

Article

Seedling Production of *Retrophyllum rospigliosii* in Nurseries and Potential Reforestation Areas Using Modeling Techniques

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Abstract: The success of reforestation and restoration projects depends on several factors, with proper seedling management and the selection of an appropriate planting area being crucial. In Peru, the populations of *Retrophyllum rospigliosii* (Pilg.) C.N.Page (Ulcumano) have been decreasing due to intensive logging of one of the most valuable woods in South America's tropical forests. There are few studies that unify the production of plants through seeds in nurseries and the identification of suitable areas to place the plants produced. Our study has two components. The first aimed to optimize the plant production process through an experiment that evaluated the effects of three doses of controlled-release fertilizer (CRF) (4.2, 8.4, and 12.6 g/L) and two container sizes (115 and 180 cc) on the morphological quality of seedlings in the nursery. The second component involved identifying potential reforestation areas using ecological niche modeling, based on climatic and edaphic variables. The results indicated that the 4.2 g/L CRF treatment for both container sizes had a significant positive effect on seedling growth. The average germination rate was 85% at 120 days. At six months after seedling transplantation, treatments of 4.2 g/L CRF in 115 cc and 180 cc containers were shown to have the best positive effect on morphological variables of seedlings, with a root collar diameter of 3.76 mm and a height of 13.25 cm. Regarding the potential niche models, an area of 6321.97 km² with ideal conditions for reforestation with *R. rospigliosii* was estimated, with the departments of Huánuco, Pasco, Junín, and Cusco showing the highest potential. Based on this, it is estimated that over three million plants are needed for large-scale reforestation projects. Integrating silvicultural studies with niche models is a valuable tool for supporting reforestation and ecosystem restoration projects.

Keywords: Podocarpaceae; fertilization; nursery; plant production; ecological niche modeling; reforestation



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1. Introduction

The Tropical Andes region stands as the most megadiverse area on the planet, yet concurrently suffers as one of the regions experiencing the greatest habitat loss [1]. The global loss of forested areas is deeply concerning, and taking measures to reverse this trajectory is imperative to safeguard the quality of life for future generations [2]. We find ourselves within the decade of restoration as proclaimed by the United Nations [3]; hence, reforestation and restoration initiatives employing native species will play a pivotal

role in realizing the objectives outlined by these global initiatives [4]. In this context, comprehending the biological facets and refining silvicultural practices for the cultivation of native species is paramount to devising successful reforestation and restoration endeavors.

Retrophyllum rospigliosii (Pilg.) C.N.Page, Podocarpaceae, stands as a widely distributed tree species in the Andes, documented in Bolivia, Peru, Ecuador, Colombia, and Venezuela. Within Peru, it thrives in tropical montane forests situated between 1200 and 3750 m above sea level, characterized by constant humidity and cloud cover [5]. It holds significance both economically and ecologically owing to the quality of its wood, its straight stem, and its facile adaptation to agroforestry systems [6]. Presently, the largest stands of this species have encountered significant reduction, with the total loss in its area of occupancy estimated to be at least 30%. The principal causes of stand reduction in Peru include indiscriminate harvesting and rampant illegal logging, as the wood of Podocarpaceae ranks among the most valuable in the tropical forests of South America. Additionally, issues pertaining to regeneration [6,7] and its low germination rate of less than 50% [8] contribute to its current population decline, warranting its classification as vulnerable by the International Union for Conservation of Nature (IUCN) [7]. Studies have reported a germination rate of 40% to 60% from seed, reaching an average height of 30 cm after 12 months in a substrate composed of three parts organic soil and one part sand, before being transplanted to the final field [9].

In reforestation programs, assessing seedling quality is a crucial element [10,11]. Consequently, conducting nursery seedling quality tests furnishes foresters with insights into potential seedling performance in the field and subsequent success after planting [12]. Accordingly, forest nurseries must yield seedlings with plant attributes conducive to successful establishment in the field [13]. Morphological parameters represent the most frequently utilized attributes for gauging seedling quality and post-planting success [11]. Thus, the use of morphological attributes can anticipate survival and growth in the field, which is variable depending on the species, particularly in arid conditions [10,14].

Similarly, the quality of nursery seedlings is affected by the type and size of containers [15]. The containers most commonly employed in seedling production include plastic bags, polyethylene bags, and polypropylene plastic tubes or containers of varying sizes and volumes [16]. An advantage of plastic tubes is their reduced substrate consumption, lower transportation costs, and reusability [17–19].

Conversely, fertilization is imperative for enhancing plant growth. The application of controlled-release fertilizers (CRF) can aid in the continuous supply of nutrients in the requisite form and quantity, aligning with the physiological demands of plants across different growth stages [20]. CRFs facilitate gradual and slow nutrient release, enhancing plant uptake, minimizing nutrient loss to the environment [21,22], augmenting fertilization efficiency [23], and curbing leaching losses [24]. In nurseries, tree seedling producers are increasingly turning to CRFs owing to reduced production costs [25].

Another factor determining the success of planting is identifying areas with optimal environmental conditions that contribute to the development of planted trees. In this regard, understanding the ecological niche of mountain forests allows for prioritizing areas for reforestation and restoration of Andean ecosystems, promoting sustainable management of fragile ecosystem resources [26]. Therefore, ecological niche modeling (ENM) is an important tool used to estimate the extent of species distribution and occupancy in the future, present, or past, through the use of presence data and environmental layers [27–31]. Maps of reforestation and restoration suitability using species distribution models have been increasingly applied as conservation planning tools for predicting impacts of climate change and land use changes on biodiversity [32,33]. These models can provide theoretical insights for systematic conservation planning, providing key information for the decision-making process [34]. On the other hand, these models must be adjusted to specific contexts to obtain more accurate predictions on the potential distribution of the species [27,34], due to the great influence of the environmental conditions of each area.

Some authors conducted research on *R. rospigliosii*; for example, studies have been conducted on the effect of chemical fertilization on a plantation in Colombia for 20 years [35], the effect of water deficit on the growth and physiological response of plants [36], and the growth and yield of pure plantations [37]. In Peru, the efficiency of vegetative propagation in a subirrigation chamber has been studied [6], and the genetic diversity and population structure of Ulcumano was examined [38]. However, studies on the propagation of this species from seeds are still lacking. Furthermore, the silvicultural techniques needed to improve its production in nurseries, as well as the appropriate selection of planting sites using modeling techniques, are unknown. This study is necessary because it addresses the conservation of *R. rospigliosii*, a vulnerable forest species due to intensive exploitation. The innovation of this study lies in its comprehensive approach, because it combines silvicultural experiments from seeds to optimize seedling production with ecological niche models to identify suitable areas for reforestation. This approach not only improves the quality of seedlings through the use of controlled-release fertilizers, but also offers precise mapping of areas with optimal climatic and soil conditions, facilitating the planning of large-scale reforestation projects and promoting ecosystem restoration.

Our study objectives were as follows: (1) to evaluate a nursery-level plant production method to reforest populations of *Retrophyllum rospigliosii* in potential areas of the Peruvian Andes, (2) to assess the effect of different doses of controlled-release fertilizer and two container volumes on the morphological quality of *R. rospigliosii* under nursery conditions, and (3) to identify potential reforestation areas with *R. rospigliosii* through ecological niche modeling.

2. Materials and Methods

2.1. First Component: Nursery Experiment

The experiment was conducted between September 2020 and June 2021 at the nursery of San José, situated in the district of Chontabamba, province of Oxapampa, Pasco region. The nursery is positioned at geographical coordinates 10°35'42" S and 75°24'91" W, at an elevation of 1800 meters above sea level, within an area spanning 91 square meters (Figure 1B). It is situated in the very humid forest–low tropical montane life zone. The mean annual temperature stands at 17.9 °C, with an average annual precipitation of 1603 mm and a mean annual relative humidity of 85.9% [39].

2.1.1. Study Material, Germination, and Transplantation

In September 2020, fruits were gathered from four *R. rospigliosii* trees located in the forest of Chontabamba, Oxapampa. Upon arrival at the nursery, the fruits underwent washing, pulping, and subsequent extraction of the seeds. These seeds were then subjected to disinfection in an aqueous solution of sodium hypochlorite and water (2:8), wherein they were immersed for a duration of 10 min. Following disinfection, the seeds were rinsed with distilled water and subsequently air-dried in the shade for a period of 15 days [40].

Subsequent to the drying process, 1000 seeds were sown (September 2020) in a seedbed containing the commercial substrate MecPlant Forestal 3. The commercial substrate was composed of composted pine bark and vermiculite expanded in a ratio of 6:1, and base fertilizer (N-P₂O₅-K₂O), with a pH of 4.8, humidity of 60%, and electrical conductivity of 1.5 mS/cm (according to the information provided by the manufacturer; Parana, Brazil). The seedbed was shielded with a black raschel mesh providing 50% illumination. Daily irrigation was administered via a pressure sprayer to facilitate germination, with no irrigation applied on days of precipitation. Germination occurred three months post-sowing, and the seedlings were maintained with a basal fertilizer (N-P₂O₅-K₂O) before being transplanted into containers filled with the same base substrate (MecPlant Forestal 3) and the addition of CRF (Basacote Plus 6M) at three escalating doses of 4.2, 8.4, and 12.6 g/L [39], alongside a control treatment (no fertilizer). The chemical composition of the CRF was 16% nitrogen (N), 8% phosphorus (P₂O₅), 12% potassium (K₂O), 2% magnesium (Mg), and 12% sulfur (S), and the micronutrients included 0.02% boron (B), 0.05% copper (Cu), 0.40% iron (Fe),

0.06% manganese (Mn), 0.015% molybdenum (Mo), and 0.02% zinc (Zn) (according to the information provided by the manufacturer; Münster/Westphalia, Germany).

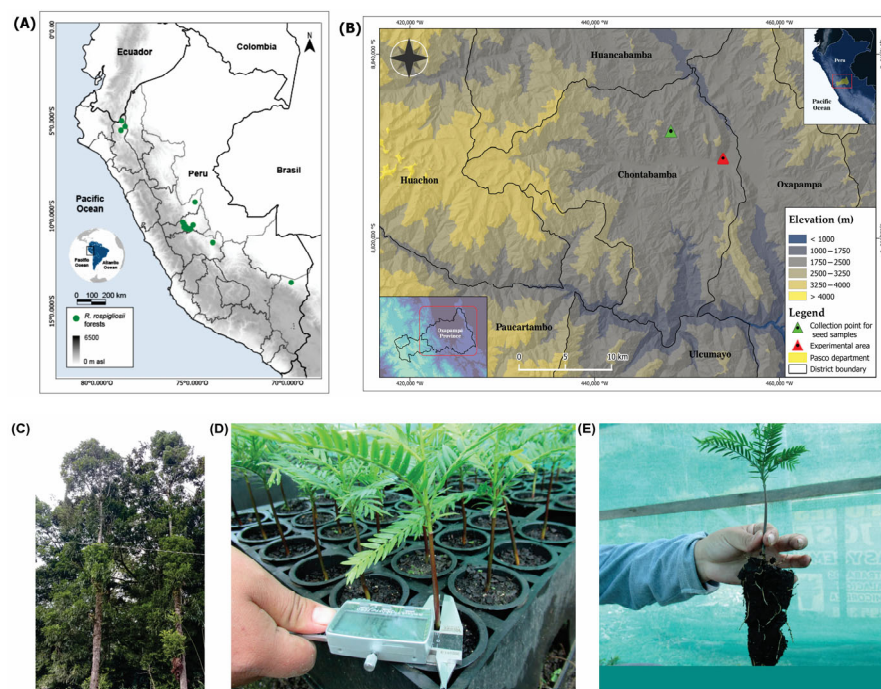


Figure 1. Study area for *Retrophyllum rospigliosii* (Pilg.) C.N.Page in the Peruvian Andes. (A) Records of the species within Peruvian territory. (B) Location of the experiment. (C) Adult trees. (D) Seedlings at six months after transplanting. (E) Root and aerial growth of seedlings in containers.

Polypropylene containers of volumes 115 and 180 cm³ were utilized, arranged in trays comprising 96 and 54 cells, respectively. After the fertilizer was applied to the commercial substrate, the containers were filled according to the treatments indicated in Table 1.

Table 1. Description of the treatments used in the study on morphological attributes of *R. rospigliosii* seedlings.

Treatments	Container Volume (cc)	Dose of CRF (g/L)	Description
T1 (control)	115	0.0	115 cc + 00 g/L de CRF
T2	115	4.2	115 cc + 4.2 g/L de CRF
T3	115	8.4	115 cc + 8.4 g/L de CRF
T4	115	12.6	115 cc + 12.6 g/L de CRF
T5 (control)	180	0.0	180 cc + 00 g/L de CRF
T6	180	4.2	180 cc + 4.2 g/L de CRF
T7	180	8.4	180 cc + 8.4 g/L de CRF
T8	180	12.6	180 cc + 12.6 g/L de CRF

After transplantation, the trays containing the containers were transferred to the experimental area, which was covered with a raschel net providing 65% illumination throughout the duration of the experiment. Consistent irrigation of the seedlings was administered daily using a pressure sprayer, with the schedule set for late afternoon (17:00 h).

2.1.2. Experimental Design

A total of 192 seedlings (24 × 8 treatments), each approximately 6–8 cm in height, were carefully chosen and grouped into sets of 24 seedlings, with each group representing

one experimental unit. The experimental design employed was completely randomized, employing a 2×4 factorial arrangement (container volume \times dose of controlled-release fertilizer, including a control), resulting in eight treatment combinations. Each treatment was studied with three replications per treatment (Table 1), and the selected seedlings were randomly allocated to each experimental unit.

2.1.3. Morphometric Evaluation

Six months post-transplantation, seedling height (H) was assessed using a graduated ruler [15], while root collar diameter (D) was measured using the VOGEL series digital vernier [41] with a range of 0–150 mm/6 inches, a resolution of 0.01 mm/0.0005, and an accuracy of ± 0.03 mm. The seedlings were removed from the containers, and washed with running water to remove all substrate residues [42]. Next, with the help of scissors, the seedlings were separated into roots and shoots to obtain the dry mass [43,44]. Roots and stems were dried in a drying oven at 65 °C for 48 h until reaching a constant weight [19,42,45]. Following this, each plant was weighed using an analytical balance to ascertain the total dry biomass (TDB), root dry biomass (RDB), and aerial dry biomass (ADB) for all samples examined [19].

The seedling growth and biomass data were used to calculate the Dickson quality index (DQI) [46], which provides a comprehensive evaluation of seedling quality [47,48]. This index was determined using Equation (1):

$$DQI = \frac{TDB}{\left(\frac{H}{D}\right) + \left(\frac{ADB}{RDB}\right)} \quad (1)$$

where TDB is total seedling dry biomass (g), H is seedling height (cm), D is root collar diameter (mm), ADB is aerial dry biomass (g), and RDB is root dry biomass (g).

Furthermore, the robustness index (RI) was calculated using the height (H)-to-neck diameter (D) ratio and the aerial dry biomass-to-root dry biomass ratio (ADB/RDB) [49].

2.1.4. Data Analysis

The data were assessed for conformity with the assumptions of normality and homogeneity of variances through the Shapiro–Wilk and Bartlett’s tests, respectively. In cases where these assumptions were not satisfied, indicating non-normality or heterogeneity of variances, the Kruskal–Wallis nonparametric statistical test was employed. Subsequently, if significant differences were observed between treatment pairs based on CRF dose and container volume, the Bonferroni test was conducted at a significance level of 5%. Data analysis was performed using SPSS v 25 statistical software.

2.2. Second Component: Ecological Niche Modeling (ENM)

2.2.1. Data Sampling for ENM

Geographical distribution data for *R. rospigliosii* were obtained from online herbarium information from the Tropicos database [50], the Global Biodiversity Information Facility database [51], and research works [52]. A total of 59 records were obtained in Peru; subsequently, incorrect georeferenced points and herbarium accessions lacking location data were checked and excluded, and one record was selected per square kilometer. Finally, 15 records were obtained (13 from GBIF and Tropicos, and 2 from the scientific literature) (Figure 1A).

2.2.2. Evaluation of ENM and Identification of Potential Reforestation Areas

The 15 identified records of *R. rospigliosii* and the 19 bioclimatic variables of temperature and precipitation obtained from the CHELSA database [53] at an approximate resolution of 1.0 km² were used. Similarly, 11 soil variables, including physical and chemical properties of the soil, were obtained from the SOILGRIDS database [54].

To ensure that the selected models met the necessary statistical and ecological requirements, decisions were made based on various factors. These included the variance inflation factor (VIF), sampling bias, and Pearson correlation (r) among the bioclimatic variables, as well as identifying variables that were not highly correlated with each other. Statistical analysis was conducted using R software version 4.1.3 in R Studio [55], as well as packages *sdm* [56], *fuzzySim* [57], and *virtualspecies* [58].

To estimate the probability distribution of occurrences, we used MaxEnt software version 3.4.4 [59] employing the KUENM package [60]. The potential distribution of the optimal model was determined by conducting 10,000 Bootstrap replicas each with 500 iterations.

The potential distribution derived from the top model was based on average performance metrics such as Area Under the Curve (AUC), partial Receiver Operating Characteristic (ROC), omission rate, Akaike Information Criterion (AIC as optimal complexity parameter), and Bayesian Information Criterion (BIC). These indicators were also subjected to cross-validation to ensure reliability [61]. Selection of scenarios and candidate models was based on the “best-evaluated” variable set. Finally, 10 iterations were performed with the predictor variables of the selected model, and the final map was generated from the average of the iterations.

To convert the resulting ecological niche map into binary data, a logistic threshold corresponding to the presence of 10% of the data was used, guided by the lowest distribution probability [62]. These maps were averaged to create spatial information reflecting present probabilities.

Potential distribution areas were calculated in km^2 , and to identify potential reforestation areas, we evaluated the generated model including proximity to populated centers (in meters), access routes to mining concessions, and distance from conservation areas (in kilometers) in order to identify areas with the greatest opportunities for reforestation and conservation. The final visualizations were created in QGIS version 3.18.3 [63].

R. rospigliosii is distributed from Venezuela to Bolivia, encompassing the montane Andean biogeographic region, with elevations ranging from 1500 to 3000 m above sea level. The study was limited to the Peruvian Yungas, delimited from the departments of Cajamarca, Huánuco, Pasco, and Junín, to the department of Puno (Figure 1A).

3. Results

3.1. Nursery Experiment

3.1.1. Seed Germination

Seed germination of *R. rospigliosii* was epigeal, occurring between 83 and 120 days after sowing in commercial substrate composed of composted pine bark and expanded vermiculite in a 6:1 ratio. At 120 days, a germination rate of 85% was achieved.

3.1.2. Morphological Characteristics of Seedlings After the Application of CRF Treatments Using Two Container Volumes

The interaction between the two factors yielded significant differences ($p < 0.05$) for morphological variables, underscoring their interdependence and the significant combined effects on root collar diameter, plant height, and dry biomass (Table 2 and Figure 2).

The combination of different doses of CRF and container volume significantly influenced the median root collar diameter ($p < 0.05$) of *R. rospigliosii*. The Bonferroni-corrected test indicated that treatments T2 and T6 reached the means of the highest diameter range compared to the other treatments (Figure 2A). The results showed a significant influence of the combination of the factors controlled-release fertilizer (CRF) dose and container volume on the growth in root collar diameter of *R. rospigliosii*.

Table 2. Results of the Kruskal–Wallis test for the effect of two container volumes (A) and three controlled-release fertilizer doses (B) plus control treatment on growth and morphological attributes related to plant quality in *R. rospigliosii*.

Variables	Factors	H-Value	p-Value
Diameter (mm)	A	2.33	0.126 ns
	B	80.34	0.000 *
	Interaction A × B	84.13	0.000 *
Height (cm)	A	1.65	0.198 ns
	B	83.15	0.000 *
	Interaction A × B	85.17	0.000 *
Aerial dry biomass (g)	A	0.30	0.581 ns
	B	118.15	0.000 *
	Interaction A × B	121.44	0.000 *
Root dry biomass (g)	A	0.11	0.735 ns
	B	139.24	0.000 *
	Interaction A × B	139.95	0.000 *
Total dry biomass (g)	A	0.28	0.592 ns
	B	132.89	0.000 *
	Interaction A × B	134.98	0.000 *
Robustness index (RI)	A	0.24	0.624 ns
	B	29.00	0.000 *
	Interaction A × B	31.15	0.000 *
Dickson quality index (DQI)	A	0.16	0.687 ns
	B	126.78	0.000 *
	Interaction A × B	127.40	0.000 *
ADB/RDB	A	0.00	0.971 ns
	B	96.32	0.000 *
	Interaction A × B	98.59	0.000 *

*—there exists a significant statistical difference, ns—there exists no significant statistical difference.

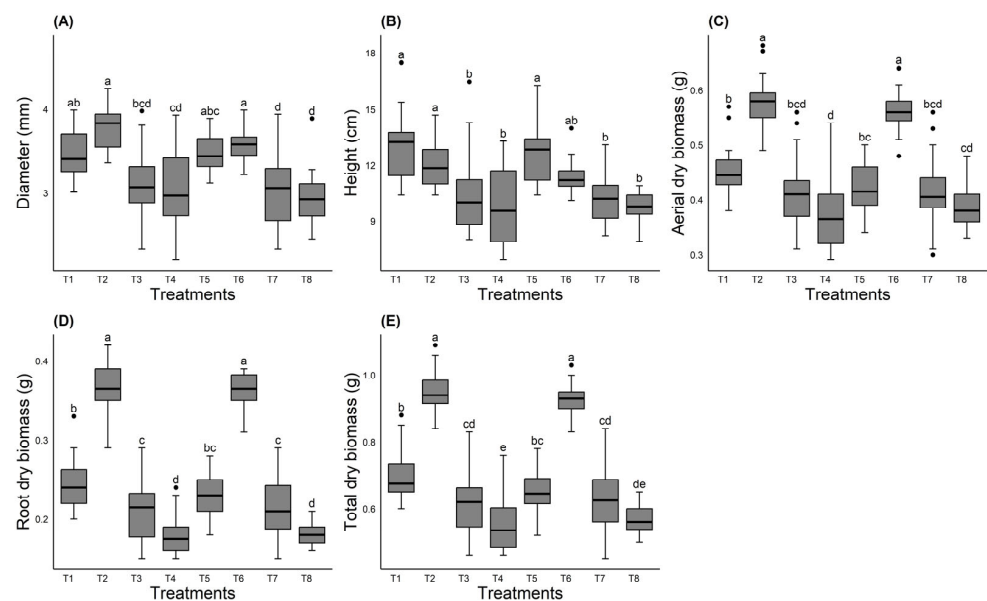


Figure 2. Growth and development of *R. rospigliosii*: root collar diameter (A); seedling height (B); aerial dry biomass (C); root dry biomass (D); and total dry biomass (E). Different letters indicate significant differences ($p < 0.05$).

Similarly, the analysis revealed that seedling height growth was significantly influenced by the combination of CRF doses and container volume ($p < 0.05$), thereby yielding

significant differences in median heights across treatment types. The box plot (Figure 2B) illustrated that the highest median heights of the seedlings were achieved by the unfertilized treatments T1 and T5, and the fertilized treatment T2. Consequently, the means and medians of plant height in treatments T1, T5, and T2 were statistically equivalent and exceeded the rest of the treatments, with height means of 12.92, 12.52, and 12.02 cm, respectively. The growth in height of the seedlings showed a different behavior than the diameter variable, with the best response found for the treatments without fertilization T1 and T5, followed by the treatment with fertilizer T2.

Additionally, the values of dry aerial biomass, root biomass, and total biomass exhibited significant differences influenced by the combination of CRF doses and container volume ($p < 0.05$). Figure 2C–E depict the outcomes of dry aerial, root, and total biomass. In summary, the combinations of treatments T2 and T6 yielded higher mean and median values for aerial biomass (0.58 and 0.56 g), root biomass (0.37 and 0.36 g), and total biomass (0.95 and 0.93 g), respectively. Doses of 4.2 g/L of CRF positively influenced the aerial dry biomass, root dry biomass, and total dry biomass of *R. rospigliosii* at 180 days after fertilization in both container volumes. The dry weight of the aerial and root parts of fertilized plants was higher at low doses (1.4 g/plant or 4.2 g/L).

Regardless of the fertilization treatments, the container volume did not significantly influence ($p > 0.05$) the morphological attributes of *R. rospigliosii*.

3.1.3. Seedling Quality Indices After the Application of CRF Treatments Using Two Container Volumes

The combination of different doses of CRF and container volumes significantly influenced the median values of the plant quality index ($p < 0.05$) in *R. rospigliosii*. Notable differences were observed between the median values of the robustness index (RI) among different treatments. The box plot (Figure 3A) illustrates those treatments, T2 and T6, that attained the highest median RI values. These treatments had an RI index mean of 3.19 and 3.18, respectively, which is below 6, indicative of high-quality plants. Furthermore, the remaining treatments had RI values below 6, indicating that all seedlings would be classified as high-quality plants. Robustness is considered a characteristic that influences the early performance of the plantation. If the robustness index is greater than 6 during cultivation under nursery conditions, aerial pruning is recommended.

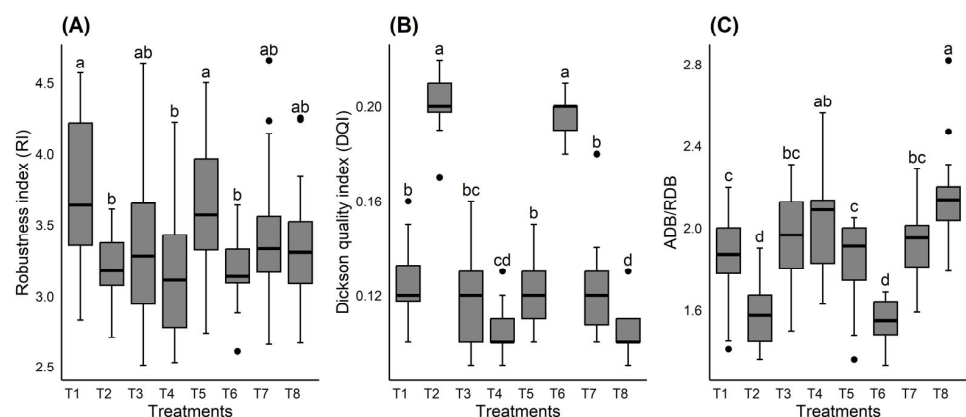


Figure 3. Morphological indices of *R. rospigliosii*: robustness index (A); Dickson quality index (B) and dry shoot biomass/dry root biomass ratio index (ADB/RDB) (C). Different letters indicate significant differences ($p < 0.05$).

The Dickson quality index (DQI) test using the Kruskal–Wallis test demonstrated significant differences ($p < 0.05$) between the medians of DQI according to the type of treatment. The box plot (Figure 3B) depicts that the highest DQI medians were achieved by treatments T2 and T6. These treatments attained higher mean and median DQI values,

and were statistically equivalent between the two treatment pairs (0.20 in both cases), classifying *R. rospigliosii* seedlings as medium quality. When analyzing the seedling quality indexes, it was found that the dose of 4.2 g/L provided the best results for the Dickson quality index (DQI), robustness index (RI), and the aerial dry biomass/root dry biomass ratio (ADB/RDB).

Moreover, the combination of CRF doses and container volume influenced the results of the aerial dry biomass/root dry biomass ratio (ADB/RDB) index, with significant differences observed between the medians of this index by treatment type ($p < 0.05$). Figure 3C illustrates that treatments T2 and T6 achieved the best results for the plant quality index, akin to RI and DQI. Pairwise comparisons indicated that T2 and T6 presented the most favorable indices expressed through the mean of the ADB/RDB index ranges. Based on the results of the means and medians of the ADB/RDB index, it is evident that treatments T2 and T6 exhibit the best plant qualities, with index means below 2 (1.57 and 1.54, respectively), classifying them as high-quality plants, followed by the untreated pairs of treatments T1 and T5, with average indices of 1.86 and 1.84, respectively.

3.2. Ecological Niche Modeling (ENM)

3.2.1. Contribution of Predictor Variables

The quadratic-linear feature class with a regularization multiplier of one was the optimal model found. On average, good predictive performance was obtained for the present model (AUC = 0.99 ± 0.03 , AICc = 547.3, Δ AICc = 0.007, W AICc = 0.001). The relative contribution of each predictor variable to the ENM was evaluated by visualizing the percentage contribution and permutation importance. For the current model, we selected eight environmental variables under the Jackknife test: primarily temperature seasonality (BIO4), mean temperature of the coldest quarter (BIO11), minimum temperature of the coldest months (BIO6), precipitation of the wettest month (BIO13), organic carbon density, elevation, mean temperature of the driest quarter (BIO9), and percentage of sand (Sand). These variables showed a high percentage of contribution to the model fit for the current period (Table 3).

Table 3. Percent contribution and the permutation importance for the present models.

Periods	Variable	Percent Contribution (%)	Permutation Importance (%)
Present	BIO4	23.7	51.9
	BIO11	22.8	21.9
	BIO6	21.2	6.9
	BIO13	11.2	6.1
	Organic carbon density	7.3	4.9
	Elevation	5.6	3.2
	BIO9	4.7	2.8
	Sand	3.9	2.5

3.2.2. Current Potential Distribution

The potential distribution of *R. rospigliosii* in Peru is approximately 6321.97 km², covering nine departments of Peru (Table 4 and Figure 4A). The regions of Pasco, Junín, and Huánuco were found to be suitable areas for *R. rospigliosii* under current climatic and soil conditions (potential distribution greater than 1000 km²). Likewise, potential areas for *R. rospigliosii* were identified in the regions of Cajamarca and Puno, with an area less than 100 km² (Table 4). On the other hand, the modeling study identified new areas of potential distribution of this species, in montane forest ecological formations in the regions of Cusco, Amazonas, Madre de Dios, and San Martín.

Table 4. Potential distribution areas for *R. rospigliosii* in Peru; data highlighted in bold correspond to departments where no known records of the species exist, although its presence is highly probable as they are within the range.

Department	Area (km ²)
Pasco	~1857.56
Junín	~1594.96
Cusco	~1230.45
Huánuco	~1085.45
Amazonas	~360.79
Cajamarca	~82.62
Madre de Dios	~72.63
Puno	~31.57
San Martín	~5.94
Total	~6321.97

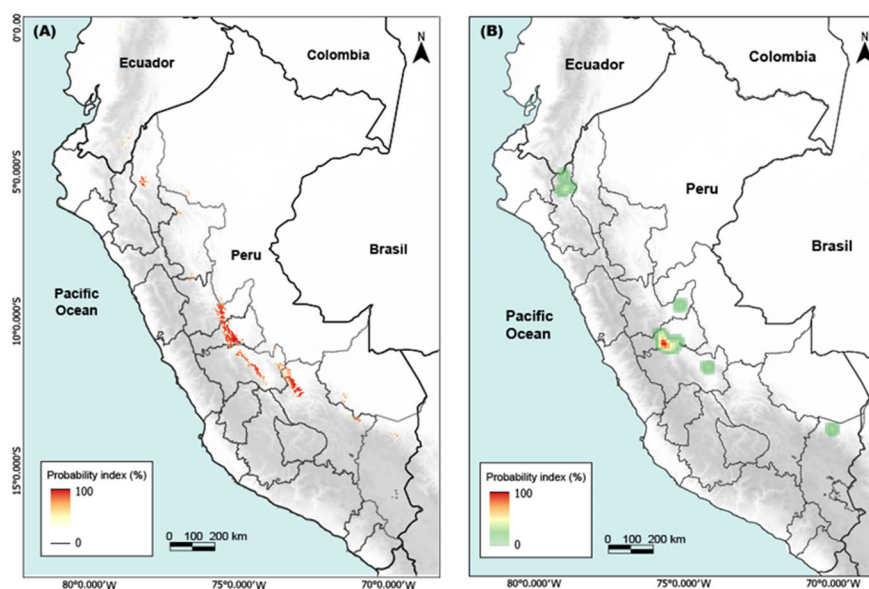


Figure 4. *R. rospigliosii* potential area in Peru: (A) potential current area, and (B) potential reforestation area.

3.2.3. Priority Reforestation Areas According Ecological Niche Modeling (ENM)

Priority areas for reforestation are concentrated mainly in the central region of Peru. The priority reforestation areas were concentrated in the departments of Pasco and Junín. The ENM projection and the kernel density for reforestation areas identified that the areas with a probability of presence less than 80% cover an area of 1045.72 km², and a significant reduction of 83.46% compared to the original distribution of high-potential areas, which covered 6321.97 km². Priority areas with high potential for reforestation are now concentrated in places where there is a high presence of *R. rospigliosii* in Peru. This suggests that the analysis prioritized areas where this native species has better conditions to grow.

In particular, potential reforestation areas could be found in Pasco (726.9 km²), Huánuco (243.15 km²), Cajamarca (49.43 km²), Junín (24.11 km²), and Puno (2.13 km²), for a total potential restoration area in Peru of 1045.72 km² (Figure 4B).

4. Discussion

4.1. Nursery Experiment

4.1.1. Seed Germination in Commercial Substrate

A germination rate of 85% and a shorter germination period (83–120 days) were achieved for *R. rospigliosii* compared to a study conducted in Colombia [64], which reported

a value of 74% using a substrate of soil: sand in a 2:1 ratio, and a period of 94 and 134 days. In Cauca, Colombia, using pre-germination treatments of scarification (removal of the seed coat testa), germination averages between 52% and 70% were reported, and without treatment, 34% germination was achieved in an average period of 124 days in substrates of black soil and sawdust [65]. This indicates that the use of the commercial substrate MecPlant Forestal 3 could improve the germination performance of the species; however, we consider that more studies are needed [43]. Another factor to take into account is the origin of the seeds, since germination rates can vary between populations, pre-germinated treatments, and storage conditions. Ceballos-Freire and López-Ríos [66] reported an average germination of 26% and 28% with seeds stored for three and six months, respectively, at room temperature, indicating that the germinative response of *R. rospigliosii* is highly variable.

4.1.2. Combined Effect of the Application of CRF Treatments and Two Container Volumes on the Morphological Characteristics of Seedlings

The results showed a significant influence of the combination of the factors controlled-release fertilizer (CRF) dose and container volume on the growth in the root collar diameter of *R. rospigliosii*. Similar results have been reported for the species *Pinus tecunumanii* [39], where a low dose of Basacote CRF was used. The results indicate that fertilization to some extent increases growth in plant diameter, and the use of CRF could contribute to the continuous supply of nutrients based on the nutritional requirements of the plants and the type of substrate used [20]. The CRF used in this experiment has a nutrient release time of 5 to 6 months according to the manufacturer's information, making silvicultural management simpler compared to conventional fertilization. Soon after application, most CRFs release a considerable percentage of macronutrients and micronutrients depending on the temperature and humidity of the substrate, and then expand and contract, controlling nutrient release and making nutrients gradually available during the plant growth period [67,68].

On the other hand, several authors point out that the initial diameter of seedlings is the most reliable indicator for survival and growth of plants in the field, and is considered an indirect morphological indicator of plant robustness and root system size [10,69]. Additionally, the initial stem diameter is considered the best morphological attribute for predicting future growth because it correlates positively with seedling weight and root system size [11,13]. Results from various research studies indicate that stem diameter is the most useful morphological measure of seedling quality, and that seedlings with larger stem diameters tend to survive better than those with a smaller stem diameter [12,70].

The growth in the height of the seedlings showed a different behavior than the diameter variable, with the best response found for the treatments without fertilization, T1 and T5, followed by the treatment with fertilizer T2. A decreasing trend in height was observed with higher doses of CRF, suggesting that *R. rospigliosii* seedlings do not respond positively to mineral fertilization. Therefore, high doses of fertilization could be harmful by affecting other soil systems such as mycorrhizae [71]; however, this depends on the nature of the species. For example, positive results in height increase have been observed in *Eucalyptus grandis* [72] with higher fertilization doses, indicating that forest species are very sensitive to the addition of CRF to the substrate [73], and that the CRF doses would be determined by the nutritional requirement of the different species and the type of base substrate used [15]. Therefore, it can be concluded that *R. rospigliosii* seedlings during the initial growth phase require a minimum addition of controlled-release fertilizers, since it is not a species that is very demanding in terms of soil requirements as it develops in soils with acidic pH (5.18), slight salinity (0.38), good organic matter content (7.03%), high availability of phosphorus (29.96 ppm), and medium availability of potassium (204 ppm).

Likewise, it was found that doses of 4.2 g/L of CRF positively influenced the aerial dry biomass, root dry biomass, and total dry biomass of *R. rospigliosii* at 180 days after fertilization in both container volumes. The dry weight of the aerial and root parts of

fertilized plants was higher at low doses (1.4 g/plant or 4.2 g/L). However, these results were different with other species. For example, *Acacia mangium* increases its dry mass as the doses of Osmocote Plus CRF increase [67], while plants fertilized with Basacote CRF registered lower average values in aerial and root dry biomass at all doses, but higher values than the control. Another behavior was registered for *Pinus cembroides*, which increased its aerial, root, and total dry biomass when treated with doses of CRF higher than those used in our study [68]. In this sense, it is important to highlight that beyond a certain dose (4.2 g/L, in our case), there is no observed increase in growth and/or biomass. This behavior demonstrates that plants have a maximum biological growth at an ideal fertilization dose [15].

The higher the total dry biomass value, the better the quality of the seedlings produced in the nursery, and the survival and growth of the plants after transplanting in the field [72]. Furthermore, root growth (dry root biomass) is a determining factor to ensure plantation development and establishment [69,73]. The results obtained in this study indicate that dry biomass values are related to the ideal nutrient dosage, which could vary during different stages of plant growth [21,72], so long-term studies are required.

In general, the combination of container volume and CRF dose influenced the morphological parameters studied, a result similar to that reported by Cabreira et al. [15] in the evaluation of *Dalbergia nigra* seedling growth. However, the use of containers with different volumetric capacities did not determine variations in plant growth and development. Several studies have shown that using larger containers results in seedlings with greater root development and better quality [19,74,75]. In this sense, we suggest that in order to optimize nursery resource use, smaller containers should be used. Nevertheless, we also suggest conducting more experiments with containers of contrasting volumes and evaluating container shape independently of volume (same volume but with different shapes) [76].

The container volume did not influence the growth of the seedlings according to the treatments applied, suggesting that both volumes were adequate for the initial production of *R. rospigliosii* seedlings. This result could be attributed to the duration of the experiment (six months), since both volumes would be sufficient for the root development of the plants, and the container volume would not be a critical factor for this species.

4.1.3. Combined Effect of the Application of CRF Treatments and Two Container Volumes on the Seedling Quality Indices

When analyzing the seedling quality indexes, it was found that the dose of 4.2 g/L provided the best results for the Dickson quality index (DQI), robustness index (RI), and the aerial dry biomass/root dry biomass ratio (ADB/RDB). In general, there have been reported cases where fertilization, combined with the use of different types of containers and other silvicultural treatments, significantly improves DQI, such as in *Austrian pine* (0.34–0.73) [14], *Swietenia humilis* (0.25–0.36) [47], *Taxus chinensis* (0.36–0.52), and *Phoebe chekiangensis* (0.72–0.86) [48].

Robustness is considered a characteristic that influences the early performance of the plantation. If the RI index is greater than 6 during cultivation under nursery conditions, aerial pruning is recommended. This can also be applied when the ADB/RDB ratio has high values, in order to compensate transpiration with water absorption [77]. Lower values are related to better plant quality and indicate that the plant is more robust with a vigorous stem. Conversely, higher values indicate a disproportion between height growth and diameter, such as elongated stems with thin diameters. Several authors found diverse values for different species, such as *Pinus devoniana* (RI = 1.6) [78], *Pinus douglasiana* (RI = 4.2), and *Pinus greggii* (RI = 10.4) [79]. In our study, treatments T2 and T6 achieved the best RI, while the other treatments also had values below 6. There are still no specific parameters for this index. It is recommended that in conifers the values be less than 6, since seedlings with a lower RI are more robust and show better adaptation, which reduces the risk of physical damage during their establishment in the final field [49].

The DQI is the best parameter to indicate plant quality, as it expresses the balance of mass distribution and robustness, avoiding the selection of disproportionate plants and discarding shorter plants with greater vigor [13,77–79]. However, it should be considered that low values of this index do not always indicate better plant quality. For example, the lowest index (0.01) was obtained when the seedling is subjected to a strong hardening level, which reflects an imbalance between the aerial and root parts and/or height and diameter, expressing low potential for both survival and height growth [79]. Another case was reported in *Cedrela odorata*, which reached values of 0.2 at only 5 months of age, without necessarily indicating that the plants are suitable for field transplantation [80]. Regarding this, previous studies report that stem diameter has a close relationship with the quality indexes of the studied seedlings, with larger diameters resulting in the best quality indexes [67]; therefore, only measuring the stem diameter could be suggested as a non-destructive method for estimating seedling quality.

Based on the results of the means and medians of the ADB/RDB index, it is evident that treatments T2 and T6 exhibit the best plant qualities, with index means below 2 (1.57 and 1.54, respectively), classifying them as high-quality plants, followed by the untreated pair of treatments T1 and T5, with average indices of 1.86 and 1.84, respectively. The aerial dry biomass/root dry biomass (ADB/RDB) ratio of *R. rospigliosii* seedlings was less than 2, classifying the plants as high quality, according to the recommendations of Sáenz et al. [79] and Rueda Sánchez et al. [78]. To ensure greater survival in the field, it is essential that seedlings have a low ratio between aerial and root biomass. This balance ensures that the roots are better developed, facilitating the absorption of nutrients and water, and reducing the physiological demands of the plant under conditions of water or nutritional stress.

4.2. Ecological Niche Modeling (ENM)

Potential Areas for Reforestation Identified Through ENM

Mitigating the effects of anthropogenic activity and climate change makes it important to identify priority reforestation areas to safeguard diversity and cohabiting species. In this context, our results show that there are potential areas to establish *R. rospigliosii* in suitable habitats with a higher likelihood of species presence. Through ecological niche modeling, it is possible to propose conservation and restoration strategies for threatened species, as it allows us to identify and locate places with probabilities of species presence that meet climatic and soil limits, thus focusing conservation and restoration efforts for the species [81].

The distribution of this species is influenced by temperature and precipitation patterns and by soil physical properties, supported by good predictive performance ($AUC > 0.99$), reflecting a good model efficiency [82]. The analyses suggest that the species could be present in the five identified departments, with Pasco representing 29.38% of the current model, which reflects the highest percentage of suitable habitats for the species, in agreement with Reynel et al. [83]. Similarly, the current model shows that the species can find a suitable habitat in four more departments; hence the need for further exploration in those places.

On the other hand, we observe that priority reforestation areas would represent 16.54% of the current model, with Pasco, Huánuco, Cajamarca, Junín, and Puno departments standing out, necessitating conservation proposals to prevent population decline of the species [84]. However, the potential distribution area of *R. rospigliosii* shows that there is 6327.97 km² of suitable habitat for the species' development; furthermore, population density shows that there are eight individuals per hectare [85], and more than 3 million plants would be needed to cover the suitable area for reforestation. Nevertheless, *R. rospigliosii* forests are characterized by being primary forests cohabiting with tree species such as *Cecropia* sp., *Prumnopitys harmsiana*, and *Hieronyma* sp., so the specific environmental conditions of *R. rospigliosii* also include the distributional range of these cohabiting species, and it is necessary to propose propagation strategies for these species to maintain ecological balance. Finally, the potential distribution area also indicates the need to increase

exploration and validation efforts for the presence of *R. rospigliosii*, in order to improve reforestation strategies for conservation and restoration of these ecosystems.

Reforestation and restoration are inherently multidisciplinary activities [86]. The integration of approaches from different scientific disciplines has been frequently suggested. In this study, we propose a novel framework that bridges improvements in silvicultural practices with ecological niche modeling. Although niche modeling has been used to identify priority restoration areas [87], it often neglects the estimation of the number of seedlings needed to reforest these areas effectively. Our work addresses this gap by linking silvicultural methodologies with computational modeling techniques. We advocate for a multidisciplinary perspective [4] to improve reforestation and restoration outcomes. We understand that this initial approach has limitations, so we consider it essential to encourage future studies that explore additional factors, which would allow improving the accuracy of the estimates and facilitate successful reforestation with greater chances of survival and growth of native species.

5. Conclusions

Our results show that *R. rospigliosii* presents ideal characteristics at 6 months of age with low fertilization treatments and the indiscriminate use of containers of different volumes. Doses of 4.2 g/L of CRF in 115 cc and 180 cc containers showed a greater effect on the growth and quality indices of *R. rospigliosii* seedlings. In this sense, we recommend opting for the use of containers with smaller volumes to optimize space and substrate use in the nursery. Likewise, we emphasize the need to use seeds as the main means of propagation since they ensure greater genetic diversity and, therefore, the establishment of more resilient populations in the face of a changing climate.

The ecological niche model used has allowed us to identify suitable areas for reforestation. We have identified five potential reforestation areas in Peru with suitable climatic and soil conditions. Therefore, it is suggested to use this method of *R. rospigliosii* plant production in these locations to reforest the forests and make them more resilient in the face of a changing climate.

The study will allow the improvement in the production of *R. rospigliosii* seedlings in the nursery through the use of controlled-release fertilizers, as well as facilitating the identification of suitable areas for reforestation through the modeling of ecological niches. This novel approach could contribute to the planning of large-scale reforestation projects and the restoration of natural forest ecosystems.

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