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Research Article

Effects of different ratios of nitrogen–calcium fertilization on growth and photosynthetic characteristics in the endangered plant *Excentrodendron hsienmu* seedlings

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Abstract

Excentrodendron hsienmu, a karst-endemic tree species of high ecological and economic value, is classified as endangered and nationally protected in China. This study aimed to establish a theoretical basis for the propagation and conservation of *E. hsienmu* by evaluating the effects of nitrogen–calcium fertilization on seedling growth and photosynthetic performance. One-year-old seedlings were subjected to nine treatment combinations of pure nitrogen (1.0, 2.0, 3.0 g/plant) and pure calcium (1.5, 3.0, 4.5 g/plant). Results showed that all fertilization treatments significantly improved seedling growth and photosynthetic traits compared to the control. The N2Ca2 treatment (2.0 g N and 3.0 g Ca per plant) yielded the highest increases in height (26.20 cm), ground diameter (6.96 mm), total biomass (85.64 g), root system development, and chlorophyll content. Additionally, photosynthetic parameters such as net photosynthetic rate (13.96 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and transpiration rate (4.21 $mmol \cdot m^{-2} \cdot s^{-1}$) were significantly enhanced under N2Ca2. All measured characteristics, except stomatal conductance, differed significantly from the control ($p < 0.05$). These findings provide a scientific foundation for the optimized cultivation, restoration, and conservation of *E. hsienmu*.

Keywords: endangered plant; *Excentrodendron hsienmu* seedlings; growth characteristics; nitrogen–calcium fertilization; photosynthetic characteristics

Introduction

Excentrodendron hsienmu, a large evergreen tree of the genus *Excentrodendron* in the family Malvaceae, is distributed in the southwestern region of Guangxi and parts of the tropical monsoon forest areas in Yunnan. Being, considered an endemic species in Karst regions, it also grows in Vietnam and Laos (Li *et al.*, 2008). It plays a crucial role in maintaining the biodiversity of karst monsoon forests and in improving the ecological environment (Tan *et al.*, 2018). As a precious tree species, prioritized for development in Guangxi, *E. hsienmu* contributes significantly to the control of karst desertification. Moreover, due to its hard texture, water and corrosion resistance, it is widely used in high-end furniture, carving, construction, industry, and manufacturing, possessing important economic and scientific research value (Hou *et al.*, 2018). However, due

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to challenges with natural regeneration and threats from illegal logging, *E. hsienmu* is endangered and has been designated a protected tree species in China. The conservation and propagation of *E. hsienmu* are therefore particularly important (Wei *et al.*, 2023).

Fertilizer is an important factor that affects the growth and development of plants, with different fertilizer elements having varying effects (Li *et al.*, 2023). Nitrogen is an essential nutrient element for plants (Tang *et al.*, 2022; Qiu *et al.*, 2024) and is used in the synthesis of proteins and enzymes for capturing and transferring photosynthetic electrons (Cassim *et al.*, 2022; Yin *et al.*, 2022), therefore, the content of nitrogen directly affects the intensity of photosynthesis (Weng *et al.*, 2021; Chrysargyris *et al.*, 2024). Calcium is a major constituent of plant cell walls, with functions in maintaining cell morphology, regulating ion balance and osmotic pressure, as well as plant growth and development, and, influences responses to various biotic and abiotic stresses (Zhou *et al.*, 2014; Doyle *et al.*, 2021). The evidence-based selection and application of an appropriate nitrogen–calcium fertilizer ratio can significantly enhance the growth and net photosynthetic efficiency of plants and improve their photosynthetic performance (Yue *et al.*, 2021; De Souza *et al.*, 2023). *E. hsienmu* is a calcium-preferring tree species. It needs to continuously absorb calcium minerals from the soil to alleviate abiotic stress and enhance its hardness, thus, calcium plays a vital role in the growth of *E. hsienmu* (Tan *et al.*, 2018).

In recent years, different ratios of nitrogen-calcium in fertilizer have been investigated. Among various studies on this topic, Ozyhar *et al.* (2022) found in their study on poplar trees where the combined application of nitrogen–calcium can promote the absorption by plants of other nutrients from the soil, thereby facilitating growth in acidic soil. Liu (2024) discovered that applying nitrogen-calcium fertilizers in an appropriate ratio can promote the growth and development of *Morus alba* L. seedlings, by applying the optimal nitrogen-calcium ratio of 2:1. Importantly, ratios that are too high or too low will inhibit their growth and physiological processes. Weng *et al.* (2023) studied the molecular mechanisms of poplar growth under nitrogen-calcium interactions, and their results showed that maximum poplar seedling growth occurred from treatment with a N:Ca ratio of 2:1, which resulted in a significantly greater of growth than the two other tested treatments. Li *et al.* (2013) showed that the different ratios of nitrogen-calcium fertilization in a specific ratio can significantly enhance the growth and development of greenhouse nectarines. The results of Weng *et al.* (2021) indicated that the different ratios of nitrogen-calcium fertilization could also markedly promote poplar growth, and there is an optimal level of calcium for maximizing the growth and development of poplars for different nitrogen levels. Evidently, the appropriate ratio of nitrogen-calcium fertilization has a distinct effect on promoting the growth and development of plants.

The studies on *E. hsienm*u are mainly focused on the growth dynamics of artificial young forests of cutting seedlings (Peng *et al.*, 2016), the phenotypic traits of seeds and their germination patterns (Hao *et al.*, 2021), factors influencing cutting propagation (Shen *et al.*, 2014), population dynamics, community structure description (Xiang *et al.*, 2013; Tan *et al.*, 2019), and biological characteristics (Tang *et al.*, 2005).

There is a lack of data regarding the impact of different ratios of nitrogen-calcium fertilization on the growth and photosynthetic characteristics of *E. hsienmu* seedlings. Currently, there are few studies on the effect of the fertilization ratio on *E. hsienmu*, and no author has conducted research on the basic principles of nitrogen-calcium ratio control for this species. Moreover, since *E. hsienmu* is a calcium-loving tree species, the basic principles of nitrogen-calcium ratio control for ordinary crops do not apply. This study determined the appropriate ratio to be 1:1.5 after investigating the nitrogen-calcium ratios in the substrates of 10 native habitats in which *E. hsienmu* grows well. A ratio higher or lower than this will inhibit the growth of *E. hsienmu* and increase its likelihood of entering an endangered state. Since *E. hsienmu* thrives only in limestone areas with abundant calcium, our study also aimed to promote its growth through evidence-based determination of the optimal nitrogen and calcium conditions for soils typical of afforested land. Consequently, applying these conditions could lead to expansion of its planting area, thereby enabling the better protection and cultivation of *E. hsienmu* and mitigating its endangered status.

In this study, one-year-old *E. hsienmu* cutting seedlings were used as the experimental material, and a two-factor experimental design involving nitrogen-calcium was employed to explore the effects of various fertilization ratios on their growth and photosynthetic characteristics. The optimal combination formula that can promote the growth and photosynthetic characteristics of *E. hsienmu* seedlings was identified, providing a scientific theoretical basis for the propagation and maintenance of *E. hsienmu* seedlings. This study also has positive implications for expanding its planting area, alleviating the endangered status, and maintaining biodiversity in karst regions.

Materials and Methods

Overview of the experimental site

The experimental site was situated within the experimental base of the Guangxi Zhuang Autonomous Region Forestry Research Institute (108°20′E, 22°55′N), with an altitude of approximately 80 m. It is characterized by a humid subtropical monsoon climate, with an annual average temperature of 21.7 ℃. Annually, there are around 160 precipitation days and the average rainfall is 1,300 mm, mainly concentrated from April to September. There is almost no frost throughout the year. The test soil is the common red soil in afforestation land, collected from the Laohuling area of the Guangxi Zhuang Autonomous Region Forestry Research Institute, with a pH 5.72 $g{\cdot}kg^{-1}$, total nitrogen 0.86 $g{\cdot}kg^{-1}$, total phosphorus 1.15 $g{\cdot}kg^{-1}$, total potassium 5.17 $g{\cdot}kg^{-1}$, total calcium 0.61 $g{\cdot}kg^{-1}$, and organic matter 14.68 $g{\cdot}kg^{-1}$. The terrain of the experimental site was flat, and equipped with a timed sprinkler irrigation device to ensure sufficient moisture for the seedlings.

Experimental design

On December 1st, 2022, one-year-old cuttings of *E. hsienmu* with excellent growth and uniform size (the average seedling height of 28.6 cm and the average ground diameter of 3.56 mm) were planted in perforated plastic pots with a diameter of 25 cm and a depth of 25 cm, with one plant per pot. The substrate was a mixture of perlite, peat soil, and laterite red soil in a weight ratio of 1:1:3, with 8 kg of substrate per pot. During the planting period, the seedlings were subjected to the same maintenance and management. The treatment commenced on March 1st, 2023, following a three-month seedling hardening period. At this time, the average seedling height was 31.3 cm, and the average ground diameter was 4.03 mm. A two-factor interaction design involving nitrogen-calcium was adopted for the experiment, with CK (control group) representing the non-fertilization control group, a total of 10 treatment groups, and 9 replicates for each treatment group.

In determining the nitrogen-calcium levels for testing the nursery substrate and seedlings, were considered the Ca levels in the native karst habitat substrate, and calculated a reasonable fertilization rate (Li, 1983), and accounted for the pre-experimental site conditions. The following nitrogen-calcium fertilization formula was determined for the experiment: the pure nitrogen (N) content was N1 = 1.0 (low), N2 = 2.0 (medium), and N3 = 3.0 (high) g/plant; the pure calcium (Ca) content was Ca1 = 1.5 (low), Ca2 = 3.0 (medium), and Ca3 = 4.5 (high) g/plant. In the experiment, fertilization was performed once a month, on the 1st of each month from March to August, 2023, with 6 applications in total. The fertilization method was irrigation fertilization, where the required fertilizer was dissolved in 500 ml of water solution before application. Soil moisture was maintained by thoroughly watering the pots until drainage occurred without causing overflow. The drainage water collected in the tray was poured back into the pot to prevent nutrient loss, particularly nitrogen and calcium. The final nitrogen-calcium levels after six rounds of fertilization were total N 1.21 $g{\cdot}kg^{-1}$ and total Ca 1.81 $g{\cdot}kg^{-1}$, which were similar to the high-calcium characteristics of the native soil of *E. hsienmu*. The nitrogen fertilizer used in the experiment was urea $CO(NH_2)_2$, with a nitrogen content of 46.4%, and the calcium fertilizer was slaked lime $Ca\ (OH)_2$, with a calcium content of 52.0%.

The experimental design for the different ratios of nitrogen-calcium fertilization is shown in Table 1.

Table 1. Experimental design for the different ratios of nitrogen-calcium fertilization

Treatment	Pure nitrogen（N）	Pure calcium（Ca）
N1Ca1	1.0	1.5
N1Ca2	1.0	3.0
N1Ca3	1.0	4.5
N2Ca1	2.0	1.5
N2Ca2	2.0	3.0
N2Ca3	2.0	4.5
N3Ca1	3.0	1.5
N3Ca2	3.0	3.0
N3Ca3	3.0	4.5
CK	0	0

Note: the data in the table represent the amount of pure nitrogen and pure calcium applied each time; unit: g/plant

Index measurement

Growth Index Measurement: For each treatment group, three *E. hsienmu* seedlings were selected based on tree height and ground diameter which were closest to the mean values of the treatment group. The seedling height and ground diameter were measured before commencement of the experiment on March 1, 2023, and after its completion on August 30, 2023. The measurement tools were a ruler and a vernier caliper.

The following formula was used: the increase in seedling height/ground diameter = the value after the experiment - the value before the experiment.

The soil attached to the roots was rinsed off with distilled water and the roots were then placed in a root scanning sample tray with a thin water layer to ensure the roots were as dispersed as possible. They were scanned using a root scanner (Espon Expression 1680 Scanner, Seiko Espon Corp, Japan) and various root indicators were analyzed, including total root length, average root diameter, total root volume, and total root surface area, using the WinRHIZO root analysis system (Regent Instruments Inc, Quebec, Canada) (Lian *et al.*, 2022; Chen *et a*l., 2021). Biomass indicators, including root biomass, stem biomass, leaf biomass, and total biomass, were determined using the oven-drying and weighing method (Wang *et al.*, 2023).

Photosynthetic Indicators: An LI-6400 portable photosynthesis system (LI-COR, USA) was employed for measurements on August 30, 2023, from 9:00 to 12:00 a.m. The photosynthetically active radiation dose was set to 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the leaf temperature, air flow rate, and CO_2 concentration were set to 30 ℃, 500 $\mu mol \cdot s^{-1}$, and 400 $\mu mol \cdot mol^{-1}$, respectively. Healthy and mature leaves were sampled from *E. hsienmu* seedlings in good physiological condition, avoiding the central vein. Indicators such as net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), transpiration rate (T_r), chlorophyll and carotenoids were determined. The content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll *a+b* (Chl *a+b*), and carotenoids (Car) was measured using the approach of Lichtenthaler (1987). The topmost mature leaves were collected and 0.1 g of fresh leaves were chopped and dipped overnight in a mixture of acetone, absolute ethanol and distilled water ($V_{acetone}/V_{ethano}l/V_{absolute\ distilled\ water}$ = 4.5:4.5:1) for the extraction of photosynthetic pigments. Then, the supernatant was centrifuged at 4,000 rpm for 10-15 minutes. Using the spectrophotometer (Thermo BioMate 3, USA), absorbance values of supernatant were measured under wavelengths of 665, 649, and 470nm, respectively. The pigment content was measured using the following formulas:

$$\text{Chl } a \text{ (mg/L)} = 13.95 \times \text{A665} - 6.88 \times \text{A649} \quad (1)$$
$$\text{Chl } b \text{ (mg/L)} = 24.96 \times \text{A649} - 7.32 \times \text{A665} \quad (2)$$
$$Y\text{Chl } a + b \text{ (mg/L)} = \text{Chl } a + \text{Chl } b \quad (3)$$
$$\text{Car (mg/L)} = (1000 \times \text{A470} - 2.05 \times \text{Chl } a - 114.8 \times \text{Chl } b)/245 \quad (4)$$

where A665, A649, and A470 are the absorbance values at the 665, 649, and 470 nm wavelengths. Then, the content of photosynthetic pigments was calculated as $mg \cdot g^{-1}$ fresh weight.

Statistical analysis

Excel 2019 was used for data processing. Data processing and significance analysis ($p < 0.05$) were performed using DPS 7.05. Range analysis was conducted using SPSS RO, with multiple comparisons based on the LSD.

Results

Effects on the height and ground diameter of E. hsienmu seedlings

The different ratios of nitrogen-calcium fertilization significantly enhanced the height and ground diameter of *E. hsienmu* seedlings (Figure 1).

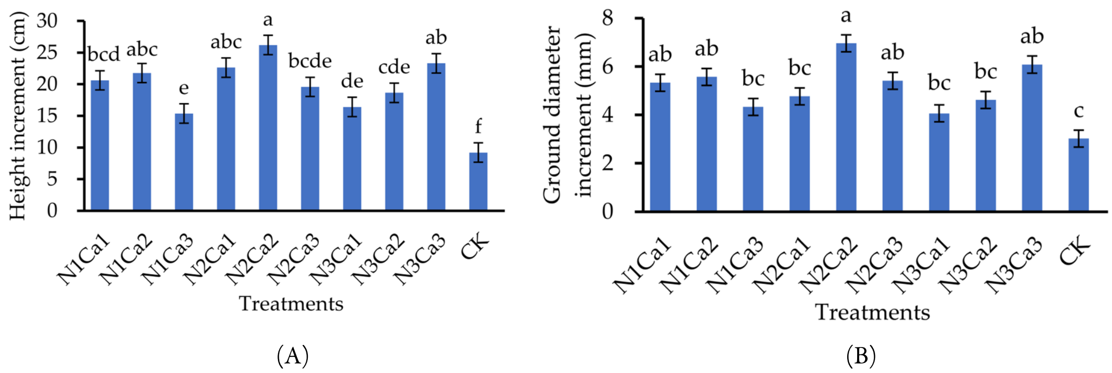

Figure 1. Effects of different ratios of nitrogen–calcium fertilization on changes in (A) height and (B) ground diameter growth of *E. hsienmu* seedlings

Bars are mean values ± standard error (n = 9). Means with different letters indicate significant differences according to Duncan's multiple range test at $p < 0.05$

The analysis of variance indicated that there were significant differences in the increases in height and ground diameter of *E. hsienmu* seedlings among different treatments ($p < 0.05$), and the influence effects of calcium were greater than those of nitrogen (Ca>N). The degree of influence for the three different fertilization rates of nitrogen–calcium on the height of *E. hsienmu* seedlings follows the order N2>N3>N1, Ca2>Ca1>Ca3, and for the ground diameter the order is N2>N1>N3, Ca2>Ca3>Ca1. After different fertilization treatments with different ratios, the height and ground diameter of *E. hsienmu* seedlings reached their maximum values in the N2Ca2 medium-strength nitrogen–calcium treatment group at 26.20 cm and 6.96 mm, respectively. The increase in seedling height after N2Ca2 treatment was not significantly different compared to that after N1Ca2, N2Ca1, and N3Ca3 treatment, but significantly increased by 184.8% compared to the CK. There were significant differences between other treatment groups and the CK. There was no significant difference in the increase in ground diameter of the N2Ca2 treatment group compared to the N1Ca1, N1Ca2, N2Ca3, and N3Ca3 treatment groups, but it significantly increased by 130.5%, 50.6%, 46%, 60.7%, and 71.4% compared to the CK, N3Ca2, N2Ca1, N1Ca3, and N3Ca1 treatment groups, respectively. The imbalanced nitrogen-calcium ratio groups N1Ca3 and N3Ca1 had lower seedling height and ground diameter increments than the other treatment groups, but these values were significantly higher than in the CK. Evidently, the different ratios of nitrogen-calcium fertilization in-creases the height and ground diameter of *E. hsienmu* seedlings to varying degrees, and the N2Ca2 treatment group with the combination of medium N and Ca doses appears to be optimal for promoting growth based on the height and ground diameter of *E. hsienmu* seedlings.

Effects on biomass of E. hsienmu seedlings

The different ratios of nitrogen-calcium fertilization significantly enhanced biomass accumulation in the *E. hsienmu* seedlings (Figure 2).

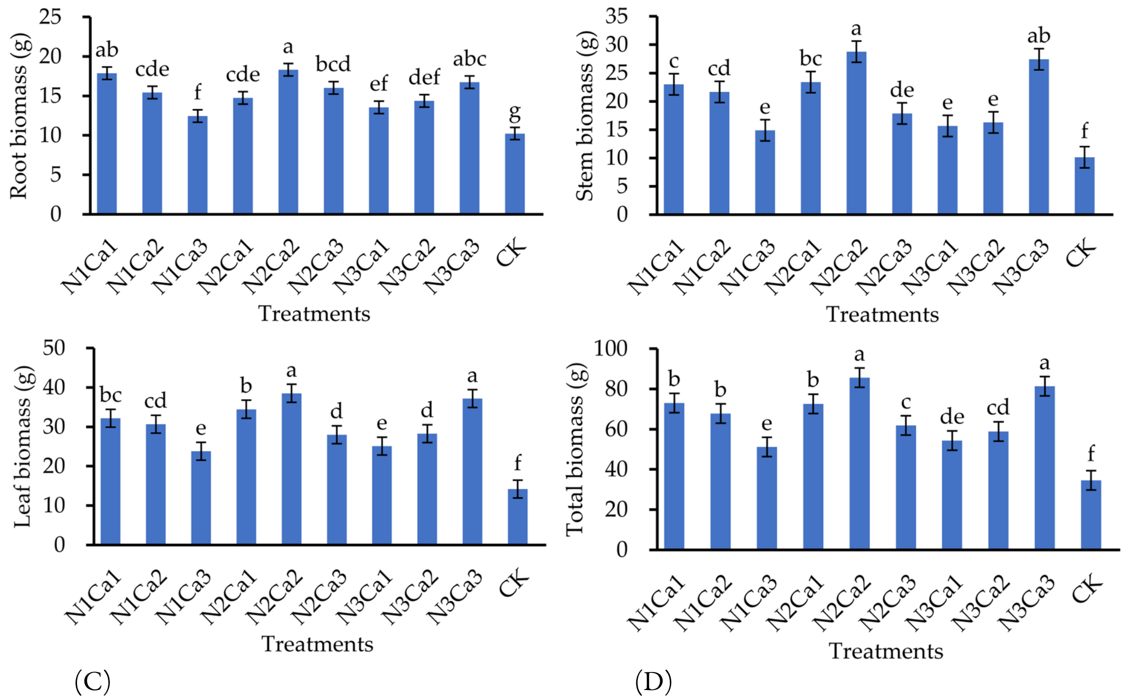

Figure 2. Effects of different ratios of nitrogen-calcium fertilization on the (A) root, (B) stem, (C) leaf, and (D) total biomass of *E. hsienmu* seedlings

Bars are mean values ± standard error (n = 9). Means with different letters indicate significant differences according to Duncan's multiple range test at $p < 0.05$

The analysis of variance revealed that there were significant differences in the biomass of *E. hsienmu* seedlings among different treatment groups ($p < 0.05$). The influence effects of nitrogen-calcium on the root, stem, leaf, and total biomass were all Ca > N. The degree of influence for the three different fertilization rates of nitrogen–calcium on both the root and stem biomass of *E. hsienm*u seedlings follows the order N2>N1>N3, Ca2>Ca1>Ca3, while for both the leaf and total biomass the order was N2>N3>N1, Ca2>Ca1>Ca3. After different fertilization ratio treatments, the biomass of *E. hsienmu* seedlings reached its highest values following the N1Ca1, N2Ca2, and N3Ca3 treatments in the synergistic treatment group under the same nitrogen conditions and was significantly higher than in the CK. Among them, the root biomass of *E. hsienmu* seedlings in the N2Ca2 treatment group reached a maximum value of 18.33 g, which was not significantly different from that of the N3Ca3 and N1Ca1 groups, but represented a significant increase of 79.0% compared to the CK. The stems, leaves, and total biomass of *E. hsienmu* seedlings in the N2Ca2 treatment group reached maximum values of 28.78 g, 38.53 g, and 85.64 g, respectively, which were significantly increased by 183.5%, 171.3%, and 147.6%, respectively, compared to the CK. The biomass of the seedlings in the imbalanced nitrogen-calcium ratio groups receiving N1Ca3 and N3Ca1 was lower than in the other treatment groups but still significantly higher than in the CK. Evidently, the different ratios of nitrogen-calcium fertilization increases the biomass of various *E. hsienmu* tissues to different ex-tents, and the N2Ca2 medium-strength nitrogen-calcium treatment group represents the optimal combination for effectively promoting biomass growth in *E. hsienmu* seedlings.

Effects on the root-shoot ratio of E. hsienmu seedlings

The root-shoot ratio refers to the ratio of the weight of the underground part (roots) to the aboveground part (stems, leaves, etc.) of a plant (Lichtenthaler, 1987). The different ratios of nitrogen-calcium fertilization significantly decreased the root–shoot ratio of *E. hsienmu* seedlings (Figure 3). The analysis of variance indicated that there were significant differences in the root-shoot ratio of *E. hsienmu* seedlings among different treatments ($p < 0.05$), with Ca>N regarding influence effects. The degree of influence for the three different fertilization rates of nitrogen-calcium on the root-shoot ratio of *E. hsienmu* seedlings follows the order N2>N1>N3, Ca3>Ca1>Ca2. The root-shoot ratio of *E. hsienmu* seedlings was lower in the N2Ca1, N3Ca3, and N2Ca2 treatment groups, and there was no significant difference among these three groups; moreover, the root–shoot ratio of those groups was 39.4%, 38.4%, and 35.1% lower than in the CK, respectively. It can thus be seen that all treatment groups could reduce the root- shoot ratio to varying degrees, and the balanced ratio of medium and high contents of nitrogen-calcium could more effectively reduce the root–shoot ratio of *E. hsienmu* seedlings.

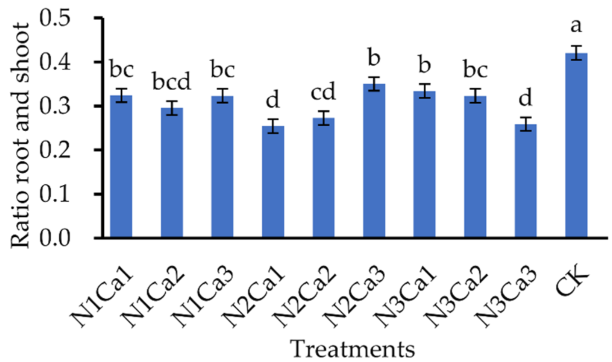

Figure 3. Effects of different ratios of nitrogen–calcium fertilization on the root–shoot ratio of *E. hsienmu* seedlings

Bars are mean values ± standard error (n = 9). Means with different letters indicate significant differences according to Duncan's multiple range test at $p < 0.05$

Effects on the root system of E. hsienmu seedlings

The effects of different ratios of nitrogen-calcium fertilization were characterized based on the total root length, average root diameter, total root volume, and total root surface area of the root system of *E. hsienmu* seedlings, and the values were higher than those of the CK in all cases (Table 2). The analysis of variance reveals that the total root length, average root diameter, total root volume, and total root surface area of *E. hsienmu* seedlings were significantly different among different treatments ($p < 0.05$), with Ca>N in all cases regarding influence effects. The degree of influence for the three different fertilization rates of nitrogen-calcium on the total root length, total root volume, and total root surface area of *E. hsienmu* seed-lings follows the order N2>N1>N3, Ca2>Ca3>Ca1, while for the average root diameter, the order is N2>N1>N3, Ca2>Ca1>Ca3. After different fertilization ratio treatments, all root system indicators in the *E. hsienmu* seedlings reached their highest values after N1Ca1, N2Ca2, and N3Ca3 treatment in the synergistic treatment groups and were significantly higher than the corresponding values for the CK. Among them, the average root diameter of the N2Ca2 treatment group was the highest, with an overall value of 2.41 mm, which was not significantly different from the N1Ca1, N2Ca3, and N3Ca3 treatment groups, but represented a significant increase of 54.5% compared to the CK. The total root length, total root volume, and total root surface area of the N2Ca2 treatment group reached the highest overall values of 396.53 cm, 18.16 cm^3, and 299.90 cm^2, respectively, which were not significantly different from N1Ca1 and N2Ca3 treatment groups, but were significant increases of 31.2%, 212.0%, and 102.6% compared to the CK, respectively. The root system indicators of the N1Ca3 and N3Ca1 imbalanced nitrogen-calcium ratio groups were lower than those of the other treatment groups but still higher than in the CK. It is evident that the fertilization treatment with interaction based on the medium contents of nitrogen-calcium increased the total root length, average root diameter, total root volume, and total root surface area of *E. hsienmu* seedlings to differing degrees, with total root volume increasing the most. Of all the combinations, the N2Ca2 treatment thus appears to be optimal for promoting growth of the root system of *E. hsienmu* seedlings.

Table 2. Effects of different ratios of nitrogen-calcium fertilization on the total root length, average root diameter, total root volume, and total root surface area of *E. hsienmu* seedlings

Treatment	Total root length (cm)	Average root diameter (mm)	Total root volume (cm^3)	Total root surface area (cm^2)
N1Ca1	381.98±4.12ab	2.33±0.16ab	16.39±2.15ab	279.13±19.32ab
N1Ca2	357.17±8.65bcd	2.03±0.10bcde	11.68±1.36cde	228.27±14.81cde
N1Ca3	328.72±4.79ef	1.68±0.08f	7.34±0.78fg	173.64±10.66fg
N2Ca1	346.55±9.50cde	1.88±0.10def	9.62±0.98defg	203.99±10.85ef
N2Ca2	396.53±7.00a	2.41±0.13a	18.16±1.88a	299.90±15.24a
N2Ca3	370.76±7.78abc	2.25±0.08abc	14.85±1.31abc	262.64±14.22abc
N3Ca1	334.18±23.23de	1.72±0.07ef	7.82±0.14efg	180.88±15.11fg
N3Ca2	350.42±3.99cde	1.96±0.05cdef	10.54±0.45def	215.21±4.04def
N3Ca3	364.51±5.90bc	2.17±0.15abcd	13.64±1.97bcd	248.70±20.02bcd
CK	302.15±3.31f	1.56±0.10g	5.82±0.70g	148.01±9.31g

The values presented in the table are mean values (n=9) across treatments. In each column, means within the same factor interaction followed by different letters indicate significant differences ac-cording to Duncan's multiple range test at $p < 0.05$

Effects on the net photosynthetic rate (P_n) and stomatal conductance (G_s) of E. hsienmu seedlings

Under the different ratios of nitrogen-calcium fertilization treatment, the net photosynthetic rate (P_n) and stomatal conductance (G_s) of the leaves of *E. hsienmu* seedlings were enhanced to varying degrees (Figure 4). The analysis of variance indicated that there were significant differences in the P_n and G_s of *E. hsienmu* seedlings among different treatments ($p < 0.05$). The influence effects of nitrogen-calcium were N>Ca for P_n and Ca>N for G_s. The degree of influence for the three different fertilization rates used on both the P_n and G_s of *E. hsienmu* seedlings follows the order N2>N1>N3, Ca2>Ca3>Ca1. After using different fertilization ratios, the *Pn* and *Gs* of *E. hsienmu* seedlings reached their highest values at N1Ca1, N2Ca2, and N3Ca3 in the balanced the nitrogen-calcium treatment groups, and were significantly higher than in the CK. Among them, the maximum P_n value of *E. hsienmu* seedlings in the N2Ca2 treatment group was 13.96 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which was significantly different from other treatment groups and represented an increase of 104.4% compared to the CK. The maximum G_s value of *E. hsienmu* seedlings in the N2Ca2 treatment group was 0.10 $mol \cdot m^{-2} \cdot s^{-1}$, which was not significantly different from that of the N1Ca1, N2Ca1, N2Ca3, and N3Ca3 treatment groups, but represented a significant increase of 120.0% compared to the CK. Evidently, the different ratios of nitrogen-calcium fertilization have varying degrees of influence on plant photosynthesis. The fertilization combination used in the N2Ca2 treatment group appears optimal for promoting increases in the P_n and G_s of *E. hsienmu*.

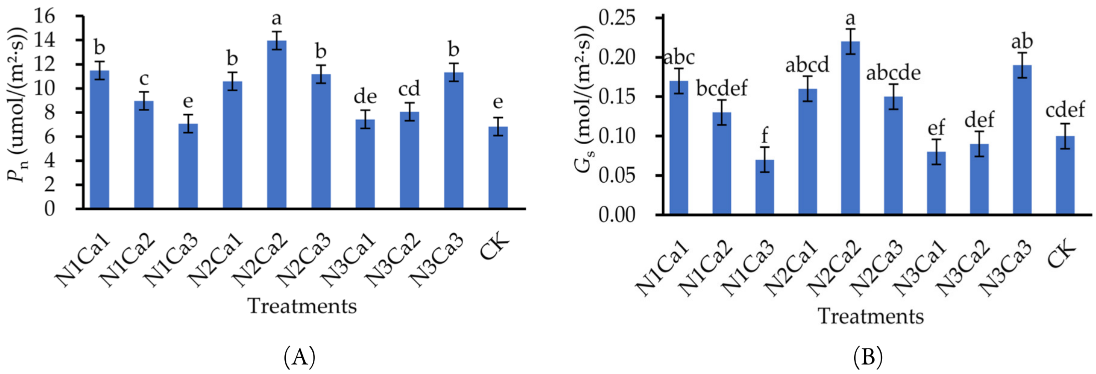

Figure 4. Effects of different ratios of nitrogen–calcium fertilization on the (A) net photosynthetic rate (P_n) and (B) stomatal conductance (G_s) of *E. hsienmu* seedlings

Bars are mean values ± standard error (n = 9). Means with different letters indicate significant differences according to Duncan's multiple range test at $p < 0.05$

Effects on the intercellular CO_2 concentration (C_i) and transpiration rate (T_r) of E. hsienmu seedlings

The different ratios of nitrogen-calcium fertilization exerted varying degrees of influence on the intercellular CO_2 concentration (C_i) and transpiration rate (T_r) of *E. hsienmu* seedlings (Figure 5). The analysis of variance demonstrated that there were significant differences in the C_i and T_r of *E. hsienmu* seedlings among different treatments ($p < 0.05$), and Ca > N in all cases regarding influence effects. The degree of influence for the three different fertilization rates tested on the C_i and T_r of *E. hsienmu* seedlings follows the order N2>N3>N1, Ca2>Ca3>Ca1. After using different fertilization ratios, the C_i and T_r of *E. hsienmu* seedlings reached their highest values in the N1Ca1, N2Ca2, and N3Ca3 synergistic treatment groups under the same nitrogen conditions and were significantly higher than in the CK. In particular, the C_i of the N2Ca2 treatment group reached the highest value of 279.51 $\mu mol \cdot mol^{-1}$, which was not significantly different from that of the N3Ca3, N2Ca1, N1Ca2, and N1Ca1 treatment groups, but a significant increase of 57.4% compared to the CK. The T_r of the N2Ca2 treatment group reached a maximum value of 1.70 $mmol \cdot m^{-2} \cdot s^{-1}$, which was significantly different from other treatment groups and an increase of 147.6% compared to the CK. The C_i and T_r values of the imbalanced nitrogen-calcium ratio groups receiving N1Ca3 and N3Ca1 treatment were lower than those of the other treatment groups. While the C_i was significantly higher than that of the CK, the T_r value showed no significant difference compared to the CK. Evidently, the fertilization combination for the N2Ca2 treatment group, with medium-strength nitrogen-calcium doses, appears optimal for promoting the C_i and T_r of *E. hsienmu* seedlings.

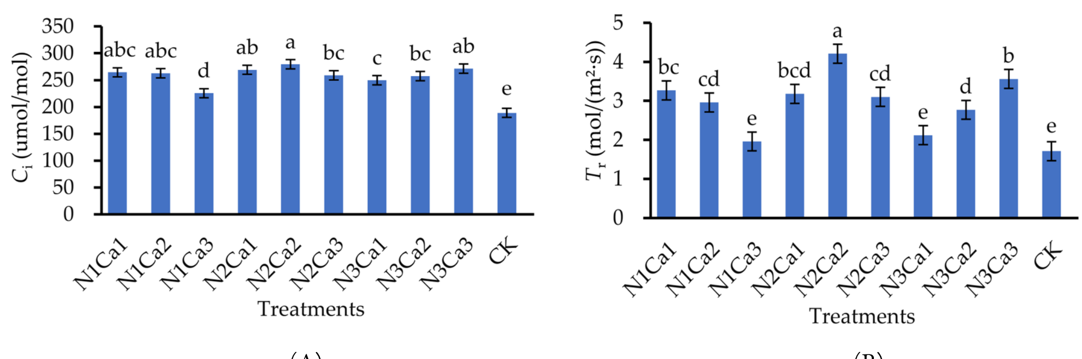

Figure 5. Effects of different ratios of nitrogen–calcium fertilization on the (A) intercellular CO_2 concentration (C_i) and (B) transpiration rate (T_r) of *E. hsienmu* seedlings

Bars are mean values ± standard error (n = 9). Means with different letters indicate significant differences according to Duncan's multiple range test at $p < 0.05$

Effects chlorophyll a, chlorophyll b, chlorophyll a+ b, and carotenoid contents in E. hsienmu seedlings

Under different nitrogen -calcium fertilization treatments, the contents of chlorophyll *a*, chlorophyll *b*, chlorophyll *a+ b*, and carotenoids in the leaves of *E. hsienmu* seedlings were all enhanced (Figure 6).

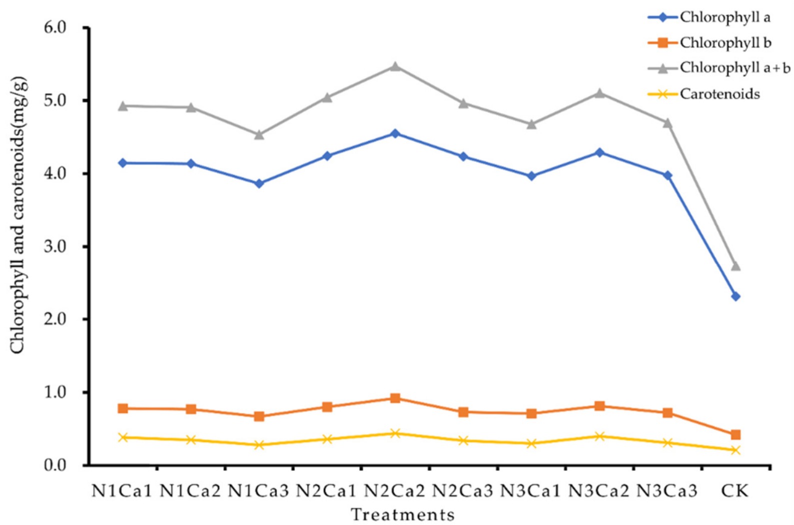

Figure 6. Effects of different ratios of nitrogen-calcium fertilization on the contents of chlorophyll *a*, chlorophyll *b*, chlorophyll *a+ b*, and carotenoids in *E. hsienmu* seedlings

The values presented in the table are mean values (n = 9) across treatments. In each column, means within the same factor interaction followed by different letters indicate significant differences ac-cording to Duncan's multiple range test at $p < 0.05$

The analysis of variance indicated that there was no significant difference in chlorophyll content between different nitrogen–calcium treatment groups ($p > 0.05$), but there were significant differences in chlorophyll content between each treatment group and the CK ($p < 0.05$). After using different fertilization ratios, the chlorophyll and carotenoid contents of *E. hsienmu* seedlings reached their highest values at N1Ca1, N2Ca2, and N3Ca3 in the balanced nitrogen-calcium treatment groups under the same nitrogen conditions, and were significantly higher than those in the CK. The highest values of chlorophyll *a*, chlorophyll *b*, chlorophyll *a+ b*, and carotenoid contents were achieved in the N2Ca2 treatment group, at 4.55 $mg \cdot g^{-1}$, 0.92 $mg \cdot g^{-1}$, 5.47 $mg \cdot g^{-1}$, and 0.44 $mg \cdot g^{-1}$, respectively. Compared with the CK (2.31 $mg \cdot g^{-1}$, 0.42 $mg \cdot g^{-1}$, 2.73 $mg \cdot g^{-1}$, and 0.21 $mg \cdot g^{-1}$), they were increased by 97.0%, 119.0%, 100.4%, and 109.5%, respectively. Evidently, the fertilization combination used in the N2Ca2 treatment group appears optimal for increasing the contents of chlorophyll *a*, chlorophyll *b*, chlorophyll *a+ b*, and carotenoids in *E. hsienmu* seedlings.

Discussion

Appropriate fertilization can alleviate deficiencies in soil nutrients, facilitate the absorption of various nutritional elements by container seedlings, enhance photosynthesis, and be conducive to the growth of container seedlings (Gong *et al.*, 2024; Wang *et al.*, 2024). However, excessive fertilization can alter the properties of the substrate. Excessive nitrogen may inhibit the activity of plant RuBP, reduce the efficiency of chlorophyll in utilizing light energy, and ultimately suppress plant growth (Guo *et al.*, 2005; Du *et al.*, 2022; Su *et al.*, 2025;). As essential mineral nutrients for plant growth and development, nitrogen and calcium can effectively promote this processes when properly balanced (Yue *et al.*, 2021). This study showed that that the ratio in medium amounts of nitrogen-calcium fertilization significantly promotes the growth and development of *E. hsienmu* seedlings. Regarding influence effects, Ca>N except in the case of the net photosynthetic rate, where N>Ca, which is consistent with *E. hsienmu* being calciphilic (Tan *et al.*, 2018).

The growth of a plant can be directly reflected by the height and ground diameter of its seedlings (Pan *et al.*, 2024). The results of this experiment showed that under different nitrogen treatments, the height and ground diameter increment of the *E. hsienmu* seedlings reached their maximum values in the N2Ca2 (medium-strength nitrogen and calcium treatment group). In contrast, the seedlings in the N1Ca3 and N3Ca1 (imbalanced nitrogen-calcium ratio groups) were lower than in the other treatment groups but significantly higher than in the CK. This indicates that increases in the plant height and ground diameter of the *E. hsienmu* seedlings are more likely with moderate-ratio nitrogen-calcium fertilization, whereas large differences in nitrogen-calcium are not conducive to the growth of *E. hsienmu* seedlings. This probably because when either nitrogen or calcium are too low, the utilization and transformation of high calcium or high nitrogen cannot be enhanced in the plants. This result is in accordance with the outcomes of fertilization research by Xiaohang Weng *et al.* (2021; 2023) and Xiangjun Li *et al.* (2021) on poplar and *Pinus Sylvestris* var. *Mongolica*.

The root system is an important organ for plants to absorb water and nutrients and, in addition to playing a very important role in the growth of plants, it can indicate occurrence of several environmental changes (Wei *et al.*, 2006). In this study it was found that the fertilization with a balanced nitrogen-calcium ratio can significantly promote the growth of root hairs, greatly increasing the contact area between seedlings and soil. On the surface of plant root hairs, proton pumps actively transport protons into the soil, thereby acidifying the soil to achieve the mildly acidic environment preferred by *E. hsienmu*. Meanwhile, proton pumps also cotransport with calcium ions, potassium ions, sodium ions, etc., enabling cations and protons to be absorbed by plants from the soil together. This fully improves the nutrient utilization efficiency of plants and promotes seedlings to accumulate more nutrients. This result was similar to the findings of Xianbang Wang *et al.* (2023) regarding the fertilization of seedlings and its effects on the growth and root system of *Cinnamomum kanehirae*.

The ratio of plant roots to stems and leaves can also better reflect the balance of water and nutrient metabolism in plants (Lian *et al.*, 2022; Tang *et al.*, 2023). Yan Ao *et al.* (2019) found that the higher the root–shoot ratio, the more developed the root system, the stronger the ability to adapt to adverse conditions, and the higher the quality of seedlings. However, in the present study it was observed that the root-shoot ratio of *E. hsienmu* seedlings treated with different nitrogen-calcium ratios were lower than in the CK indicating that moderate fertilization can reduce the root-shoot ratio of plants. Moreover, the treatment groups with smaller root-shoot ratios had better growth trends, which is contrary to other research conclusions (Ao *et al.*, 2019). This may be because as the root weight increases, more nutrients are absorbed and transported, thus promoting the rapid growth of stems and leaves, and resulting in a much greater increase in the stem-to-leaf weight ratio than in the root weight, so the root-shoot ratio is smaller.

Biomass comprehensively reflects various growth indicators of seedlings, serving as the main manifestation of energy acquisition in plants. It holds significant importance for the growth, development, and structural formation of plants (Li *et al.*, 2023). The results showed that the biomass of *E. hsienmu* seedlings presented higher values in the treatment groups with balanced nitrogen-calcium fertilization ratios (N1Ca1, N2Ca2, and N3Ca3). Among them, N2Ca2 was the optimal treatment group, indicating that the synergistic nitrogen–calcium fertilization ratio helps protect plants, enhance organic matter metabolism, provide energy for plant growth and development, and affect plant trait characteristics. This result is in accordance with the results of fertilization research on peanuts and *Pinus yunnanensis* by Zhaoyang You *et al.* (2024) and Yalin Li *et al.* (2021).

Photosynthesis, as the fundamental process of nutrient synthesis in plants, essentially refers to the efficient conversion of light energy into chemical energy. It can indirectly reflect the growth rate of plants, affect the synthesis, accumulation, and distribution of photosynthetic products, and ultimately influence plant growth (Zhang *et al.*, 2019). The analyses reveald that the photosynthesis of *E. hsienmu* seedling leaves under nitrogen-calcium interaction was significantly higher than that of the CK. Each photosynthetic index showed higher values in the treatment groups with balanced ratio fertilization (N1Ca1, N2Ca2, and N3Ca3), among which N2Ca2 the optimal was the treatment group. The results of this study indicate that there was significantly higher photosynthesis in *E. hsienmu* seedling leaves under the influence of different ratios of fertilization than for the CK. With different nitrogen treatments, the highest values of various photosynthetic indicators were achieved in the groups that received N1Ca1, N2Ca2, and N3Ca3 synergistic treatment, with N2Ca2 being the optimal treatment. This may be because appropriate nitrogen-calcium synergy maintains the balance of nutrients in the plant and increases the activity of soluble proteins and RuBp carboxylase, thereby maintaining a high level of photosynthesis, being ~~is~~ more conducive and promoting the operation and accumulation of plant photosynthetic products (Zhang *et al.*, 2019; Zhou *et al.*, 2023). The photosynthetic indicators of the seedlings in the N1Ca3 and N3Ca1 nitrogen–calcium ratio treatment groups were lower than in the other treatment groups but significantly higher than in the CK, indicating that the more calcium is applied under low nitrogen treatment, the less favourable it is for the photosynthesis of *E. hsienmu* seedlings. This might be because if nitrogen cooperation could not keep up with high calcium concentrations, the excess calcium can have a toxic effect on *E. hsienmu* seedlings, causing their stomata to close, and damaging the inner membranes of chloroplasts, destroying the thylakoid membrane, interfering with leaf photosynthesis and ultimately inhibiting plant growth (Long *et al.*, 2005). However, excess nitrogen can also stress the cells of *E. hsienmu*, inhibiting the growth of *E. hsienmu* seedlings (Li *et al.*, 2013). This is in accordance with the results of Xiaohang Weng *et al.* (2021) on the synergy of nitrogen-calcium in poplar.

Stomata function to control the inflow of inorganic carbon into chloroplasts, reaching the carboxylation center where Rubisco is located. In the Calvin-Benson cycle, the absorbed carbon dioxide is converted into glyceraldehyde 3-phosphate (G3P). Plants utilize G3P to synthesize sucrose and starch, which are used to build biomass (Ma *et al.*, 2022; Zhen *et al.*, 2022). In this study, there was no significant difference in the effects of the N3Ca1 and N1Ca3 treatments on stomatal conductance compared to the control, and the leaf biomass was expected to lower than in the control group. However, in this experiment, there was a significant increase in leaf biomass compared to the control group. This may be because the calcium can promote the synthesis of soluble sugars and starch (Li *et al.*, 2021). Moderate nitrogen can also help plants maintain high levels of sucrose, fructose, and glucose, which are beneficial for starch synthesis (Yuan *et al.*, 2023), thereby generating more biomass and increasing leaf biomass. This result was similar to the research results of Xiangjun, Li *et al.* (2021) and Hailong Liu *et al.* (2009). Chlorophyll, as an important photosynthetic pigment, and its index values directly reflect the ability of leaves to absorb and transform light energy (Yuan *et al.*, 2023). Chlorophyll absorbs light energy and converts carbon dioxide and water into organic matter and oxygen under light irradiation, thereby promoting the accumulation of plant biomass. The results of this study show that there was no significant difference in chlorophyll content between the treatment groups, though they all exhibited significantly higher values than in the CK. The highest value was achieved in the synergistic N2Ca2 treatment group. Combined with the net photosynthetic rate of *E. hsienmu* seedling leaves in Figure 4(a), it indicates that appropriate nitrogen-calcium interaction is conducive to the increase of chlorophyll content in *E. hsienmu* seedlings. Through chlorophyll, plants efficiently absorb light energy in the range of 400-700 nm, exciting electrons to an excited state, which then enter the transfer of Photosystem Ⅰ (PSⅠ) and Photosystem Ⅱ (PSⅡ). Through a series of electron transfer chains, electrons not only drive proton pumps to transport protons from the stroma side to the lumen side, thereby forming a proton gradient, but also provide energy for ATP synthase to synthesize ATP. Ultimately, these electrons are received by electron acceptors (Gan *et al.*, 2023). After a series of reduction reactions, CO_2 was successfully converted into organic substances such as glucose, thereby promoting the accumulation of dry matter in *E. hsienmu* seedlings. This result is consistent with the findings of Zhenxing Wang *et al.* (2012) on *Phoebe bournei* young trees.

Conclusions

Based on the characteristics of various indicators in this experiment, it can be inferred that fertilization with an appropriate ratio of nitrogen-calcium can exert a remarkable promoting effect on the growth and photosynthetic characteristics of *E. hsienmu* seedlings. In the N2Ca2 treatment group, where the levels of nitrogen-calcium were 2.0 g N and 3.0 g Ca per plant (medium-strength nitrogen-calcium), the one-year-old *E. hsienmu* seedlings exhibited the highest rates of growth and photosynthesis based on the indicators. Extreme ratios of high nitrogen and low calcium, or low nitrogen and high calcium, were not beneficial for the growth and development of *E. hsienmu* seedlings. Studying the fertilization ratio of nitrogen-calcium can aid in the production of fertilizer that enables *E. hsienmu* to grow normally in ordinary afforestation areas, thereby expanding its planting area and alleviating its endangered status. This fertilization formula is universal and can also be used as a reference for introducing and cultivating other trees growing in limestone areas in conventional afforestation soil.

Authors' Contributions

Conceptualization, X.L. and J.P.; methodology, X.L; validation, X.L., Y.H. and X.H.; investigation, X.L. and J.P.; data curation, X.L., Y.H. and X.H.; writing-original draft preparation, X.L.; writing-reviewand editing, J.P.; Supervision, J.P.

All authors have read and agreed to the published version of the manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Ao Y, Liu J, Chen H, Yuan F, Liu J, Zhang X (2019). Annual growth rhythm and character correlation analysis of 1-year-old *Xanthoceras sorbifolium* seedlings from different provenances. Journal of Northwest Forestry University 34(3):91-97. *https://doi.org/10.3969/j.issn.1001-7461.2019.03.14*

Cassim BMAR, Besen MR, Kachinski WD, Macon CR, Almeida Junior JHV, Sakurada R, … Batista MA (2022). Nitrogen fertilizers technologies for corn in two yield environments in South Brazil. Plants 11:1890. *https://doi.org/10.3390/plants11141890*

Chen FY, Mo P, Kong Z, Tian L, Duan H, Li M, … Sheng G (2021). Deep placement of nitrogen fertilizer affects grain field, nitrogen recovery efficiency, and root characteristics in direct-seeded rice in South China. Journal of Plant Growth Regulation 40(1):379-387.

Chrysargyris A, Xylia P, Zengin G, Tzortzakis N (2024). Purslane (*Portulaca oleracea* L.) growth, nutritional, and antioxidant status under different nitrogen levels in hydroponics. Horticulturae 10:1007. *https://doi.org/10.3390/horticulturae10091007*

De Souza M, Barcelos JPdQ, Rosolem CA (2023). Synergistic effects of subsoil calcium in conjunction with nitrogen on the root growth and yields of maize and soybeans in a tropical cropping system. Agronomy 13:1547. *https://doi.org/10.3390/agronomy13061547*

Doyle JW, Nambeesan SU, Malladi A (2021). Physiology of nitrogen and calcium nutrition in blueberry (*Vaccinium* sp.). Agronomy 11:765. *https://doi.org/10.3390/agronomy11040765*

Du K, Zhang Y, Qin S, Wang L, Zhang B, Wang S (2022). Effects of nitrogen fertilization on physiological response of maize to soil salinity. Agriculture 12:877. *https://doi.org/10.3390/agriculture12060877*

Esteves E, Maltais-Landry G, Zambon F, Ferrarezi R, Kadyampakeni D (2021). Nitrogen, calcium, and magnesium inconsistently affect tree growth, fruit yield, and juice quality of huanglongbing-affected orange trees. HortScience 56(10):1269-1277. *https://doi.org/10.21273/HORTSCI15997-21*

Gan Y, Guo L, Gao C, Song W, Wu J, Liu L, Chen X (2023). Light-driven CO_2 conversion system: construction, optimization and application. Chinese Journal of Biotechnology 39(06):2390-2409. *https://doi.org/10.13345/j.cjb.221008*

Gong X, Wang X, Dang K, Zhang Y, Ji X, Long A, … Feng B (2024). Nitrogen availability of mung bean in plant-soil system and soil microbial community structure affected by intercropping and nitrogen fertilizer. Applied Soil Ecology 203:105692. *https://doi.org/10.1016/j.apsoil.2024.105692*

Hao H, Huang Z, Peng Y, Cao Y, Shen W, Tan C (2021). Phenotypic characters and germination rule of *Excentrodendron hsienmu* fruit and seed. Journal of Fujian Forestry Science and Technology 48(4):40-46. *https://doi.org/10.13428/j.cnki.fjlk.2021.04.009*

Hou Y, Shen W, Huang X, Ou Z (2018). Research status and development trend of *Excentrodendron hsienmu*. Forestry and Environmental Science 34(5):113-117.

Li H, Liu S, Wang Y, Liu J, Feng L, Chen X (2013). Effects of calcium on photosynthetic characteristics and quality of garlic. Acta Horticulturae Sinica 40(06):1169-1177. *https://doi.org/10.16420/j.issn.0513-353x.2013.06.023*

Li K, Jiang Z, Huang Y, Xiang W, Lu S, Ye Z, Su Z (2008). Dynamics of dominant population and its influence on karstification in Southwest Guangxi, China. Acta Geoscientica Sinica (02):253-259.

Li K, Xiao X, Li B, Wang L, Long S, Yang B, Yang Y (2023). Effects of combined application of nitrogen, phosphorus and potassium fertilizers on the growth of *Toona ciliata* Roem. Journal of Central South University of Forestry & Technology 43(1):50-56.

Li R (1983). Determine the economically reasonable fertilization amount based on the fertilizer effect function. Chinese Journal of Soil Science (3):24-29. *https://doi.org/10.19336/j.cnki.trtb.1983.03.008*

Li X, Zhang G, Li H, Sun Y, Huo Y, Huang S, … Zhou Y (2021). Effects of exogenous calcium on the growth and physiological characteristics of *Pinus sylvestris* var. *Mongolica* seedlings in sandy land. Chinese Journal of Soil Science 52(5):1095-1103. *https://doi.org/10.19336/j.cnki.trtb.2021032903*

Li Y, Wang J, Du Y, Chen Q (2024). Optimization of an aerated fertilizer irrigation application scheme for tomato photosynthesis, yield and quality in Xi'an, China. Scientia Horticulturae 338:113743. *https://doi.org/10.1016/j.scienta.2024.113743*

Li Y, Xu Y, Tang J, He Y, Wang D, Li J, Cai N (2023). Effect of different nitrogen and phosphorus combinations on seedling growth and nutrient accumulation of *Pinus yunnanensis* seedlings. Journal of Zhejiang A&F University 40(1):115-125. *https://doi.org/10.11833/j.issn.2095-0756.20220223*

Li Z, Zhang Y, Han L, Xu J (2013). The interactive effects of nitrogen and calcium on photosynthetic characteristics and chlorophyll fluorescence parameters of nectarine under protected culture. Journal of Plant Nutrition and Fertilizers 19(4):893-900. *https://doi.org/10.11674/zwyf.2013.0415*

Lian H, Qin C, Zhao Q, Begum N, Zhang S (2022). Exogenous calcium promotes growth of adzuki bean (*Vigna angularis* Willd.) seedlings under nitrogen limitation through the regulation of nitrogen metabolism. Plant Physiology and Biochemistry 190:90-100. *https://doi.org/10.1016/j.plaphy.2022.08.028*

Lichtenthaler HK (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148:350-382. *https://doi.org/10.1016/0076-6879(87)48036-1*

Liu H, He P, Jin J, Li W, Zhang K, Wang X, … Hou Y (2009). Effects of nitrogen nutrition on sugar and starch accumulation of high starch maize and common maize. Journal of Plant Nutrition and Fertilizers 15(03):493-500.

Liu M (2024). Effects of combined application of nitrogen and calcium on growth and physiological characteristics of *Morus alba* L. seedlings. MSc thesis. Shenyang Agricultural University, China.

Long M, Tang X, Yu W, Liao Y, Huang W, Qin R (2005). Effects of different calcium levels on photosynthesis and protective enzyme activities of melon leaves. Guihaia (01):77-82.

Ma X, Xuan Z, Yang H, Zhang K, Gao Y, Tan Z, Wang X (2022). Effects of water-nitrogen coupling on photosynthetic physiological characteristics of sand-cultured cucumber. Shandong Agricultural Sciences 54(05):110-119. *https://doi.org/10.14083/j.issn.1001-4942.2022.05.016*

Ozyhar T, Marchi M, Facciotto G, Bergante S, Luster J (2022). Combined application of calcium carbonate and NPKS fertilizer improves early-stage growth of poplar in acid soils. Forest Ecology and Management 514:120211. *https://doi.org/10.1016/j.foreco.2022.120211*

Pan Y, Yang M, Liu W, Xia Y, Zeng S, He R, … Yan B (2024). Effects of different formula fertilization on the growth and physiological characteristics of *Quercus chungii* seedlings. Journal of Central South University of Forestry & Technology 44(06):69-80. *https://doi.org/10.14067/j.cnki.1673-923x.2024.06.007*

Peng Y, Huang Z, Hao H, Shen W, Cao Y, Tan C, Ou Z, Zhen W (2016). The growth rhythm of young plantation of *Excentrodendron hsienmu* cutting seedling. Guangxi Forestry Science 45(3):248-252. *https://doi.org/10.19692/j.cnki.gfs.2016.03.003*

Qiu S, Zhu X, Yue Z, Tao X, Zhou K (2024). Correlation analysis and estimation of chlorophyll content with color characteristics for *Ginkgo biloba* under water-nitrogen interactions. Journal of Northwest Forestry University 39(4):15-23. *https://doi.org/10.3969/j.issn.1001-7461.2024.04.02*

Shen W, Li Z, Peng Y, Cao Y, Huang Z, Hao H (2014). Study on impact factors of cutting propagation of *Excentrodendron hsienmu*. Journal of West China Forestry Science 43(6):24-28. *https://doi.org/10.16473/j.cnki.xblykx1972.2014.06.017*

Su L, Bai T, Yu H, Wu G, Tan L (2025). The effect of nitrogen nutrition on the growth and photosynthesis fluorescence characteristics of jackfruit seedlings. South China Fruits 34(2):163-173. *https://doi.org/10.13938/j.issn.1007-1431.20180502*

Tan C, Shen W, Ou Z, Peng Y, Cao Y, Hao H (2018). Effects of nitrogen application on the photosynthetic physiology of *Excentrodendron hsienmu* in karst area under Cd stress. Journal of Northwest Forestry University 33(1):26-30. *https://doi.org/10.3969/j.issn.1001-7461.2018.01.04*

Tan Y, Shen W, Fu Z, Zheng W, Ou Z, Tan Z, … He F (2019). Effect of environmental factors on understory species diversity in Southwest Guangxi *Excentrodendron tonkinense* forests. Biodiversity Science 27(9):970-983. *https://doi.org/10.17520/biods.2019133*

Tang X, Kang Y, Liang X, Ma D, Wang L (2022). Effects of N, P and K proportional fertilization on the physiological and photosynthetic characteristics of *Tsoongiodendron odorum* seedlings. Journal of Northwest Forestry University 37(04):37-42. *https://doi.org/10.3969/j.issn.1001-7461.2022.04.05*

Tang Y, Mao L, Gao H (2005). Over-exploitation and lack of protection is leading to a decline of a protected calcicolous tree species *Excentrodendron hsienmu* (Tiliaceae) in China. Biological Conservation 126(1):0-23. *https://doi.org/10.1016/j.biocon.2005.04.016*

Tang Y, Yang P, Lu N, Liu Z, Zhong S, Huang J, … Ye Y (2023). Effects of shading on growth and photosynthetic characteristics of *Cunninghamia lanceolata* seedlings. Chinese Journal of Applied and Environmental 29(5):1084-1092. *https://doi.org/10.19675/j.cnki.1006-687x.2022.06037*

Wang C, Ma Y, He W, Kuzyakov Y, Bol R, Chen H, Fan M (2024). Soil quality and ecosystem multifunctionality after 13-year of organic and nitrogen fertilization. Science of the Total Environment 931:172789. *https://doi.org/10.1016/j.scitotenv.2024.172789*

Wang Q (2017). The influence of nitrogen supply level on photosynthesis of larch seedlings. Technology Innovation and Application 8:289.

Wang X, Zhou Z, Han Q, Pan D, Wang D, Huang G (2023). Effect of nitrogen application methods on the growth and root development of *Cinnamomum kanehirae* seedlings. Journal of Plant Nutrition and Fertilizers 29(12):2381-2388. *https://doi.org/10.11674/zwyf.2023213*

Wang Z, Zhu J, Wang J, Wang Y, Lu Y, Zheng Q (2012). The response of photosynthetic characters and biomass allocation of *P. bournei* young trees to different light regimes. Acta Ecologica Sinica 32(12):3841-3848. *https://doi.org/10.5846/stxb201112021842*

Wei L, Shangguan Z (2006). Specific root length characteristics of three plant species, *Bothriochloa ischaemum*, *Hippophae rhamnoidess* and *Quercus liaotungensis* in the Loess Plateau. Acta Ecologica Sinica 26(12):4164-4170

Wei Y, Wang B, Li D, Lu F, Huang F, Li J, … Li X (2023). Prediction of potential suitable areas for endangered karst obligate plant *Excentrodendron hsienmu* in China. Guihaia 43(3):429-441. *https://doi.org/10.11931/guihaia.gxzw202204081*

Weng X, Li H, Zhou Y, Ren C, Zhang S, Liu L (2023). Relative availability of nitrogen and calcium regulates the growth of *Poplar* seedlings due to transcriptome changes. Forests 14: 1899. *https://doi.org/10.3390/f14091899*

Weng X, Li H, Zhou Y, Ren C, Zhang S, Zhu W, … Yang C (2021). Effects of nitrogen-calcium synergy on growth, photosynthetic characteristics and chlorophy I fluorescence of *Populus* L. Journal of Shenyang Agricultural University 52(3):356-361. *https://doi.org/10.3969/j.issn.1000-1700.2021.03.014*

Xiang W, Wang B, Ding T, Huang Y, Nong Z, Liu S, Li X (2013). Age structure and quantitative dynamics of *Excentrodendron hsienmu* population in a karst seasonal rain forest in South China. Chinese Journal of Ecology 32(4):825-831. *https://doi.org/10.13292/j.1000-4890.2013.0185*

Yin M, Li Z, Yang Y, Li C, Tang Y, Tang J, … Wu X (2022). Effects of fertilization on growth and photosynthetic characteristics of *Gardenia jasminoides* seedlings. Journal of Northeast Forestry University 50(05):32-36. *https://doi.org/10.13759/j.cnki.dlxb.2022.05.005*

You Z, Wang J, Liu Y, Yan Z, Zhang J, Wan S (2024). Interactive effect of N and Ca on the nitrogen metabolism enzyme activity, nitrogen utilization and calcium accumulation of *Peanut*. Chinese Journal of Oil Crop Sciences 46(4):904-912. *https://doi.org/10.19802/j.issn.1007-9084.2023017*

Yuan J, Wang L, Zhang Q, Zhang J, Tian S, Chang F, … Liang Z (2023). The effect of pre harvest spraying of calcium chloride on the photosynthetic function of Apple leaves and fruit quality. Jiangsu Agricultural Sciences 51(20):152-161. *https://doi.org/10.15889/j.issn.1002-1302.2023.20.022*

Yue Y, Jin C, Zhang M, Li Z (2021). Effects of different nitrogen and calcium levels on the quality of peach fruit in facilities. China Fruits (4):55-58. *https://doi.org/10.16626/j.cnki.issn1000-8047.2021.04.011*

Zhang H, Zheng Y, Ye J, Gao W, Qiao Y, Dai C, … Shi S (2019). Effects of exogenous Ca2+ on stomatal traits, photosynthesis, and biomass of maize seedings under salt stress. Chinese Journal of Applied Ecology 30(03):923-930. *https://doi.org/10.13287/j.1001-9332.201903.020*

Zhang Q, Zhou Z, Wang X, Huang G, Liang K, Yang G, Liu G (2021). Effects of fertilization on photosynthetic and chlorophyll fluorescence characteristics of *Tectona grandis*. Journal of Central South University of Forestry & Technology 41(4):31-38. *https://doi.org/10.14067/j.cnki.1673-923x.2021.04.004*

Zhen K (2022). A model for the environmental response of photosynthesis and photorespiration rates of cucumber. MSc Thesis, Nanjing Agricultural University, Nanjing, China.

Zhou S, Jiang J, Gao L, Wang L, Li S, Li X (2014). Effects of calcium on anti-oxidant enzymes and membrane lipid peroxidation of Brazil Banana seedling under NaCl stress. Southwest China Journal of Agricultural Sciences 27(6):2354-2359. *https://doi.org/10.16213/j.cnki.scjas.2014.06.018*

Zhou Y, Li X, Chen G, Zhuo W (2023). Research progress in plant RuBisCO. Scientia Sinica Vitae 53(09):1213-1229. *https://doi.org/10.1360/SSV-2022-0275*