



Nitrogen Source and Rate Influence on Growth and Physiology of *Cinchona micrantha* Seedlings

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ABSTRACT

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Cinchona micrantha, an Andean species with high medicinal value due to its alkaloids such as quinine, faces propagation difficulties due to a lack of knowledge about its initial nutritional requirements. This study evaluated the differential effect of nitrogen (N) sources and levels on the morpho-physiological development of *Cinchona micrantha* under nursery conditions. Twelve treatments resulting from the combination of three sources of nitrogen fertilization (conventional urea, urea + *N*-(*n*-butyl)thiophosphoric triamide (NBPT), and urea + 3,4-dimethylpyrazole phosphate (DMPP)) and four dose levels (0, 0.3, 0.6, and 0.9 g/plant) were analyzed using a completely randomized factorial design. Growth parameters (height, stem diameter, biomass, leaf area), relative chlorophyll content (SPAD), and survival were measured every two weeks for four months, as well as physicochemical changes in the soil at the beginning and end of the trial. The T22 treatment (urea + NBPT, 0.6 g/plant) showed the best performance, with increases of 67% in height, 20% in diameter, 61% in leaf area, 2.5-fold in above-ground biomass, and 2.4-fold in root biomass, as well as 13% more chlorophyll, maintaining low mortality and no phytotoxicity. In contrast, higher doses (0.9 g/plant) generated abiotic stress and high mortality (> 80% in T31 and T33). This study provides scientific evidence applicable to the design of balanced fertilization schemes in nurseries, with direct implications for reforestation programs, ecological restoration, and conservation of medicinal species native to the Andes.

1. INTRODUCTION

The genus *Cinchona*, which includes species of high medicinal value such as *C. micrantha*, faces critical challenges in its propagation due to scarce information on its nutritional requirements at early stages [1, 2]. Nitrogen (N) is an essential macronutrient that regulates key physiological processes, from chlorophyll synthesis to alkaloid formation [3-9]. However, under nursery conditions, the morpho-physiological response of the *Cinchona* genus species to different sources (organic or mineral) and levels of N remains poorly explored [10]. Despite recent advances in understanding mycorrhizal associations that enhance germination and initial growth in related *Cinchona* species [11], there is a notable absence of studies discriminating between N sources, such as conventional urea

versus inhibitor-enriched formulations, and their dose-dependent effects on seedling physiology. This gap limits the development of efficient protocols for seedling production, crucial for reforestation and conservation programs [1, 10, 12-14].

Recent studies show that N use efficiency (NUE) in tropical tree species varies significantly depending on the applied source, with marked differences between organic and inorganic compounds [15-18]. For example, the application of urease-enriched urea or nitrification inhibitors could result in strategies that reduce nitrogen losses and optimize its uptake by plants [19]. The addition of urease inhibitors such as *N*-(*n*-butyl)thiophosphoric triamide (NBPT) slows the activity of this enzyme, which slows the hydrolysis of urea to ammonia (NH₃). This delay decreases the localized increase in pH

around urea molecules and reduces the concentration of NH_3 in the soil, leading to less volatilization and greater retention of the applied nitrogen, thus prolonging its availability to plants [20].

On the other hand, the use of nitrogen fertilizers enriched with nitrification inhibitors, such as 3,4-dimethylpyrazole phosphate (DMPP), has been shown to be effective in retarding the microbial conversion of ammonium to nitrate. This effect is due to the specific inhibition of the enzyme ammonium monooxygenase, responsible for the first step in the nitrification pathway [21]. As a consequence, the permanence of nitrogen in the form of ammonium ($\text{NH}_4^+\text{-N}$) in the soil is prolonged, which improves its availability to plants for a longer period and reduces potential losses by leaching and denitrification [22]. In *C. micrantha*, whose phenology is linked to seasonal pulses of nutrient availability [2], this variability could modulate biomass partitioning and secondary metabolite synthesis [23, 24].

At the physiological level, N directly influences photosynthetic capacity through its role in Rubisco and thylakoid structure [25]. Work on related species reveals that N deficiencies reduce specific leaf area and water use efficiency [8, 26-28]. However, these studies do not discriminate between nitrogen sources, nor do they evaluate optimal thresholds for *Cinchona*, a genus of species with higher light requirements and lower tolerance to excess fertilization [10, 13]. The lack of these data prevents the optimization of resources in Andean nurseries, where the availability of inputs is limited [12, 29].

The use of nitrogen fertilizers represents a key agronomic practice to increase crop productivity [30]. Numerous studies have documented that adequate N application contributes significantly to increased agricultural yield [31] by improving the availability of this essential nutrient for plant development. However, when application rates exceed the actual crop needs, the effect can be counterproductive, generating a decrease in growth [32] due to nutritional imbalances, phytotoxic effects, and alterations in soil properties [33].

Methodologically, the integrated evaluation of morpho-physiological parameters, from growth variables (height, diameter, leaf area) to photochemical indicators (chlorophyll content), allows a robust characterization of nutritional efficiency [34-36]. In *Cinchona*, however, most studies focus on adult stages, ignoring the seedling stage, where phenotypic plasticity is maximal as it occurs in other forest species [37-39]. This gap is critical, as the rootstocks established in the nursery determine field establishment success [29, 40-42]. This study seeks to fill these gaps through a factorial design evaluating (1) three N sources (urea, urea + NBPT, urea + DMPP), (2) four application levels (0, 0.3, 0.6, and 0.9 g/plant), and (3) their impact on morpho-physiological parameters of *C. micrantha* in the nursery. Specifically, the research question posed is: How do nitrogen sources and their application levels influence the growth, photosynthetic efficiency, and survival of *C. micrantha* seedlings? The results will provide scientific bases for balanced fertilization protocols, with direct applications in conservation and sustainable agroforestry programs in the tropical Andes.

2. METODOLOGY

2.1 Location of study area

The study was carried out in the locality of La Cascarilla,

located in the province of Jaén, Cajamarca region, Peru, at an altitude of 1810 meters above sea level ($5^\circ 40' 21.12''$ S; $78^\circ 53' 55.65''$ W). This area is characterized by a humid subtropical climate, with an average annual rainfall of approximately 1730 mm. Temperatures fluctuate between an average minimum of 13.0°C and a maximum of 20.5°C , conditions that directly influence the physiological and developmental processes of the plant species present in the study area [14]. The experiment was conducted in a nursery covered with 70% shade raschel mesh to simulate natural light conditions in Andean forests, using “5 × 8” polyethylene bags filled with forest substrate extracted from areas where *Cinchona* trees grow and daily manual irrigation to maintain substrate moisture at 70-80% of field capacity [12].

2.2 Experimental design and treatments

A completely randomized design (CRD) with 10 treatments was used, consisting of combinations of nitrogen source type (urea, urea + NBPT, urea + DMPP) and nitrogen dose levels (0.3, 0.6, and 0.9 g/plant), with an additional control group (Control) to which no nitrogen was applied (Figure 1). Each treatment had three replicates and 20 plants per replicate. This experimental structure allowed for independent and combined analysis of the effect of fertilizer type and dose on the morphophysiological variables of *C. micrantha*.

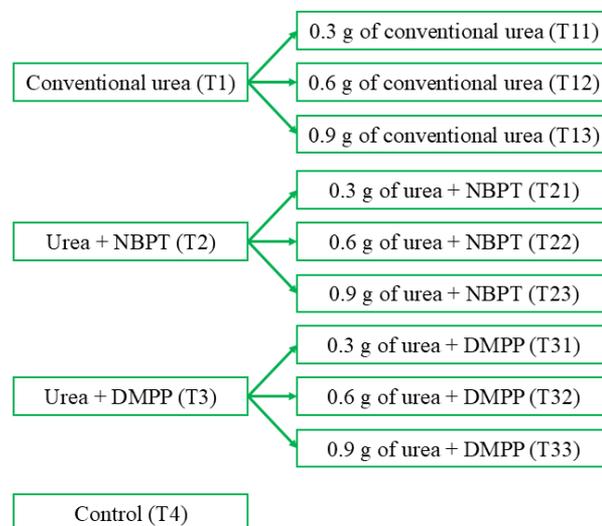


Figure 1. Arrangement of nitrogen sources (conventional urea, urea + NBPT, urea + DMPP) and doses (0, 0.3, 0.6, 0.9 g plant⁻¹) in a completely randomized design

2.3 Measurements and calculations

2.3.1 Experiment setup

The trial was implemented using *Cinchona micrantha* seedlings with at least four months of nursery growth after replanting. Nitrogen fertilizer treatments included a control (T4) to which no fertilizer was applied and three formulations: T1 = conventional urea, T2 = urea added with NBPT (urease inhibitor), and T3 = urea enriched with DMPP (nitrification inhibitor). The application was carried out manually on the substrate, taking care that the granules did not come into direct contact with the stem or remain exposed on the surface, according to the doses established for each treatment. The assignment of treatments to plants was carried out using a random number generator to ensure an unbiased distribution

of treatments in the nursery, following standard methods for CRD designs [43].

2.3.2 Measurement of chlorophyll content

Relative leaf chlorophyll content was quantified using a portable SPAD-502 meter (Konica Minolta). Measurements were taken every 15 days for a period of four months. Readings were taken at 4:00 p.m., the time selected to minimize diurnal variations in photosynthetic activity and ensure the stability of chlorophyll values, since photosynthetic rates tend to be more consistent in tropical species in the afternoon. In each evaluation, two pairs of intermediate leaves were selected per plant, excluding the main vein, and four measurements were taken and averaged to obtain a representative value per individual [44].

2.3.3 Evaluation of height increase

Plant height was measured with a 60 cm ruler, complemented with a square to ensure an accurate reading perpendicular to the substrate. Measurements were taken from the base of the stem, at the point of contact with the substrate, to the apical meristem. Assessments were made every 15 days throughout the four-month monitoring period.

2.3.4 Stem diameter determination

Basal diameter was measured using a digital caliper (vernier), selecting a uniform point on the stem, free of irregularities or knots. The vernier jaws were positioned perpendicular to the stem axis and adjusted gently to avoid deformation of the plant tissue. Readings, expressed in millimeters, were recorded biweekly for the duration of the trial.

2.3.5 Evaluation of dry biomass

At the end of the experiment, the aerial (leaves and stems) and subways (roots) structures of each seedling were harvested. Subsequently, each component was dried in an oven at 80°C for 48 hours. Once dehydrated, the samples were weighed to determine the total dry weight. In the case of the leaves, before drying, the leaf area was estimated [14].

2.4 Statistical analysis

The data obtained were subjected to tests of normality assumptions (Shapiro-Wilk) and homogeneity of variances (Levene's test) to validate the application of parametric models. When the assumptions were met, two-factor analysis of variance (ANOVA) was performed to detect significant effects ($p < 0.05$) of both individual factors (fertilizer type and dose) and their interaction on morphological (height, stem diameter, leaf area, above and below ground biomass) and physiological (relative chlorophyll content (SPAD)) variables.

Where significant differences were detected, a Duncan mean comparison test ($p < 0.05$) was applied to identify homogeneous groups. For variables related to temporal dynamics, such as the SPAD index and height, a repeated measures analysis was used to evaluate the evolution over time, considering the dependence structure between serial observations.

3. RESULTS

Figure 2(a) shows a clear pattern of stabilization of mortality around day 30 for most treatments, indicating that

death events occurred mainly during the initial phase of the trial (after fertilization was applied). Treatments T13 and T12 recorded 100% mortality from the first 15 days, suggesting extremely unfavorable conditions for survival. Similarly, T33 and T31 quickly reached mortality levels above 80%, although in slightly longer times. On the other hand, treatments T21, T22, and T4 showed considerably lower mortality rates, with T22 and T4 standing out with values close to 6.7%, indicating more favorable conditions for survival or less exposure to lethal factors.

Figure 2(b) compares the final impact of each treatment. The values indicate that treatments T12 and T13 were the most lethal, reaching 100% mortality. In contrast, treatments T21, T22, T23 and T4 presented mortality rates below 15%, suggesting that these conditions could be the most suitable for future experimental or productive applications. It is also notable that treatment T31, with 80% mortality, treatments T32 and T33 with 73.3% and 86.7% mortality, respectively. Taken together, the data evidence a strong influence of treatment type on individual survival, highlighting the need for careful selection of experimental conditions to optimize results in similar trials.

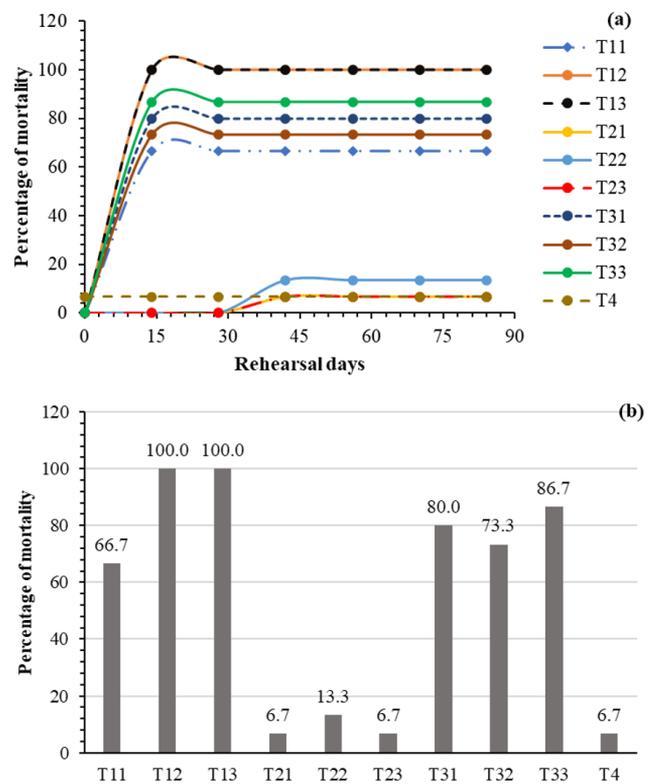


Figure 2. Cumulative mortality dynamics (%) of *Cinchona micrantha* under different nitrogen fertilization treatments during the evaluation period, with (a) Temporal evolution of mortality per treatment, (b) Final mortality (%) at the end of the experiment

Figure 3 shows the comparison of height increment (cm) between plants subjected to different treatments (T11 to T4), through a bar graph with standard errors, and superscript letters indicating significant statistical differences ($p < 0.05$) according to a Duncan post-hoc analysis. Treatments T21, T22, and T23 stand out for presenting the highest height increments (15.0, 15.5, and 16.0 cm, respectively), grouping themselves in the statistical categories “a” and “ab”, indicating that they do not differ significantly among them, but they do differ

significantly with respect to the lower-yielding treatments. T21 is identified as statistically superior (letter “a”) compared to T11, T31, and T32, suggesting that this dose and type of fertilizer significantly favor the increase in height of *C. micrantha*.

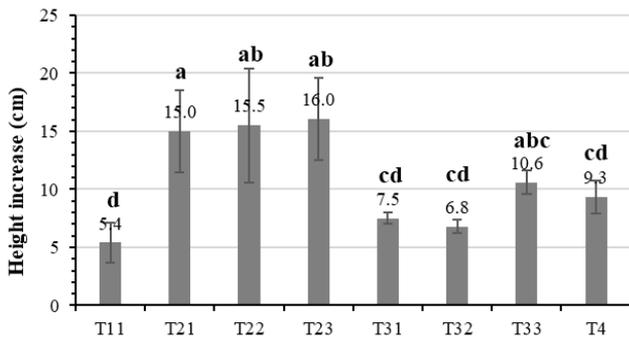


Figure 3. Average increase in height (cm) of *Cinchona micrantha* in response to different sources and levels of nitrogen

The bars represent the mean ± standard error. Superscript letters indicate significant differences ($p < 0.05$) according to Duncan's test.

In contrast, treatment T11 exhibits the lowest growth (5.4 cm) and is grouped in category “d”, indicating its statistically significant difference with respect to most of the other treatments. Similarly, T31 (7.5 cm) and T32 (6.8 cm) show reduced performance, grouping under the “cd” category. Treatment T33, with an increase of 10.6 cm, is in an intermediate category (“abc”), suggesting a moderate yield, not significantly different from T4 (9.3 cm, “cd” group) or the higher treatments. This overlap between statistical categories suggests that while there are clear trends, differences between certain treatments may be marginal.

This analysis suggests that height growth is highly sensitive to the conditions imposed by treatments, with T21, T22, and T23 being the most promising in promoting vigorous development. These findings have direct implications for the selection of cultivation or management protocols under experimental or commercial conditions, particularly if height increment is considered a critical parameter for the successful establishment or competitiveness of *C. micrantha*.

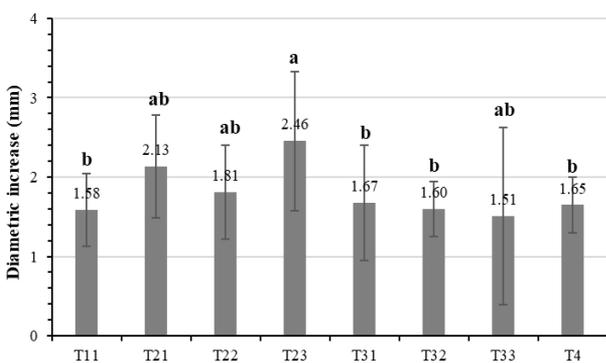


Figure 4. Average increase in basal diameter (mm) of *Cinchona micrantha* according to nitrogen fertilization treatments

The bars represent the mean ± standard error. Superscript letters indicate significant differences ($p < 0.05$).

Figure 4 represents the increase in diameter (cm) of *C. micrantha* plants. Treatment T23 presented the greatest

diameter increase with 2.46 mm, significantly higher than several treatments, being classified in statistical category “a”. This result suggests an optimal physiological response to nitrogen fertilizer + NBPT. In contrast, treatments T11, T31, T32, T33, and T4, whose increments varied between 1.51 and 1.67 mm, are grouped in category “b”, indicating a significantly lower diameter growth compared to T23.

Treatments T21 (2.13 mm) and T22 (1.81 mm) achieved intermediate increments, without differing significantly from T23, which places them in the “ab” category. This grouping suggests that, although their performance is not the highest, it is competitive and statistically indistinguishable from the superior treatment, which makes them potential alternatives for optimizing diameter growth under certain conditions.

Treatment T11, with the second lowest value (1.58 mm), coincides with its poor performance in height growth, which reinforces its characterization as a suboptimal treatment. On the contrary, T23, which also presented one of the highest increases in height, consolidates its position as the most effective treatment in terms of integral vegetative development.

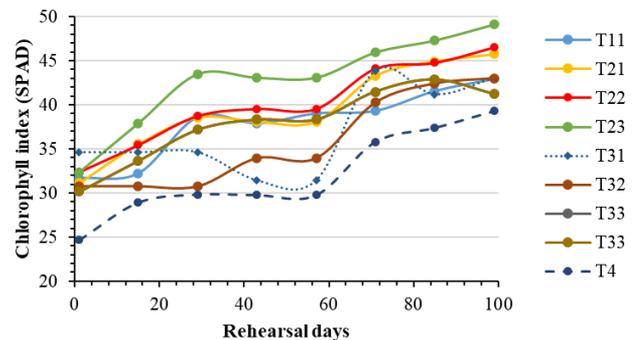


Figure 5. Evolution of relative chlorophyll content (SPAD) over time under different nitrogen fertilization treatments during the experiment

Figure 5 shows the evolution of SPAD as a function of time (trial days) under different doses and types of fertilization. A general upward trend is observed in all treatments, with a marked differentiation in the physiological behavior of the plants in response to the different experimental conditions.

Initially, SPAD values were between 25 and 33 units, increasing progressively as the trial progressed. Treatment T23 evidences a superior and sustained performance over time, reaching a value close to 48 SPAD at day 96, suggesting a higher photosynthetic capacity or better nutrient assimilation compared to the other treatments. T22 and T21 also exhibit consistent increases, stabilizing around 46 and 45 SPAD, respectively, towards the end of the evaluated period, which positions them as efficient alternatives.

In contrast, treatments T31, T32, and T4 show the lowest values over time. T4, in particular, shows limited behavior, remaining below 39 SPAD at the end of the trial. T32, with consistently low values, stands out for a suboptimal response. It is remarkable that the inflection point that occurs in several treatments around day 60, where a more pronounced increase in the SPAD index is evidenced, especially in T22, T23, and T33.

Figure 6 represents the effect of fertilizer doses and type on *C. micrantha* plant growth, disaggregated into three components: leaf weight (a), stem weight (b), and root weight (c), expressed in grams. The bars are accompanied by standard errors and letters indicating significant differences between

means ($p < 0.05$), determined by a Duncan post hoc test.

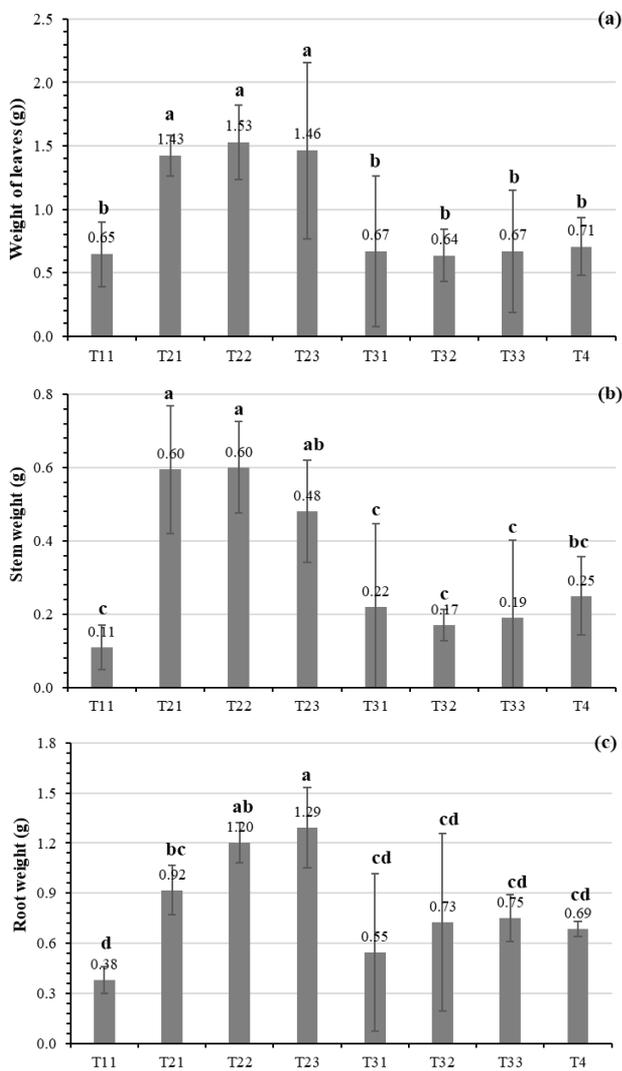


Figure 6. Dry biomass (g) of vegetative organs of *Cinchona micrantha* as a function of the sources and levels of nitrogen applied: (a) Dry weight of leaves, (b) Dry weight of stem, and (c) Dry weight of roots. Bars represent mean \pm standard error; letters indicate significant differences ($p < 0.05$) according to Duncan.

From Figure 6(a), it is observed that treatments T21, T22, and T23 present the highest values (1.43, 1.53, and 1.60 g, respectively), with significant differences compared to the rest of the treatments, placing them in a homogeneous group "a". This indicates that these treatments favor greater leaf development, possibly due to better nutrient availability, hormonal regulation, or photosynthetic efficiency. In contrast, T11, T31, T32, T33, and T4 form a homogeneous group "b" with significantly lower leaf weights (between 0.55 and 0.71 g), reflecting a suboptimal response in leaf biomass accumulation.

Stem response also exhibits a favorable trend in T21 and T22 (both with 0.60 g), followed by T23 (0.48 g), although the latter shares a statistical group with intermediate performance treatments such as T31 and T4. Notably, T11 presents the lowest value (0.11 g) and differs significantly from all treatments (Figure 6(b)). This pattern reaffirms the competitive advantage of the T2 treatments in promoting stem elongation and thickening.

The analysis of the root system shows greater variability.

T23 stands out as the treatment with the highest root weight (1.29 g), followed closely by T22 (1.20 g), both in the top statistical groups ("a" and "ab"). The treatment T11 and T31, are grouped in the lower levels with weights ranging from 0.38 to 0.55 g. Treatments T31, T32, T33, and T4 present homogeneous results in the "cd" group (Figure 6(c)).

Taken together, these results show a clear superiority of treatments T21, T22, and T23 in the integral development of plant biomass, covering both photosynthetic organs and support and absorption structures.

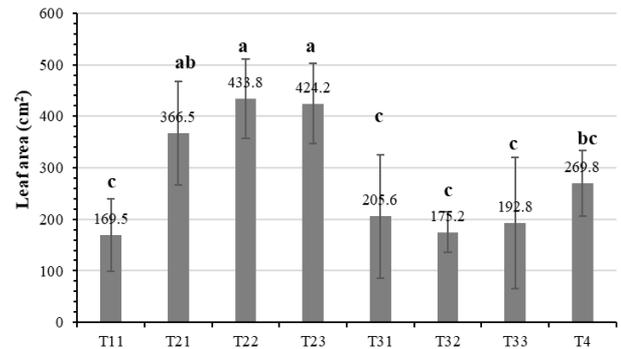
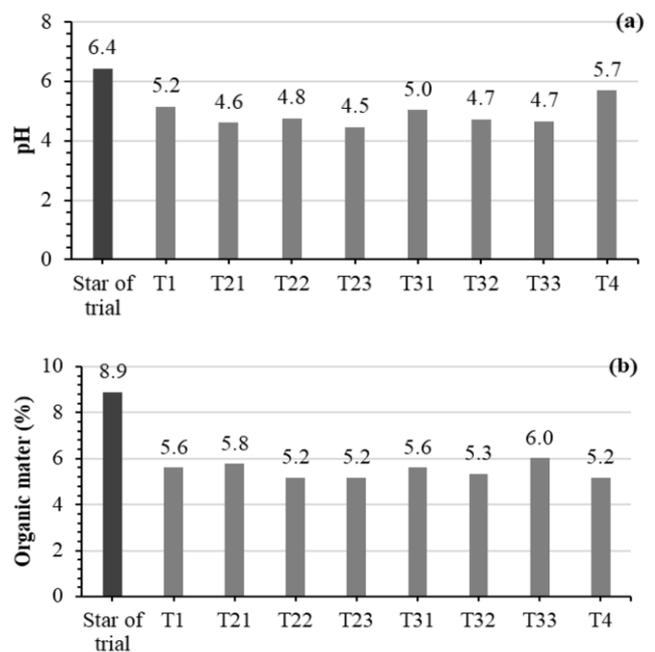


Figure 7. Average leaf area (cm²) of *Cinchona micrantha* under different nitrogen fertilization treatments. Bars indicate mean \pm standard error. Superscript letters represent significant statistical differences ($p < 0.05$).

The results in Figure 7 show that treatments T22 (433.8 cm²) and T23 (424.2 cm²) have the largest leaf areas, with significant differences with respect to most of the other treatments, grouped in statistical category "a". This suggests that these treatments promote optimal leaf development. This is followed by T21 (366.5 cm²), which is placed in the "ab" group, indicating a positive trend, although not significantly higher than all the others. In contrast, T11 (169.5 cm²), T31 (205.6 cm²), T32 (175.2 cm²), and T33 (192.8 cm²) show the lowest leaf areas and are grouped in statistical group "c", evidencing a suboptimal leaf growth response. T4, with an intermediate leaf area (269.8 cm²), is positioned in group "bc", with significant differences with respect to the most productive treatments.



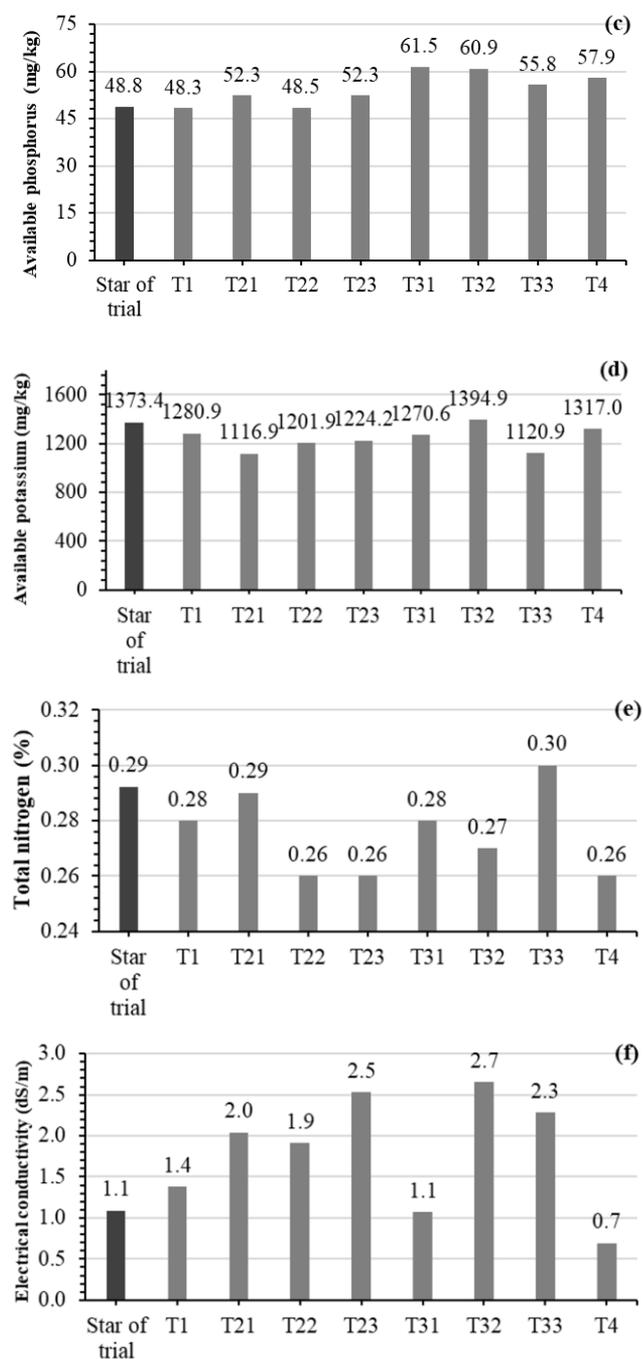


Figure 8. Changes in soil physicochemical properties at the beginning and end of the nursery fertilization experiment: (a) Soil pH, (b) Organic matter (%), (c) Available phosphorus (mg/kg), (d) Available potassium (mg/kg), (e) Total nitrogen (%), (f) Electrical conductivity (dS/m)

Figure 8 shows the evolution of various physicochemical properties of the soil at the beginning and at the end of the trial. Regarding soil pH (Figure 8(a)), a general decrease in pH from the initial value of 6.4 to more acidic levels is observed in the intermediate treatments (T21 to T33), with a partial recovery in T4 (5.7). Organic matter (Figure 8(b)) decreased from an initial value of 8.9% to a range of 5.2-6.0% in most treatments, suggesting an accelerated mineralization of the organic fraction. However, T33 managed to maintain a content of 6.0%, suggesting that this treatment could favor the stabilization of organic carbon in the soil. Available phosphorus (Figure 8(c)) showed a substantial increase in phosphorus availability, especially in T31 (61.5 mg/kg) and

T32 (60.9 mg/kg), compared to the initial value (48.8 mg/kg). Regarding available potassium (Figure 8(d)), initially at 1373.4 mg/kg, it showed a slight reduction in most treatments, with the exception of T32 (1394.9 mg/kg), which exceeded the initial value. Total nitrogen levels (Figure 8(e)) remained relatively stable, with slight fluctuations between 0.26 and 0.30%. T33 presented the highest value (0.30%). Finally, with respect to electrical conductivity (Figure 8(f)), an indicator of salinity, it increased significantly in treatments T21, T22, T23, T32 and T33, reaching a maximum of 2.7 dS/m in T32. However, T4 showed a marked reduction to 0.7 dS/m.

4. DISCUSSIONS

The research provides robust empirical evidence on the differential effects of N sources and levels on the morpho-physiological development of *Cinchona micrantha* in the nursery, an emblematic species of ecological and medicinal value in the Andes. The findings reaffirm the hypothesis that N source and concentration significantly affect growth variables, photosynthetic efficiency, and survival, which is consistent with previous studies in tropical forest species [34, 35].

First, treatments enriched with inhibitors (NBPT and DMPP) showed superior results in most of the variables evaluated. Treatment T23 (urea + NBPT, 0.9 g/plant) consistently stood out for inducing the highest growth in height, diameter, chlorophyll content, and aerial biomass. This behavior suggests a better synchrony between N availability and plant physiological demand, reducing loss by volatilization and leaching, as also reported by [19, 20]. In tropical tree seedlings, such N fertilization regimes often redirect C from structural carbohydrates toward photosynthetic machinery, elevating Rubisco content and net CO₂ assimilation rates, which in turn amplify leaf expansion and root proliferation as evidenced in the elevated leaf area (up to 424.2 cm² in T23) and root biomass (1.29 g in T23) [25, 45].

Urease and nitrification inhibitors prolong the permanence of N in assimilable forms (NH₄⁺), improving NUE. These results agree with those reported by researchers [21, 22], who reported significant increases in N retention and plant productivity under systems fertilized with DMPP. In this study, the better physiological performance in NBPT treatments was also reflected in SPAD values, which showed a sustained upward curve, in agreement with higher photosynthetic activity [8].

From the survival point of view, the lowest mortality was recorded in treatments T21, T22, T23, and T4. Intriguingly, the unfertilized control (T4) exhibited low mortality rates (6.7%) and intermediate growth metrics (e.g., height increment of 9.3 cm, leaf area of 269.8 cm²), potentially attributable to adaptation to nutrient-poor substrates typical of Andean soils, where native species like *C. micrantha* have evolved mechanisms for efficient nutrient scavenging and minimal osmotic stress [12]. In nursery contexts for tropical plants, controls without fertilizer often perform adequately due to residual soil nutrients, reduced salinity-induced toxicity, and promotion of mycorrhizal associations that enhance water and nutrient uptake without the phytotoxic risks of over-fertilization [46, 47]. This resilience in T4 underscores the species' inherent tolerance to low-N environments, possibly mediated by conserved C allocation toward root exploration rather than rapid aboveground growth [45].

Surprisingly, the treatment without fertilization (T4) showed low mortality rates, which could be attributed to lower osmotic load or salt toxicity in the substrate, in contrast to treatments such as T13 and T12, where high doses and type of fertilizer caused severe abiotic stress. This agrees with reports by Li et al. [32], who documented that excessive N doses can induce phytotoxic effects and affect soil microbial structure. In terms of biomass, it was observed that urea + NBPT treatments (T21-T23) significantly increased leaf and root production. This finding suggests efficient nutrient mobilization to functional and supporting organs, possibly mediated by increased Rubisco activity and light uptake efficiency [25, 36]. The higher root biomass observed at T22 and T21 could also reflect an indirect effect on root architecture induced by N type, as has been evidenced in other woody species [26].

Leaf area, a key parameter in photosynthesis and biomass accumulation, showed maximum values at T22 and T23. This implies that NUE not only promotes linear growth but also leaf expansion, which favors plant energy balance. The positive relationship between chlorophyll and leaf area in these treatments indicates a functional coupling between N availability and photosynthetic performance, as has been documented in rice and maize under nutritional stress conditions [8, 28].

In relation to the physicochemical properties of the soil, a progressive acidification was observed in urea treatments, especially in the enriched ones, probably as a result of intensified nitrification. This phenomenon may reduce the availability of certain nutrients and affect soil biota [7], although the decrease was moderate. The reduction in organic matter suggests accelerated fertilization-induced mineralization, while the increase in available phosphorus, particularly in treatments T31 and T32, raises questions about the N-P interaction and its dynamics in Andean soils [17]. Finally, electrical conductivity increased significantly in treatments with higher nitrogen loading, especially T33, suggesting a potential risk of substrate salinization under intensive fertilization schemes. This finding is relevant in tropical nursery contexts, where salt accumulation can limit water and nutrient uptake, compromising field establishment [29].

Notwithstanding these insights, the study harbors limitations warranting acknowledgment. The experimental duration of four months, while sufficient for initial morphophysiological assessments, precludes elucidation of long-term effects, such as sustained biomass partitioning or alkaloid accumulation in mature stages, which may diverge under prolonged N exposure [45, 48]. Furthermore, the measurement scope, confined to morphophysiological and edaphic parameters, omits direct quantification of metabolic intermediates (e.g., amino acids, carbohydrates) or microbial dynamics, constraining inferences on C/N metabolism and rhizosphere interactions [35, 47]. Future investigations should extend temporal horizons and incorporate metabolomic profiling to validate these mechanisms in field-transplanted seedlings.

5. CONCLUSIONS

The results showed that the source and dose of nitrogen fertilization exerted a significant differential effect on the morpho-physiological performance of *Cinchona micrantha*

under nursery conditions. In particular, the application of urea enriched with urease inhibitor (NBPT) at an intermediate dose (0.6 g/plant, treatment T22) is positioned as the most efficient strategy, combining high survival rates with remarkable increases in height, diameter, chlorophyll content, leaf area, and total biomass. This treatment optimizes nitrogen availability for the plant, improves photosynthetic efficiency, and promotes balanced growth of aerial and subterranean organs, while minimizing risks such as salinity or mortality. Likewise, the use of inhibitors such as NBPT and DMPP showed clear advantages over conventional urea, reducing potential losses by volatilization and leaching, and favoring greater nitrogen stability in the soil system. However, higher doses (0.9 g/plant) in some treatments led to phytotoxic effects and high levels of mortality, which highlights the need to establish physiologically safe thresholds for sensitive species such as *C. micrantha*.

From an applied perspective, these results provide solid technical foundations for the development of balanced fertilization protocols in nurseries of native tropical forest species, contributing to optimizing the production processes of seedlings with high physiological quality and a greater probability of success in the field. In addition, the findings have direct implications for reforestation programs, ecological restoration, and conservation of medicinal species endemic to the Andes.

Finally, it is recommended that future research evaluate the interaction of these fertilizers with other edaphic variables (microbiota), as well as the monitoring of the plants in the transplant stage in the field, in order to validate the persistence of the benefits observed in the nursery under real conditions of establishment.

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