data. It is essential to increase the sample size in seedling quality studies to reduce the impact of random fluctuations and thus improve the reliability of the results [50]. However, one limitation is the high cost associated with plant tissue analysis, which makes it difficult to carry out studies with a larger number of samples. Also, it is important to mention that with the measured variables (Table 2), it is not possible to differentiate the nutritional enhancements of N and P as a consequence of a specific microorganism's activity, or just the immediate nutrient availability of the NMC solution. In order to tackle this issue, further research should incorporate an analysis that identifies specific fungi and bacterial genres in the NMC stock solution and also in the rhizosphere at the end of the trial.

In order to adequately answer the question of the optimal NMC dose for each species, it is essential to identify the most useful indicator to anticipate plant survival under field conditions. Each of the four indices evaluated has specific advantages and disadvantages. The robustness index provides information on the ratio between shoot height and its diameter, which is crucial for plant stability [53]. However, its limitation lies in the fact that it does not include data on root development [50]. On the other hand, the shoot–root index and the root height/length ratio do consider the root system [54]. In fact, the shoot–root index could be a better indicator because it is based on both root and aboveground biomass unlike its counterpart, which focuses on growth distances without considering lateral development and biomass supporting the plant [55]. On the other hand, the Dickson quality index has the advantage of predicting plant performance in the field; however, its effectiveness has mainly been proven in conifers [56], and a long-term study would be necessary to evaluate its validity in the three species studied [57].

If we take the shoot–root ratio as a reference, we observe that high doses of NMC do not harm it, allowing up to 640 mL to be applied in all treatments without affecting this indicator (Figure 9). Having said that, we must consider the effects on nutritional parameters. We know that the potassium content remained stable for all species even with the highest dose (Figure 11), so it can be discarded from the analysis. As for Nitrogen, we know that its concentration increased significantly for all species with doses of 160 mL of NMC. On the other hand, Phosphorus did not show increases with increasing doses of NMC in Bolaina and Marupa, while in Capirona, it decreased with doses higher than 160 mL. These data suggest that the most suitable dose for the three species would be 160 mL per seedling, since this volume avoids unnecessary applications in Bolaina and Marupa and prevents a reduction in Phosphorus absorption in Capirona. Finally, when analyzing the survival rates (Figure 1), we observed that Bolaina was not affected by higher doses of NMC while Capirona was; thus, a dose of 160 mL per seedling is the safest option for the latter if the yield is to be maximized. In the case of Marupa, the incorporation of NMC reduced its survival rate, possibly due to the stress of bare-root transplantation. Although the surviving plants showed vigor, it should be noted that the mortality rate will be relatively high.

5. Conclusions

In this study, the optimal dose of native microbial consortium (NMC) for the three forest species evaluated in their nursery phase was determined to be 160 mL per seedling, corresponding to the second incremental level applied. It did not negatively affect the structural robustness or reduce the survival rate of the seedlings. The shoot–root index stood out as the preferred indicator to determine this dose due to its ability to reflect the balance between the root system and the aerial part. Except for the Capirona species, whose values were close to the optimal limit, the index remained within the appropriate ranges for the other species. Regarding structural robustness, it was observed that Bolaina blanca species presented indices above the optimal range, suggesting a possible structural fragility, while Capirona showed a value close to the optimal limit. The ratio between plant height and root length remained within the ideal ranges in all species with the applied doses of NMC. On the other hand, the Dickson quality index did not show a linear increase with higher doses of NMC, remaining statistically equal to the controls in the three species. The potassium content in the tissues did not present significant variations with the increase in the NMC dose, while the Nitrogen content increased significantly at the 160 mL dose compared to the control, without higher doses resulting in higher concentrations. Finally, it was observed that the Phosphorus content did not change in Bolaina blanca and Marupa species, while in Capirona, the absorption of Phosphorus decreased with doses greater than 160 mL of NMC. These results suggest that the incremental dose of 160 mL of NMC per seedling is the most appropriate to promote balanced and healthy growth in the studied species, without compromising their nutritional or structural development.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/f16020309/s1, Supplementary Material S1: R scripts; Supplementary Material S2: Results of statistical analyses; Supplementary Material S3: Data matrix; Supplementary Material S4: Prompts asked to ChatGPT during the coding process.

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Data Availability Statement: All data generated during the study can be found in the Supplementary Materials.

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