Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2024.056429

ARTICLE

Effects of Water-Fertilizer Coupling on Growth Characteristics and Water Use Efficiency of *Camellia petelotii* Seedlings

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Received: 23 July 2024 Accepted: 21 October 2024 Published: 30 November 2024

ABSTRACT

Camellia petelotii (Merr.) Sealy is an endangered Chinese native species that originates from Guangxi Zhuang Autonomous Region, China. Previous research demonstrated that proper water and fertilizer treatments could improve the growth and quality of *Camellia* species. This study uses a three-factor, five-level quadratic rotational combination experimental design to investigate the impact of water-fertilizer coupling on plant growth characteristics and the most suitable treatment for 24-month-old grafted *C. petelotii* seedlings. The experimental design includes irrigation levels [30%, 40%, 55%, 70%, 80% of field capacity (FC)], nitrogen application (0, 2.17, 5.43, 8.70, 10.87 g·plant^{-1}), and phosphorus application (0, 0.96, 2.40, 3.85, 4.81 g·plant^{-1}). The results indicated that: (1) Water-nitrogen and water-phosphorus interactions significantly affected ground diameter, chlorophyll content and specific leaf weight (SLW), while water-nitrogen interactions significantly affected plant height and photosynthesis; (2) Application of nitrogen (8.70 g·plant^{-1}) and phosphorus (3.85 g·plant^{-1}) fertilizers under appropriate irrigation conditions (40% FC and 70% FC) improved growth. Applying fertilizers containing either nitrogen (10.87 g·plant^{-1}) or phosphorus (4.81 g·plant^{-1}) under adequate irrigation (55% FC) increased the Chl content. However, high nitrogen levels (10.87 g·plant^{-1}) reduced photosynthesis. Conversely, it was enhanced under appropriate phosphorus (4.81 g·plant^{-1}) when the irrigation level was 55% FC, indicating the sensitivity of *C. petelotii* seedlings to nitrogen fertilizer. (3) Under specific conditions of 40% FC or 70% FC irrigation and 8.70 g·plant^{-1} or 2.17 g·plant^{-1} nitrogen fertilizer application, 3.85 g·plant^{-1} phosphorus addition boosted the SLW whereas, 0.96 g·plant^{-1} phosphorus addition inhibited it. Under W = 55% FC, deficiencies in either nitrogen (N = 0 g·plant^{-1}) or phosphorus (P = 0 g·plant^{-1}) significantly decreased leaf growth, affecting SLW. In summary, *C. petelotii* was more sensitive to nitrogen fertilizer at W = 55% FC, and nitrogen deficiency inhibited *C. petelotii* growth in terms of ground diameter more than phosphorus deficiency. The *C. petelotii* seedlings performed best when treated with 55% FC, 5.43 g·plant^{-1} nitrogen, 2.40 g·plant^{-1} phosphorus per plant. These parameter estimates could optimize water and fertilizer application for *C. petelotii* seedlings.

KEYWORDS

Camellia petelotii; growth characteristics; photosynthesis; water-fertilizer coupling; water use efficiency

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1 Introduction

Camellia petelotii (Merr.) Sealy (family Theaceae) is an evergreen Chinese native shrub or tree species that belongs to *Camellia* sect. chrysantha Chang. It shows a high tolerance for shade and commonly inhabits shaded gullies, valleys, and under-forest environments. The International Union for Conservation of Nature (IUCN) Red List of Threatened Species has listed this species as Endangered (EN) [1,2]. The plant has a high aesthetic, medicinal, and nutritional value. It is an ornamental tree whose flowers are valued as a precious raw material for health drinks. It has recently spread significantly throughout southern China (including Guangdong, Yunnan, Fujian, and numerous other provinces) and a few others (such as Japan, USA, Australia, and France) [3,4]. Therefore, *C. petelotii* has high development value and immense market potential.

Proper applications of water and nutrients are the most significant factors during the cultivation of *C. petelotii* seedlings. Previous research has shown that *C. petelotii* requires high moisture levels and is drought intolerant at 15.04% to 16.54% of drought stress [5]. Sufficient water can increase the nutrient uptake of seedlings and plant cell elongation [6,7]. For example, proper irrigation (80%–85% field capacity, FC) can enhance the leaf water transport efficiency and photosynthesis of *C. oleifera* seedlings [8]. Furthermore, *C. petelotii* favors appropriate nutritional conditions in addition to medium-fertile soil. For example, potassium and compound nutrients [(N/P/K: 16/8/6) 150 g] could improve the growth of 24-month-old *C. petelotii* cutting seedlings [9]. Regarding nutrients, nitrogen is an essential constituent of proteins and chlorophyll, and it can also affect photosynthesis in some *Camellia* species. 22 g of nitrogen fertilizer [$(NH_4)_2SO_4$] and 8 mmol·L^{-1} N (4 mmol·L^{-1} NH_4Cl + 4 mmol·L^{-1} KNO_3) per pot were found to enhance ideal leaf growth and photosynthetic conditions for *Camellia sinensis* and *Camellia oleifera*, respectively [10,11]. Another nutrient, phosphorus, was also shown to promote plant quality. Phosphorus fertilizer use boosted floral bud formation and fruit ripening in *Camellia oleifera*, significantly improving yield and quality [12]. The plant responses to phosphorus levels vary depending on the nitrogen level and species under consideration. *C. oleifera* may show higher nutrient accumulation with moderate phosphorus (0.4, 0.8 mmol·L^{-1}) under nitrogen deficit, but the change was not significant with phosphorus under adequate nitrogen supply (8 mmol·L^{-1}) [13]. Thus, nitrogen and phosphorus exhibit considerable complementarity, and promoting plant growth and fertilizer utilization requires a rational fertilizer mix. Additional research has shown that the interplay of water and nutrients can significantly affect growth, physiology, and economic yields. *C. sinensis* produced the highest amount of dry matter and grew optimally with 0.485 g·pot^{-1} N, 0.274 g·pot^{-1} P, and watering every 4~5 days [14]. To summarize, appropriate water-fertilizer coupling can promote water absorption, alleviate moisture stress, and enhance the nutritional status of seedlings. Water can improve the transport efficiency of nutrients in plants, and the right combination of water and nutrients is crucial to plant growth and development during the seedling stage.

C. petelotii cultivation research is currently focused on breeding [15,16], resistance [17,18], and fertilization studies [19,20]. However, it is still unclear how water-fertilizer coupling affects the growth of *C. petelotii* seedlings. *C. petelotii* requires more nitrogen fertilizer than other nutrients to promote leaf development and metabolite synthesis [21]. Research has shown that while the phosphorus content of tea is significantly lower than nitrogen content, the use of phosphorus fertilizer is highly correlated with improved tea growth and is crucial for respiration, photosynthesis, and other mechanism processes [22,23]. To summarize, it is critical to investigate appropriate water-fertilizer coupling conditions to promote growth and water utilization in *C. petelotii* and other Theaceae family species. Therefore, the objectives of this study were to (1) determine the effect of water and fertilizer coupling on growth characteristics and water use efficiency in the seedling stage of *C. petelotii* and (2) identify the best combination of water and fertilizer for seedling growth in this valuable shrub.

2 Materials and Methods

2.1 Test Site and Materials

2.1.1 Test Site

The experiment was conducted from 25 February to 25 December 2015, at the teaching nursery of the College of Forestry, Guangxi University (22°52′ N, 108°17′ E). The test site has a subtropical monsoon climate that is mild and humid, with a mean annual temperature of 21.6°C and a mean annual precipitation of 1304 mm, ideal for the growth of C. *petelotii* seedlings.

2.1.2 Seedlings

Guangxi State-owned Liuwan Forestry (Yulin, China) provided 24-month-old grafted seedlings of *C. petelotii* (plant height 16.38 ± 5.18 cm, ground diameter 3.71 ± 0.72 mm), with *Camellia oleifera* rootstock and *C. petelotii* scion.

2.1.3 Substrates

Each pot measured 23 cm (top diameter) × 17 cm (bottom diameter) × 25 cm (height) and had a soil bulk density of 1.54 g·cm^{-3}. Each pot included 9 kg of mixed substrate [fine sand: yellow soil = 1:2 (v/v)]. The College of Forestry's Teaching Nursery supplied the Fine Sand and Yellow Soil. The basic physicochemical properties of the substrate were: soil bulk density of 1.54 g·cm^{-3}, field water holding capacity (FC) of 25.1%, pH of 5, total nitrogen of 0.79 g·kg^{-1}, total phosphorus of 0.17 g·kg^{-1}, and total potassium of 1.03 g·kg^{-1}.

2.1.4 Fertilizers

Tianjin Damao Chemical Reagent Factory produced the fertilizers used. They included nitrogen (urea: N 46%, analytical pure reagent), phosphate (calcium superphosphate: 16% P_2O_5, experimental reagent), and potassium (potassium sulfate: K_2O 60%, analytical pure reagent).

2.2 Experimental Design

This experiment has three components, each with five levels. A full factorial design requires 125 treatments but an orthogonal experimental design involves 25 treatment groups. Therefore, the study employed a quadratic regression generalized rotational combinatorial design, widely acknowledged as the most efficient approach for examining multifactorial interaction effects. This approach can lower the number of required tests while maintaining efficiency [24,25]. The experiment used a three-factor (watering, nitrogen, and phosphorus), five-level quadratic rotational combination design. Five levels (−γ, −1, 0, 1, and γ) were established for each factor, with γ representing the highest level. The quadratic equation for the same can be stated as follows:

$$y = \mathrm{a} + \sum_{j=1}^{m} b_j x_j + \sum_{k<j} b_{kj}\, x_k x_j + \sum_{j=1}^{m} b_{jj}\, x_j^2, k = 1, 2, \ldots, m-1 (j \neq k) \quad (1)$$

where m represents the experiment factor (m = 3 in this experiment), x_j (j = 1, 2, ..., m) represents the independent variable, and y represents the experiment indication (dependent variable) [26].

The regression coefficients, denoted as a, b_j, b_{kj}, and b_{jj}, are represented as $1 + m + m(m-1)/2 + m = \frac{(m+1)(m+2)}{2}$ term. Estimating regression coefficients requires n number of trials, $n \geq \frac{(m+1)(m+2)}{2} = 10$. According to the orthogonal rotation central combination design, n can be defined as:

$$\mathrm{n} = \mathrm{m_c} + \mathrm{m}_\gamma + \mathrm{m_0} \quad (2)$$

where $\mathrm{m_c} = 2^{\mathrm{m}}$ is the experiment times of the orthogonal test with each factor at 2 levels (treatment 1–8 in Table 1), $\mathrm{m}_\gamma = 2^{\mathrm{m}}$ is the experiment times of the orthogonal test related to level γ (treatment 9–14 in Table 1), and $\mathrm{m_0}$ is the experiment times of the zero-level trials. This experiment used five irrigation levels

[30%, 40%, 55%, 70%, and 80% of the field capacity (FC, 25.1%)]. Nitrogen and phosphorus were each set at five levels, totaling 20 treatments. Each treatment had five biological replicates (one basin per replicate), totaling 100 plants (Tables 1–4). Phosphorus fertilizer was applied twice, at a constant rate of 0.75 g·plant^{-1} for each treatment. For the test, seedlings with uniform growth and good health, arranged in completely randomized blocks, were placed in a shed covered in a layer of shade net (Anhui Nongfeng Shade Equipment Co., Ltd., Hefei, China) with 67.5% natural light. The soil moisture content was determined using a TDR soil moisture tachymeter, and the average of the four directions of east, west, north, and south was measured every three days. If the results were less than the value set for the experiment, watering was replenished, with watering amount = (field capacity − soil moisture content) * potting soil weight.

Table 1: Test structure matrix

Treatment	W (x_1)	N (x_2)	P (x_3)
T1	1	1	1
T2	1	1	−1
T3	1	−1	1
T4	1	−1	−1
T5	−1	1	1
T6	−1	1	−1
T7	−1	−1	1
T8	−1	−1	−1
T9	1.682	0	0
T10	−1.682	0	0
T11	0	1.682	0
T12	0	−1.682	0
T13	0	0	1.682
T14	0	0	−1.682
T15	0	0	0
T16	0	0	0
T17	0	0	0
T18	0	0	0
T19	0	0	0
T20	0	0	0

2.3 Determination of Indexes

2.3.1 Growth

The plant height and ground diameter were measured on 25 February 2015, and 25 December 2015, respectively, using a steel tape to within 0.1 cm and an electronic Vernier caliper to within 0.01 mm. All the seedlings should be measured (with three biological replicates for each plant). relative increments in plant height and ground diameter were calculated using the following formulas:

$$\mathrm{Rph} = (\mathrm{A2} - \mathrm{A1})/\mathrm{A1} \tag{3}$$

$$\mathrm{Rgd} = (\mathrm{B2} - \mathrm{B1})/\mathrm{B1} \tag{4}$$

Rph and Rgd represent the relative increment of plant height and ground diameter, respectively. A1 and B1 represent the plant height and ground diameter before the experimental treatment, while A2 and B2 represent the plant height and ground diameter at the end of the experiment.

Table 2: Actual levels of factors

Treatment	W%	N g·plant^{-1}	P g·plant^{-1}
T1	70	8.70	3.85
T2	70	8.70	0.96
T3	70	2.17	3.85
T4	70	2.17	0.96
T5	40	8.70	3.85
T6	40	8.70	0.96
T7	40	2.17	3.85
T8	40	2.17	0.96
T9	80	5.43	2.40
T10	30	5.43	2.40
T11	55	10.87	2.40
T12	55	0	2.40
T13	55	5.43	4.81
T14	55	5.43	0
T15	55	5.43	2.40
T16	55	5.43	2.40
T17	55	5.43	2.40
T18	55	5.43	2.40
T19	55	5.43	2.40
T20	55	5.43	2.40

Table 3: Coded and physical values of experimental factors

Level	Irrigation levels (%FC) W	Amount of nitrogen (urea) applied (g·plant $^{-1}$) N	Amount of phosphorus (superphosphate) applied (g·plant $^{-1}$) P
−1.682	30	0	0
−1	40	2.17	0.96
0	55	5.43	2.40
1	70	8.70	3.85
1.682	80	10.87	4.81

Note: −1.682 and 1.682 are level-coded values representing the lowest and highest levels of treatment.

Table 4: Fertilizer application frequency and application rate

Dates	N	P	K
25th Feb	1/3	1/2	0.75 g
25th May	1/3		0.75 g
25th Aug	1/3	1/2	

2.3.2 Leaf Moisture Content

For each treatment, we selected three seedlings with similar growth status and collected the second to fifth mature functional leaves from the top bud of the upper branch of the plant. We used three biological replicates for each treatment. The leaf area (LA) was measured using a Li-3000A portable leaf area meter. After selecting the leaves, the fresh weight (M0) was measured using an electronic induction balance with an accuracy of 0.0001 g. The leaves were then left at room temperature and weighed at specific intervals (0.5, 4.5, 8.5, 20.5, 32.5, 44.5, 56.5, and 68.5 h) to determine their water-holding capacity. The leaves were dried at 65°C~70°C until a constant weight was reached. We measured the final dry mass (Md), and calculated the leaf water content (LWC). Leaf water loss rate, specific leaf weight (SLW) and LWC were calculated using the following formulas:

$$\text{Leaf water loss rate} = (M0 - M)/(M0 - Md) * 100\% \quad (5)$$

where M is the fresh weight of leaves measured at each time point [27–29].

$$SLW = Md/LA \quad (6)$$

$$LWC = (\text{leaf fresh mass} - \text{leaf dry mass})/\text{leaf fresh mass} \quad (7)$$

2.3.3 Photosynthesis

The photosynthesis indexes were calculated using three biological replicates for each treatment. The chlorophyll content was determined using a combined acetone-ethanol extraction method [30]. The second to fifth mature functional leaves were randomly collected from the top bud of three seedlings with similar growth status. About 0.2 g of fresh leaf tissue (excluding the vein) was cut into pieces and added to the extraction solution (80% acetone: 95% ethanol = 1: 1 [v/v]) and placed in the dark for more than 24 h. We measured the absorbance at 645 and 663 nm and calculated the chlorophyll content in the samples using the formulas:

$$\text{Chl a} = 12.7 * A663 - 2.69 * A645 \quad (8)$$

$$\text{Chl b} = 22.9 * A645 - 4.68 * A663 \quad (9)$$

$$\text{Chl a} + \text{b} = \text{Chl a} + \text{Chl b} \quad (10)$$

At the end of December 2015, the Pn, Gs, Tr, and Ci of *C. petelotii* seedlings were measured from 9.30 a.m. to 11.30 a.m. using the Lico-6400 Convenient Photosynthesis Analyser System.

2.3.4 Statistical Analysis

Microsoft Excel 2019 was used to organize the experimental data and create tables. SAS 9.4 is employed to conduct main effects analysis and assess the relationships among factors using the response surface regression analysis (RSREG procedure). One-way analysis of variance (ANOVA) and Tukey's test with a significance level set at 0.05 were used to conduct significance analysis and *post hoc* comparisons and perform significance analysis in IBM SPSS 26.0. Finally, Origin 2022 was used for data visualization and charting. The study employed the following regression analysis model to examine the relationship

between several variables and soil moisture content, nitrogen application rate, and phosphorus application rate:

$$y = k + k_1x_1 + k_2x_2 + k_3x_3 + k_{12}x_1x_2 + k_{13}x_1x_3 + k_{23}x_2x_3 + k_{11}x_1^2 + k_{22}x_2^2 + k_{33}x_3^2 \quad (11)$$

In the above equation, y is the response variable; x_1, x_2, and x_3 represent the level-coded values of irrigation levels (W), nitrogen fertilizer application (N), and phosphorus fertilizer application (P), respectively; x_1x_2, x_1x_3, and x_2x_3 is the level-coded values of water-nitrogen interactions, water-phosphorus interactions, and nitrogen-phosphorus interactions, respectively; and k denotes the regression coefficient derived from the model. Table A1 shows the level-coded values and regression coefficients. The partial regression coefficients were standardized in the test, and the factor levels were substituted with dimensionless linear coding. Non-significant factors were excluded using regression coefficient significance tests. The positive and negative signs of each factor's coefficients indicate how it affects the variables (positive: positive impact, negative: negative impact). Directly comparing the absolute values of these coefficients allows one to estimate the extent of influence each component has on the response variable. The bigger the absolute value, the greater the degree of impact.

3 Results

3.1 Growth

Distinct treatments showed highly significant differences ($p < 0.01$) in the relative increments of plant height and ground diameter. Water, nitrogen, and phosphorus exhibited significant effects on growth indexes ($p < 0.01$), with the water-nitrogen interaction affecting plant height and the water-nitrogen, water-phosphorus interaction affecting ground diameter ($p < 0.01$) (Fig. 1 and Table A2). T13 (W = 55% FC + N = 5.43 g·plant^{-1} + P = 4.81 g·plant^{-1}) treatment resulted in the highest relative increments of plant height (52.8%, increase by 9.25 cm, 15% higher than the control). In comparison, T2 (W = 70% FC + N = 8.70 g·plant^{-1} + P = 0.96 g·plant^{-1}) treatment resulted in the highest relative increments of ground diameter (53.7%, increase by 2.04 mm, 70.5% higher than the control). The relative growth increment initially increased and then decreased with increasing irrigation levels (T10→T15–T20→T9). While the relative increment of plant height first increased and then decreased, the relative increment of ground diameter gradually increased with the increase in nitrogen application (T12→T15–T20→T11). Increased phosphorus treatment resulted in a progressive increase in plant height but an increase followed by a decrease in ground diameter (T14→T15–T20→T13). In summary, 30%–40% FC restricted the growth of *C. petelotii* seedlings. However, nitrogen (8.7 g·plant^{-1}) and phosphorus (3.85 g·plant^{-1}) fertilizers with W = 40% or W = 70% can boost height growth. Nitrogen deficiency had a more significant impact on *C. petelotii* seedlings growth than phosphorus deficiency.

The regression models were examined and analyzed with soil moisture content (x_1), nitrogen application rate (x_2), and phosphorus application rate (x_3) as independent variables and plant height relative increment (y_{ph}) and ground diameter relative increment (y_{gd}) as response variables, respectively. Both regression models were highly significant ($p < 0.01$) (Table A1). The R^2 values were 0.861 and 0.880, respectively, indicating a strong correlation between water and fertilizer factors on the relative increment of plant height and ground diameter. After excluding non-significant factors, the regression models of the response variables with water and fertilizer factors were as follows:

$$y_{ph} = -1.634 + 0.209x_1 + 0.189x_2 + 0.299x_3 + 0.011x_1x_2 - 0.008x_1^2 - 0.074x_2^2 - 0.104x_3^2$$

$$y_{gd} = -0.661 + 0.097x_1 - 0.123x_2 + 0.389x_3 + 0.017x_1x_2 - 0.012x_1x_3 - 0.003x_1^2 - 0.079x_3^2$$

The regression coefficients for x_1 and x_3 in both models were positive, with $|x_3| > |x_1|$. This suggests that soil moisture content and phosphorus application were the primary influence factors. The coupling

effects of soil moisture content and nitrogen fertilizer on the relative increment of plant height were highly significant. The coupling effects between soil moisture content and nitrogen fertilizer and between soil moisture content and phosphorus fertilizer on the relative increment of ground diameter were also highly significant. The coefficient of x_1x_3 was negative, indicating that soil moisture content and phosphorus fertilizer negatively impacted ground diameter growth.

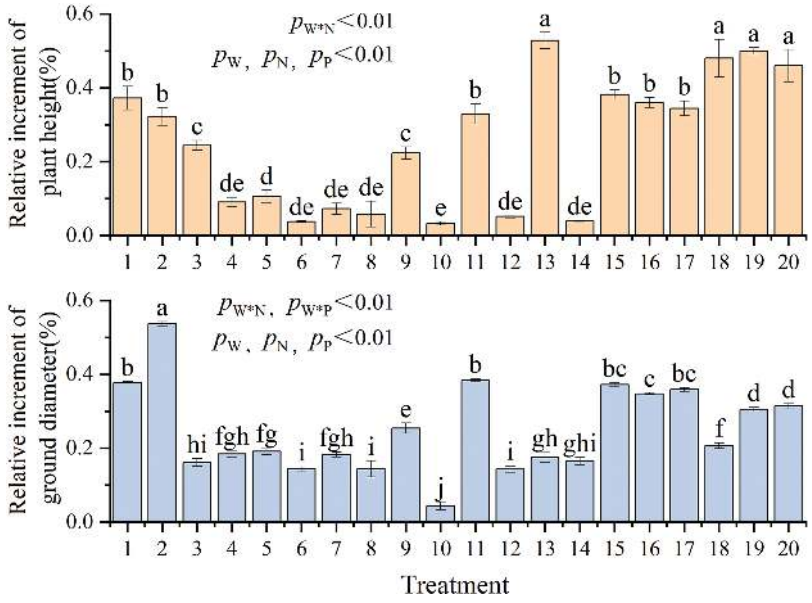


Figure 1: Relative increment of plant height and ground diameter under different coupling conditions of water and fertilizer

Note: Different lowercase letters indicate that the differences between treatments are significant at the $p < 0.05$ level, similarly hereinafter.

3.2 Chlorophyll Contents

There were highly significant differences ($p < 0.01$) between the various treatments regarding Chl a, Chl b, and Chl a + b. Fig. 2 shows the results of the multifactor analysis of variance. T2 (W = 70% FC + N = 8.70 g·plant^{-1} + P = 0.96 g·plant^{-1}) treatment resulted in maximum Chl a (4.52 mg·g^{-1}, 35.7% higher than the control), and Chl a + b content (6.08 mg·g^{-1}, 36.3% higher than the control), while T4 (W = 70% FC + N = 2.17 g·plant^{-1} + P = 0.96 g·plant^{-1}) treatment resulted in the highest Chl b content (1.58 mg·g^{-1}, 39.8% higher than the control). The chlorophyll content first increased and then decreased with the increasing irrigation levels (T10→T15–T20→T9). Chl a, Chl b, and Chl a+b content decreased and then increased when nitrogen application increased (T12→T15–T20→T11). Chl a and Chl a + b initially increased, then declined, while Chl b decreased and then increased as phosphorus application increased (T14→T15–T20→T13). Irrigation with 30% FC significantly inhibits the photosynthetic potential of *C. petelotii* seedlings. When irrigation level was adequate (55% FC), independently applying nitrogen (10.87 g·plant^{-1}) or phosphorus (4.81 g·plant^{-1}) fertilizer had a significant favorable effect on chlorophyll production. Nitrogen and phosphorus fertilizers have a complementary impact on chlorophyll synthesis. Applying nitrogen and phosphorus fertilizers simultaneously at constant water content (W = 40% FC or W = 70% FC) either insufficiently (N = 2.17 g·plant^{-1}, P = 0.96 g·plant^{-1}) or excessively (N = 8.70 g·plant^{-1}, P = 3.85 g·plant^{-1}) inhibited the synthesis of chlorophyll. However, an appropriate combination of nitrogen and phosphorus can help boost chlorophyll levels.

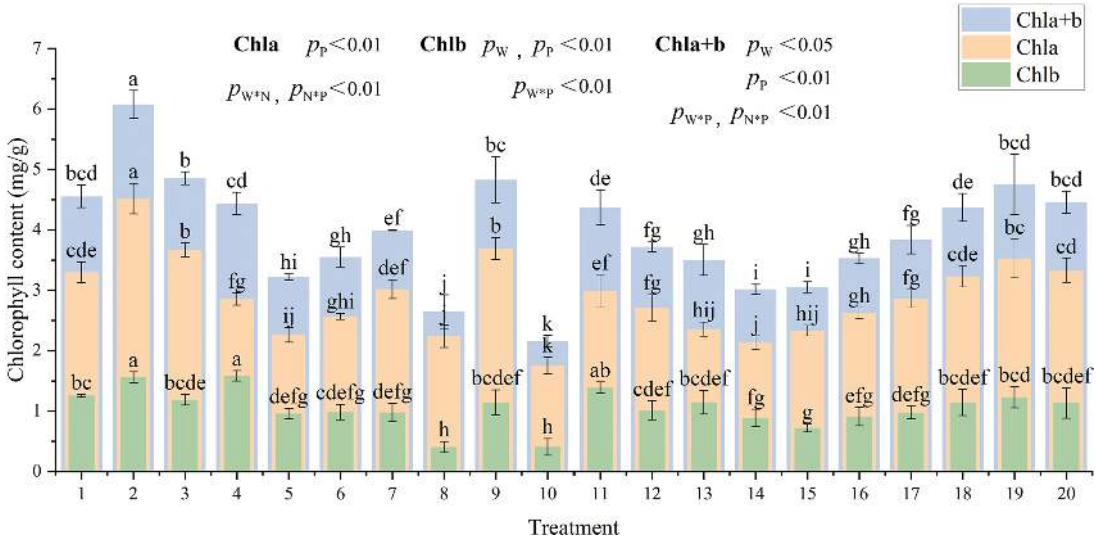


Figure 2: Changes of chlorophyll content under different coupling conditions of water and fertilizer

The regression model was examined and analyzed with soil moisture content (x_1), nitrogen application rate (x_2), and phosphorus application rate (x_3) as independent variables, and Ch l a + b content (y_{a+b}) as the response variable. The regression model was highly significant ($p < 0.01$). The R^2 value was 0.761, indicating a strong correlation between water and fertilizer factors on the chlorophyll content. The following are the regression models of the response variables with water and fertilizer factors after non-significant factors were removed:

$$y_{a+b} = -2.024 + 0.368x_1 + 3.103x_3 - 0.098x_1x_3 - 0.576x_2x_3$$

The regression coefficients for x_1 and x_3 in both models were positive, with $|x_3| > |x_1|$. This implies that soil moisture content and phosphorus application both have a positive influence on chlorophyll content, with phosphate fertilizer having the most significant impact. The negative coupling effects of soil moisture content and phosphorus fertilizer, as well as nitrogen fertilizer and phosphorus fertilizer on chlorophyll content, were found to be highly significant, with nitrogen-phosphorus > water-phosphorus.

3.3 Photosynthetic Characteristics

Between the various treatments, Pn, Tr, Gs, and Ci showed highly significant differences ($p < 0.01$), and Fig. 3 displays the multifactor variance analysis results. The values of Tr (5.29 mmol·m^{-2}·s^{-1}, 163.2% higher than the control) and Gs (0.30 mol·m^{-2}·s^{-1}, 500% higher than the control) reached the maxima under T15 (W = 55% FC + N = 5.43 g·plant^{-1} + P = 2.40 g·plant^{-1}) treatment. The values of Pn (5.59 umol·m^{-2}·s^{-1}, 133.9% higher than the control) and Ci (314.33 umol·mol^{-1}, 58.2% higher than the control) reached the maxima under T12 (W = 55% FC + N = 0 g·plant^{-1} + P = 2.40 g·plant^{-1}) and T18 (W = 55% FC + N = 5.43 g·plant^{-1} + P = 2.40 g·plant^{-1}) treatments, respectively. Overall, Pn, Tr, and Gs first increased and then decreased while Ci showed a decreased-increased-decreased trend with increasing irrigation level (T10→T5–T8→T11–T20→T1–T4→T9). Tr, Gs, and Ci initially increased before decreasing, but Pn gradually and continuously declined as nitrogen application increased (T12→T15–T20→T11). However, increased phosphorus application caused Tr, Gs, Ci, and Pn to increase initially, then decrease (T14→T15–T20→T13). Higher nitrogen (8.7 g·plant^{-1}) and phosphorus (3.85 g·plant^{-1}) levels led to enhanced Pn, Gs, and Ci with 70% FC. Low irrigation levels (40% FC) inhibited the photosynthesis in *C. petelotii* seedlings. Though under W = 55% FC, adding more phosphorus fertilizer

(4.81 g·plant^{-1}) increased photosynthesis, adding nitrogen fertilizer (10.87 g·plant^{-1}) decreased it. This suggests that *C. petelotii* seedlings need more phosphorus and are more sensitive to nitrogen fertilizers.

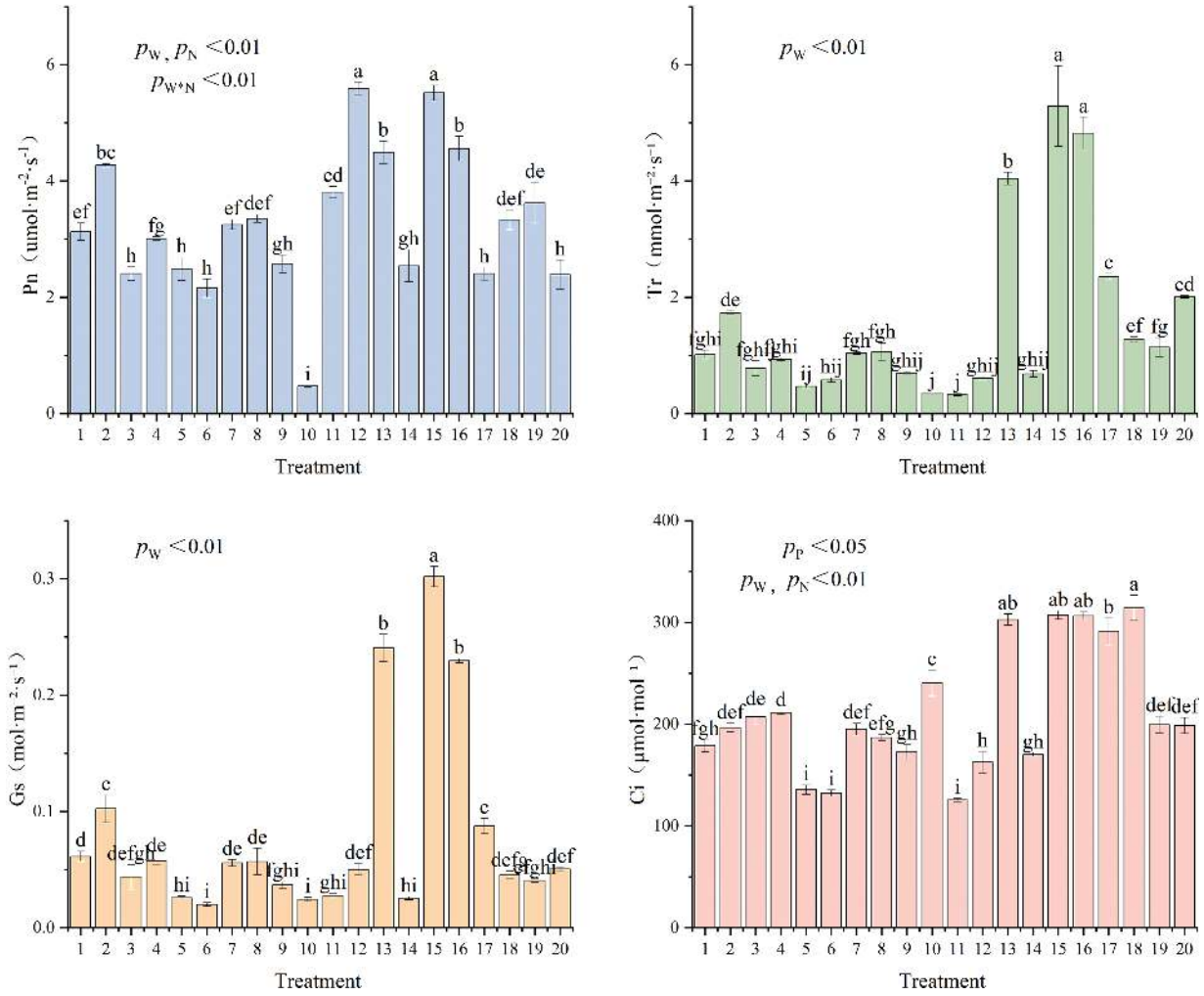


Figure 3: Effects of different water and fertilizer coupling on photosynthetic characteristics of leaves

The regression models were examined and analyzed with soil moisture content (x_1), nitrogen application rate (x_2), and phosphorus application rate (x_3) as independent variables. At the same time, Pn (y_{Pn}), Tr (y_{Tr}), Gs (y_{Gs}), and Ci (y_{Ci}) were used as response variables. All the regression models were highly significant ($p <$ 0.01). The R^2 values were 0.588, 0.498, 0.346, and 0.632, indicating that water and fertilizer levels influence Pn, Tr, Gs, and Ci. After excluding non-significant factors, the regression models of the response variables with water and fertilizer factors were as follows:

$$y_{Pn} = -6.279 + 1.563x_1 - 2.872x_2 + 0.12x_1x_2 - 0.057x_1^2 + 0.285x_2^2$$

$$y_{Tr} = -11.36 + 1.576x_1 - 0.058x_1^2 - 0.721x_2^2$$

$$y_{Gs} = -0.476 + 0.069x_1 - 0.003x_1^2 - 0.028x_2^2$$

$$y_{Ci} = -216.37 + 46.5x_1 + 103.7x_2 + 113.02x_3 - 1735x_1^2 - 40x_2^2 - 26.8x_3^2$$

The regression coefficients for x_1 were positive. This suggests that soil moisture content positively impacted photosynthesis, which is the primary function of Tr and Gs. Phosphorus fertilizer had a negative effect on Pn, with $| x_2 | > | x_1 |$, indicating that nitrogen fertilizer is the primary influencing factor. However, in the case of Ci, $| x_3 | > | x_2 | > | x_1 |$ was observed, suggesting that phosphorus fertilizer is the main factor. The soil moisture content and phosphorus fertilizer effects on the Pn were highly significant.

3.4 Leaf Moisture Content and Specific Leaf Weight

The leaf water content (LWC) did not differ significantly ($p > 0.05$) across treatments, but we observed significant differences ($p < 0.01$) in specific leaf weight (SLW) between treatments. The multifactorial analysis of variance indicated that the water main effect significantly impacted the LWC of *C. petelotii* seedlings at 44.5 h ($p < 0.05$) (Fig. 4). The LWC reached its maximum under T15 (W = 55% FC + N = 5.43 g·plant^{-1} + P = 2.40 g·plant^{-1}) treatment (64%, 18.5% higher than the control), while the SLW reached its maximum under T13 (W = 55% FC + N = 5.43 g·plant^{-1} + P = 4.81 g·plant^{-1}) treatment (1.73%, 58.7% higher than the control). The LWC increased and then decreased with increasing soil water content (T10→T15–T19). The SLW increased and subsequently reduced as the irrigation level increased (T10→T15–T20→T9). At W = 17.57%, SLW increased progressively as nitrogen and phosphorus levels rose. *C. petelotii* seedlings had higher water-holding capacity in dry conditions (30% FC) than in settings with higher irrigation levels. When fertilizer levels were constant, the SLW increased with increasing irrigation levels. The SLW increased with increasing phosphorus application at a specific irrigation and nitrogen fertilizer application level. Leaf growth was severely restricted by insufficient nitrogen or phosphorus under appropriate irrigation, impacting the SLW (T11–T12, T13–T14).

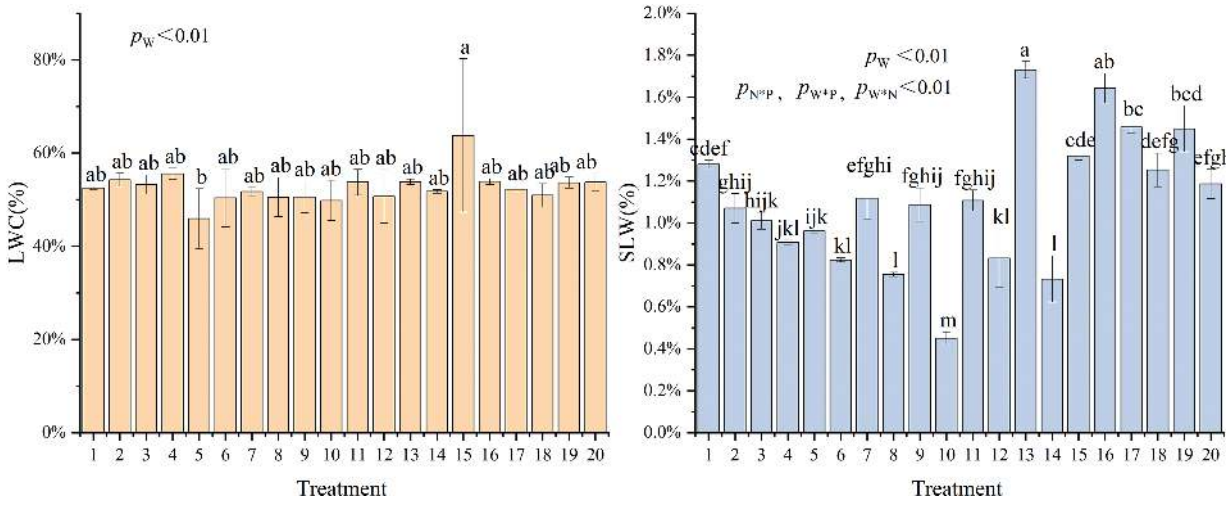


Figure 4: Effects of different water-fertilizer coupling on leaf water content and specific leaf weight

The regression model was examined and analyzed using soil moisture content (x_1), nitrogen application rate (x_2), and phosphorus application rate (x_3) as independent variables, and SLW (y_{SLW}) as the response variable. The regression model demonstrated high significance ($p < 0.01$). The R^2 value was 0.615, indicating a strong correlation between water and fertilizer factors affecting SLW. After excluding non-significant factors, the regression models of the response variables with water and fertilizer factors were as follows:

$$y_{SLW} = -0.083 + 0.011x_1 - 0.002x_1x_2 - 0.004x_1x_3 + 0.013x_2x_3$$

The regression coefficients for x_1 were positive. This implies that soil moisture content had a positive effect on the SLW. The coupling effects of nitrogen and phosphorus fertilizers on the SLW were highly significant. The negative coupling effects between soil moisture content and nitrogen fertilizer, and between soil moisture content and phosphorus fertilizer on the SLW were also significant, with nitrogen-phosphorus > water-phosphorus > water-nitrogen.

3.5 Integrated Evaluation

We summarized and comprehensively analyzed the data using the membership function. The mean of the membership function of each growth index was used as a comprehensive evaluation index for *C. petelotii* seedlings under various water-fertilizer coupling treatments. Table 5 shows that T15 (W = 55% FC + N = 5.43 g·plant^{-1} + P = 2.40 g·plant^{-1}) achieved the highest composite score, indicating that T15 is the optimal water and fertilizer condition for the growth of *C. petelotii* seedlings.

Table 5: Membership function analysis of growth indexes of *C. petelotii* seedlings under water-fertilizer coupling

Treatment	Plant height	Ground diameter	Chl a + b	Pn	Tr	Gs	Ci	LWC	SLW	Overall assessment	Sort
1	0.685	0.677	0.611	0.581	0.137	0.137	0.281	0.370	0.648	0.452	9
2	0.584	1.000	1.000	0.742	0.282	0.284	0.377	0.468	0.484	0.580	4
3	0.427	0.238	0.688	0.377	0.090	0.074	0.433	0.410	0.438	0.353	11
4	0.116	0.286	0.581	0.495	0.121	0.123	0.454	0.539	0.359	0.342	12
5	0.147	0.299	0.270	0.394	0.027	0.009	0.054	0.000	0.398	0.178	18
6	0.009	0.203	0.355	0.329	0.049	0.000	0.035	0.251	0.289	0.169	19
7	0.080	0.281	0.468	0.542	0.143	0.117	0.369	0.325	0.523	0.316	14
8	0.051	0.203	0.124	0.563	0.148	0.121	0.325	0.259	0.234	0.225	16
9	0.384	0.427	0.682	0.411	0.074	0.049	0.252	0.262	0.500	0.338	13
10	0.000	0.000	0.000	0.000	0.005	0.004	0.609	0.218	0.000	0.093	20
11	0.598	0.690	0.565	0.651	0.000	0.015	0.000	0.438	0.516	0.386	10
12	0.037	0.201	0.398	1.000	0.055	0.097	0.197	0.268	0.297	0.283	15
13	1.000	0.266	0.342	0.785	0.748	0.780	0.939	0.443	1.000	0.700	3
14	0.013	0.246	0.218	0.404	0.071	0.007	0.236	0.327	0.219	0.193	17
15	0.704	0.666	0.228	0.985	1.000	1.000	0.963	1.000	0.680	0.803	1
16	0.661	0.616	0.350	0.797	0.906	0.740	0.959	0.447	0.930	0.712	2
17	0.629	0.639	0.428	0.377	0.408	0.231	0.878	0.354	0.789	0.526	5
18	0.904	0.331	0.564	0.559	0.190	0.081	1.000	0.281	0.625	0.504	7
19	0.943	0.529	0.663	0.615	0.163	0.063	0.392	0.432	0.781	0.509	6
20	0.862	0.549	0.587	0.375	0.338	0.098	0.387	0.440	0.578	0.468	8

3.6 Relevance Analysis

Fig. 5 depicts the correlation analysis among the indicators. Photosynthetic characteristics showed a highly positive significant link with a relative increment of plant height and ground diameter, as well as LWC and SLW. LWC indicators had a highly positive considerable influence on other indicators, except for Chl a + b. The leaf photosynthetic parameters showed a high correlation, with Gs having a more substantial impact on Pn than chlorophyll content. Higher Gs allowed more carbon dioxide into the leaves, resulting in an increased rate of photosynthesis. Higher rates of photosynthesis imply that the leaves may use water more efficiently to produce nutrients, which may help maintain or increase the LWC. On the other hand, increased of photosynthetic products can promote leaf growth and thickness, ultimately affecting SLW. Thicker leaf structure boosts photosynthetic efficiency, which aids overall plant growth and encourages plant height and ground diameter. The relative increment in plant height and ground diameter positively correlated with other indicators. The interactions between total chlorophyll, photosynthesis, LWC, and SLW promoted the growth in height and ground diameter of *C. petelotii* seedlings.

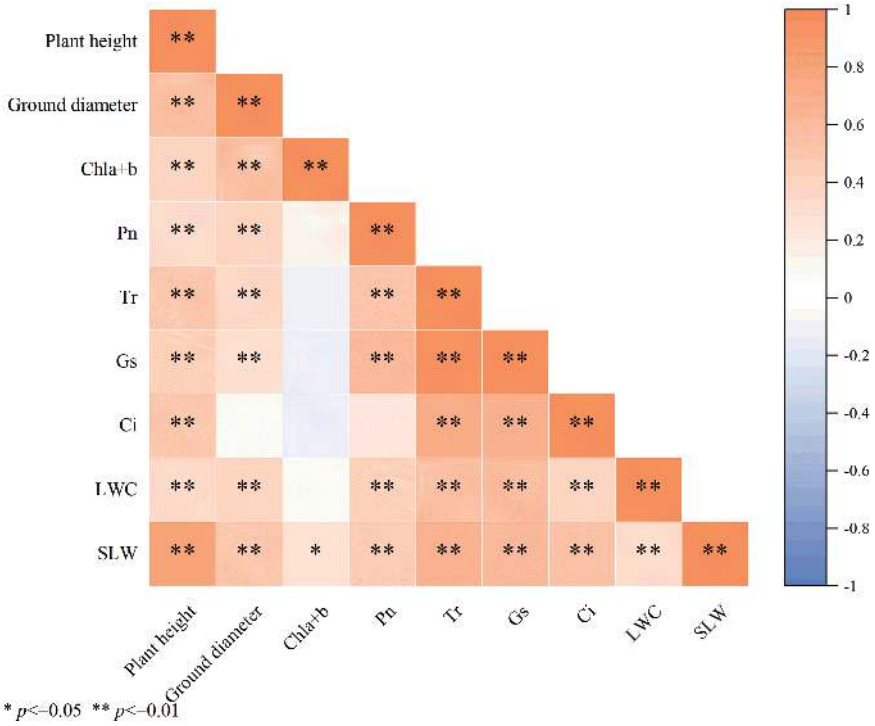


Figure 5: Correlation heat map of various indexes

Note: Orange ($0 < r \leq 1$) indicates positive correlation, blue ($-1 \leq r < 0$) indicates negative correlation, white indicates no correlation ($r = 0$), and the darker the colour indicates the stronger the correlation; * and ** indicate that the difference is significant at the levels of $p < 0.05$ and $p < 0.01$, respectively.

Principal component analysis was performed using relative increments of plant height and ground diameter, Chl a + b content, photosynthetic indexes, SLW, and LWC of *C. petelotii* (Fig. 6). The 20 treatment groups were scattered throughout the axes at various points, suggesting considerable treatment variability, with all nine markers positioned at the positive end of the *x*-axis. T18 had the most significant impact on the relative growth of plant height and ground diameter. T16 had the most significant impact on Ci, Gs, and Tr growth. T17 had the greatest effect on Pn, SLW, and LWC growth while T20 had the biggest impact on Chl a + b levels. The two primary components exhibited contribution rates of 52.6%

and 20.1% for a cumulative contribution rate of 72.7%. It indicates that the two primary components listed above can cover most of the attributes associated with developing *C. petelotii* seedlings.

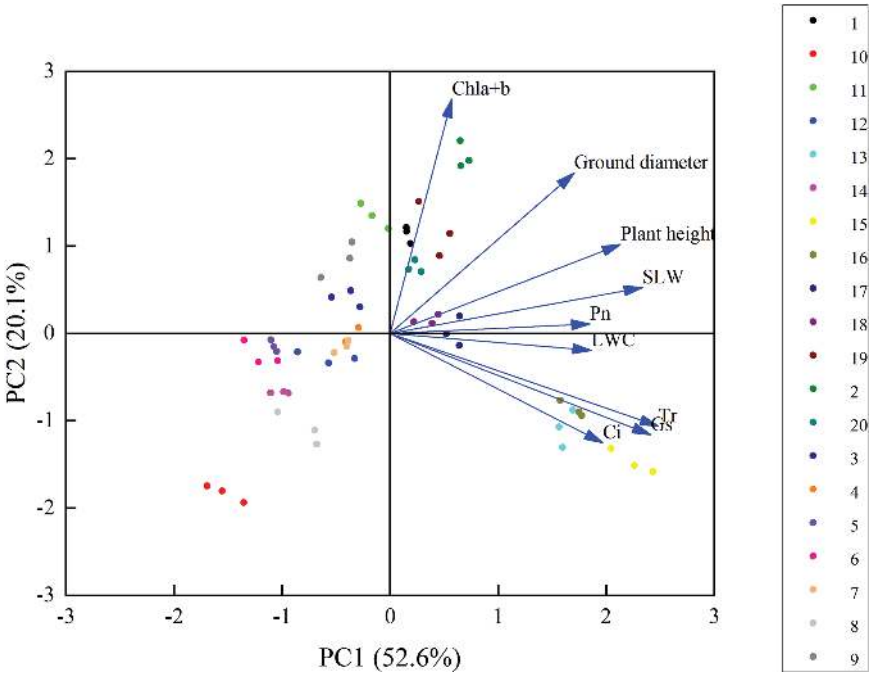


Figure 6: Principal component analysis of the growth characteristics

4 Discussion

4.1 Effect of Water-Fertilizer Coupling on C. petelotii Growth

Plant height and ground diameter can reflect seedling growth and quality, whereas optimal water-nutrient interaction can boost plant growth at the seedling stage. This study found that water deficit (30%~40% FC) significantly decreased seedling growth. However, appropriate fertilization (N = 8.7 g·plant^{-1}, P = 0.96 g·plant^{-1}) might mitigate the adverse effects. Similar research on *Robinia pseudoacacia* and *Gossypium hirsutum* seedlings revealed severe growth limitations and biomass reduction when treated with water-fertilizer coupling under water deficiency at 30% FC and −0.2 MPa, respectively. *G. hirsutum* treated with 85 mmol·L^{-1} N effectively mitigated the inhibitory effects of drought on plant height and ground diameter [31,32]. We observed that water-nitrogen interaction significantly promoted seedling growth, and applying nitrogen fertilizer (10.87 g·plant^{-1}) promoted relative increments in plant height and ground diameter in *C. petelotii* seedlings at reduced irrigation levels (55% FC). Seedlings of *Populus tomentosa* carr and *Macleaya cordata* (Willd) R.Br. also exhibit increased height and ground diameter growth under treatments of 55% FC + 5 g·plant^{-1} N and 30%~40% FC + 0.2 g·kg^{-1} N, respectively [14,33]. This shows that drought inhibits *C. petelotii* growth, whereas nitrogen application increases the available soil nitrogen. The absorption of this increased exogenous nitrogen by the root system promotes the growth and development of *C. petelotii* seedlings, thereby mitigating the damage to seedlings caused by drought stress [34]. Previous studies have shown that nitrogen promotes plant cell division and growth, whereas phosphorus accelerates cell division and stimulates aboveground growth [35]. Nutrients dissolved in water reach the root system's surface via mass flow or diffusion, and the appropriate amount of irrigation and fertilizer application stimulates ion diffusion of K^+ and $H_2PO_4^-$, which promotes nutrient uptake and plant growth [36]. We hypothesized

that *C. petelotii* seedlings reduced the energy required for survival by slowing plant height and ground diameter growth under low irrigation levels and that adding appropriate nitrogen/phosphorus fertilizers can alleviate the inhibitory effects of low irrigation levels on plant height and stem diameter. Meanwhile, fertilizer application may boost soil water potential and efficacy by transforming "ineffective" water for plant growth into "effective" water [37].

4.2 Effects of Water-Fertilizer Coupling on C. petelotii Photosynthesis

Photosynthesis is a source of energy and material for plant growth, and it plays a significant part in plant growth and development. In contrast, chlorophyll is the action ingredient for photosynthesis in plants, which impacts photosynthetic capacity [38,39]. Studies have indicated that providing plants with sufficient water and nutrients may promote a rise in chlorophyll levels, improving photosynthesis and promoting their nutritional development [40,41]. This study found that water and phosphorus, water-nitrogen. and nitrogen-phosphorus interactions significantly affected the chlorophyll content. Similar results were reported in a study on water and fertilizer in *Accinium corymbosum* seedlings [42]. Chlorophyll content decreased with decreasing irrigation levels, as demonstrated in the study on *Aglaia duperreana* [43]. Low water stress caused changes in the morphological structure of chloroplasts and photosynthetic parameters such as Pn, Ci, and Gs in *C. petelotii* seedlings [44]. It indicates that *C. petelotii* leaves that received adequate water and fertilizer had higher Gs leaves. Conversely, a low water environment reduced the Gs, chlorophyll content, and light energy absorption. It weakened the Pn of *C. petelotii* leaves, inhibiting the growth and development of the seedlings. The high nitrogen application group (8.7 $g \cdot plant^{-1}$) had a substantially higher chlorophyll content than the low nitrogen application group (2.17 $g \cdot plant^{-1}$) under low irrigation (40% FC) and low phosphorus application (0.96 $g \cdot plant^{-1}$). Similarly, high nitrogen (0.8 $g \cdot kg^{-1}$ N) treatment significantly reduced the inhibitory effects of drought and phosphorus deficit on the chlorophyll synthesis in *Robinia pseudoacacia* [45]. It implies that nitrogen can promote chlorophyll accumulation and that high nitrogen applications may reduce the inhibitory effect of low water and low phosphorus on chlorophyll production in *C. petelotii* leaves. Under low phosphorus conditions, the phosphorus uptake efficiency of seedlings may be higher, ensuring an adequate supply of phosphorus to the plant, which stimulates the nitrogen uptake required by *C. petelotii*. Low moisture inhibits chlorophyll biosynthesis and accelerates the decomposition of the original chlorophyll. Nitrogen application can boost chlorophyll synthesis and raise the nitrogen content of plant leaves, thus effectively regulating the light and performance, alleviating the light damage caused by drought stress on plants, and promoting *C. petelotii* leaf growth [46,47]. This study found that regardless of the irrigation level (40% FC or 70% FC), applying a decreased phosphorus administration rate (0.96 $g \cdot plant^{-1}$) resulted in higher chlorophyll content when supplemented with a relatively high nitrogen application rate (8.7 $g \cdot plant^{-1}$). In contrast, higher phosphorus administration (3.85 $g \cdot plant^{-1}$) increased chlorophyll content when the nitrogen application rate was lower (2.17 $g \cdot plant^{-1}$). *Zea mays* and *Oryza sativa* research followed the same trend as *C. petelotii* [48,49]. In *Oryza sativa*, lowering the phosphorus level below 45.0 $kg \cdot hm^{-2}$, the yield first increases and subsequently decreases as the nitrogen content increases. We postulated that the nitrogen-phosphorus interaction negatively affects the total chlorophyll content. Therefore, it is critical to maintain a proper balance between nitrogen and phosphorus applications. Our study found that lower moisture reduced photosynthetic characteristics regardless of nitrogen fertilizer content. Applying nitrogen fertilizer (8.7 $g \cdot plant^{-1}$) accelerated photosynthesis under sufficient water (70% FC) conditions but limited photosynthesis under low-water (W < 40%) conditions. A similar water-nitrogen coupling study in *Picea asperata* showed that nitrogen application boosts Pn under well-watered (W > 60% FC) conditions but has a detrimental under heavy water stress (40% FC) [50]. Nitrogen application can increase chlorophyll content, which leads to enhanced photosynthetic activity and light absorption by plant chloroplasts, resulting in higher Pn and increased photosynthetic capacity, which contributes to photosynthetic product accumulation and promotes plant growth [51]. Water significantly affected Pn, Tr,

Gs, and Ci, and applying nitrogen fertilizer in low moisture conditions, enhanced water stress. This resulted in a drop in *C. petelotii*'s leaf water content (LWC) and increased water loss in the guard cells, closing the stomata and limiting CO_2 entrance into the leaves. It subsequently increased in inter-cellular CO_2 levels, limiting the rate of photosynthesis. T9 (W = 80% FC) did not exhibit a higher photosynthetic rate, most likely because the tissue was saturated with moisture and the stomata closed passively, which hindered the entry of CO_2 into the chloroplasts, lowering the photosynthetic rate. Pn, Tr, and Gs values for T10 decreased. At the same time, Ci increased, which revealed that stomatal restriction values reduced at this moisture level, with non-stomatal limitation becoming the primary factor affecting *C. petelotii* photosynthesis. Consequently, both insufficient and excessive moisture significantly impacted photosynthesis and impeded the capacity to accumulate nutrients, limiting the development of *C. petelotii* seedlings.

4.3 Effects of Water-Fertilizer Coupling on Water Use Efficiency in C. petelotii

Plants respond to water stress by altering their water use efficiency, and LWC and SLW are key indices of plant adaptation to environmental changes [52]. This study demonstrated that water has a more significant impact than nitrogen. Treating the SLW of *Populus tomentosa* and *Toona sinensis* with water-fertilizer coupling yielded similar results. Their SLW increased with increasing irrigation levels but decreased when treated with drought conditions [33,53]. Low irrigation levels (30% FC) resulted in compacted soil due to the lack of moisture, causing cell membrane damage and lowering the SLW. Conversely, SLW significantly increased when irrigation was at 55% FC, indicating an increase in dry matter per unit leaf area and leaf thickness. These alterations are beneficial for resisting excessive transpiration and enhancing the water storage capacity of *C. petelotii* leaves. A positive correlation exists between the dry matter per unit leaf area (SLW), the CO_2 conversion rate, and water usage efficiency [54]. This study showed that the water-nitrogen interaction significantly affected the SLW, with a trade-off effect. Applying nitrogen fertilizer (5.43 g/plant) at lower water content (55% FC) significantly increased the SLW, but decreased with the increase of nitrogen fertilizer level. Similarly, the SLW of *Machilus pauhoi* seedlings attained a maximum with the fertilizer application (N:P = 8) at 40% FC moisture condition and decreased after that as nitrogen levels increased [55]. We postulated that increases in nitrogen fertilizer cause the saturation of the plant's total nitrogen absorption, resulting in increased nitrogen accumulation without commensurate rational usage. Applying nitrogen within the recommended range can stimulate cell division in leaf mesophyll cells, delay the formation and enlargement of spaces between cells, and enhance the leaf, palisade, and spongy tissue thickness. However, there was a significant positive correlation between leaf thickness, leaf dry matter content, and leaf nitrogen content [56]. Nitrogen fertilizer application facilitates nitrogen transport through the roots, increasing leaf nitrogen content. This enhances the plant's photosynthetic efficiency and stimulates the production of a significant amount of chlorophyll and photosynthetic proteins in the leaves. Consequently, the leaf nitrogen content further increases, which causes the leaves to get thicker. Our study found that the nitrogen-phosphorus interaction considerably impacted the SLW. The administration of nitrogen, especially in combination with phosphorus fertilizer, had a similar impact on the SLW of *Glycine* max [57]. The simultaneous application of nitrogen and phosphorus improves phosphorus availability in the soil, resulting in less nitrogen consumption and a higher nitrogen content in plant leaves [58]. Hence, the proper application of nitrogen or phosphorus fertilizer improved the nutritional composition of the soil, resulting in increased nutrient transfer to the above-ground section. Consequently, this could amplify the buildup of organic matter in the leaves, boosting the SLW. The rise in nutrient content will simultaneously impact plant photosynthesis, enhancing the rate of photosynthesis and facilitating plant growth and development. Consequently, this will also lead to an increase in SLW. In the present study, neither low nor high moisture levels significantly affected the LWC of *C. petelotii*. A similar experiment on water-fertilizer coupling in *Acer rubrum* 'Red Champions' showed no significant differences among treatments in LWC during the early

stage of color change [59]. All treatments showed higher LWC, which, according to a prior study, shows that maintaining higher LWC mitigates the negative effects of low water levels on seedling cell membranes. This indicates a high water-holding capacity and resistance to drought stress [60]. Nevertheless, *C. petelotii* naturally prefers a humid environment and has a high water requirement. Transgenic wheat exhibited higher LWC and water utilization efficiency when subjected to mild water stress [61]. This was attributed, in part, to higher levels of proline, soluble sugar, and soluble protein, along with lower malondialdehyde levels. The investigation of *C. petelotii* growth characteristics suffers from a few flaws due to the study's restrictive focus. Thus, more research and investigation into the physiological features of LWC are required.

4.4 Shortcomings and Prospects

This study investigated the growth characteristics, leaf water utilization, and correlation between indicators of *C. petelotii* seedlings under various water-fertilizer coupling treatments. It reveals the growth mechanism of *C. petelotii* seedlings under different water and fertilizer conditions. It provides a theoretical basis and practical guidance for the future cultivation and production of *C. petelotii* seedlings. This work only examined the growth mechanisms for the growth indicators. Therefore, more research is needed to investigate the mechanisms of water and nutrient uptake and utilization. It failed to elucidate the molecular mechanisms of water and nutrient interactions. In-depth research into these aspects, such as plant hydraulics and nutrient uptake mechanisms, could be included in future studies. Due to the trial constraints, certain discrepancies were observed between the potting trials and the *C. petelotii* grown naturally outdoors. Future research should combine pot trials with field trials.

5 Conclusion

This study investigated the effects of water-fertilizer coupling on the growth and water use efficiency of 24-month-old potted *C. petelotii* seedlings. Applying an appropriate amount of water and fertilizer increased the plant height and ground diameter of *C. petelotii*. However, the different water fertilizer treatments did not significantly affect leaf water content and water-holding capacity. In summary, T15 (W = 55% FC + N = 5.43 g·plant^{-1} + P = 2.40 g·plant^{-1}) was the optimum treatment, providing technical support for the investigation of the water-fertilizer coupling effect and cultivation of *C. petelotii* seedlings.

Acknowledgement: The authors acknowledge the following institutions for supporting this work: (1) College of Forestry, Guangxi University; (2) Key Laboratory of National Forestry and Grassland Administration on Cultivation of Fast-Growing Timber in Central South China; (3) Guangxi Key Laboratory of Forest Ecology and Conservation.

Funding Statement: Financial assistance project: Guangxi Forestry Science and Technology Project (Guilin kezi [2012] No. 25).

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Liangyu Luo and Ru Li. Experiments were done by Ru Li and Yijin Wang. The first draft of the manuscript was written by Liangyu Luo and Daocheng Ma. Daocheng Ma and Linghui Wang revised the original manuscript. All authors commented on previous versions of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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Appendix A

Table A1: Regression models and coefficient tests for growth indicators of *C. petelotii*

Variate.	y_{ph}	y_{gd}	y_{a+b}	y_{Pn}	y_{Tr}	y_{Gs}	y_{Ci}	y_{SLW}
Intercept	−1.634	−0.661	−2.024	−6.279	−11.364	−0.476	−216.374	−0.083
W(x1)	0.209	0.097	0.368	1.563	1.576	0.069	46.497	0.011
N(x2)	0.189	−0.123	−0.095	−2.872	1.822	0.051	103.749	0.009
P(x3)	0.299	0.389	3.103	1.881	1.908	0.072	113.023	0.016
W * W($x1^2$)	−0.008	−0.003	−0.004	−0.057	−0.058	−0.003	−1.735	0.000
N * N($x2^2$)	−0.074	−0.006	0.125	0.285	−0.721	−0.028	−39.996	0.001
P * P($x3^2$)	−0.104	−0.079	−0.265	−0.172	−0.325	0.001	−26.803	0.003
W * N(x1x2)	0.011	0.017	0.037	0.120	0.064	0.004	2.157	−0.002
W * P(x1x3)	0.006	−0.012	−0.098	−0.091	−0.034	−0.003	−1.566	−0.004
N * P(x2x3)	−0.008	−0.020	−0.576	−0.016	−0.103	−0.004	−3.013	0.013
R2	0.8614	0.8801	0.7611	0.5878	0.4976	0.3458	0.6319	0.6151
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0071	<0.0001	<0.0001

Table A2: The significance of factors and interactions on each indicator

Variate	Plant height	Ground diameter	Chl a + b	Pn	Tr	Gs	Ci	LWC	SLW
W	<0.0001	<0.0001	0.0249	<0.0001	<0.0001	0.0038	0.0009	0.0086	0.0094
N	0.0047	0.0034	0.8287	0.0004	0.0772	0.4158	0.0059	0.6961	0.3936
P	0.0031	<0.0001	<0.0001	0.1063	0.2171	0.4519	0.0435	0.2924	0.3280
W * N	0.0042	<0.0001	0.1322	0.0058	0.2576	0.2727	0.2837	0.5183	0.0003
W * P	0.3007	0.0005	0.0099	0.1543	0.6869	0.6199	0.6055	0.6517	<0.0001
N * P	0.6696	0.0847	<0.0001	0.9408	0.7245	0.8360	0.7735	0.6095	0.0001