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The Effect of Soaking Root Fertilizer on Promoting the Seedling Early Growth and Root Development of *Eucalyptus urograndis*

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Abstract: We examined the feasibility of applying soaking root fertilizer to *Eucalyptus urograndis* seedlings by dipping the roots, and the effectiveness of the method for improving the overall growth quality of the seedlings by affecting root growth. The seedlings of *E. urograndis* in the Southern National Forest Seedling Demonstration Base in China were dipped with seven kinds of soaking root fertilizer formulas, while another group of clear water was set as a control. We regularly investigated the relationship between root traits and other seedling traits, and the formulas conducive to the high-quality growth of seedlings in one month and two months were screened by principal component analysis (PCA). The feasibility of applying soaking root fertilizer by dipping the roots was analyzed by comparing the difference between the formula and the control. The F2 formula had the best promoting effect on the growth and biomass of seedlings and the highest ground diameter within one month. The biomass, cumulative height growth, and average crown width were also the highest in the two months, but the decreasing growth height in F2 was also obvious, along with reduced photosynthesis and root morphology. The F8 and F4 formulas as compound fertilizers showed the potential of a high growth rate and high quality. The seedlings on which they were all applied had an excellent photosynthetic capacity and a well-developed root system. A correlation analysis showed that root traits were significantly correlated with some aboveground indices of seedlings (growth, biomass and nutrients, etc.), among which the root K concentration was very significantly correlated with photosynthetic capacity, and the root P was very significantly correlated with seedling biomass. Dipping roots mainly promoted the uptake of P and K nutrients by roots, which had a positive effect on seedling photosynthesis and the root system, and thus improved the growth increment and growth quality of seedlings. A principal component analysis showed that dipping roots significantly promoted the growth traits of seedlings, and the best-performing formula for dipping roots was F8 at one month of growth. F4 was the best choice for growth at two months. F4 showed excellent performance in all trait indices and was the only treatment where an increase in the rhizome-to-stem ratio occurred, indicating that the nutrient distribution of F4 seedlings was uniform and efficient, which compensated for the deficiency of the follow-up fertility of the quick-acting complex fertilizer. The scientific formula of soaking root fertilizer can be economically applied by dipping roots to synchronously promote early growth and quality of seedlings, which can provide a theoretical basis for the early large-scale cultivation of *E. urograndis* and other plants.

Keywords: *Eucalyptus urograndis*; soaking root fertilizer; seedling early growth; root development

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Citation: Zhang, S.; Yang, J.; Ouyang, L.; Chen, S. The Effect of Soaking Root Fertilizer on Promoting the Seedling Early Growth and Root Development of *Eucalyptus urograndis*. *Forests* **2023**, *14*, 2013. https://doi.org/10.3390/f14102013

Academic Editor: Mulualem Tigabu

Received: 12 August 2023
Revised: 23 September 2023
Accepted: 6 October 2023
Published: 7 October 2023

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1. Introduction

The external determinants of the quality of seedlings in a plantation mainly include the site index and the plantation managerial decisions [1–3]. In areas of low fertility, low nutrient availability limits the growth of seedlings [4]. Fertilization is an important way to regulate the growth and development of plant seedlings and improve soil fertility in such areas [5]. Fertilization can be classified according to fertilization time, application mode,

and the location of the effect in the plant. For plantation seedlings, the status of early root growth fundamentally affects the subsequent survival and growth of the seedling [6]. Plants need roots to absorb water and minerals, but these resources are unevenly distributed in the soil [7]. More developed roots give the plant stronger root-absorption properties and enhance seedling resistance, growth and development [8–10]. Root fertilization is one of the methods used to promote the early growth of seedling roots. The root system growth of seedlings treated with different root soaking fertilizer formulas generally shows differences due to the chemical composition [11,12], total nutrient uptake [13,14], and elemental ratio of the fertilizer [15,16]. Root traits are also strongly correlated with the aboveground traits (such as leaf traits and biomass allocation traits) [17]. The application of soaking root fertilizers can increase fertilizer utilization, promote the rapid growth of seedlings and root development and improve the total nutrient uptake in soil [18,19].

Eucalyptus urograndis is a hybrid of *E. urophylla* and *E. grandis*, and has the advantages of an excellent stem shape, rapid growth and strong stress resistance [20–22]. It was successfully obtained by cross breeding in China in the 1980s and gradually became one of the most prominent fast-growing eucalyptus hybrids in southern China [23,24]. Seedlings of *E. urograndis* have a strong water and nutrient absorption capacity, due to their well-developed root system, and the species is therefore a good choice for short-term plantation management [25]. However, the roots of *E. urograndis* seedlings developed in southern China have a lower biomass and turnover [26], which may cause soil degradation and nutrient loss in the long-term [27]. In addition, the acid soil in southern China has nutrient limitations in terms of nitrogen (N) and phosphorus (P) [28,29], and the plant seedlings grow poorly under these nutrient deficient conditions [30]. Potassium (K), N, and P are the main nutrient components required by seedlings [31]. There are various types of soil N, and the N that is easily absorbed and utilized by plants referred to as available N (i.e., ammonium N, nitrate N, and amide N) [32]. The action mechanism and effects of different types of N fertilizer are different. For example, ammonium bicarbonate is soluble in water and can be effectively used by plants due to its rapid absorption and fertilization efficiency, but ammonium bicarbonate is volatile under moisture-rich conditions. Nitrate N can promote the absorption of calcium (Ca), magnesium (Mg) and K by plants, but it is easily leached [33]. Amide N, such as urea, is not volatile at room temperature, and can only be absorbed by plants after microbial transformation. Soil K fertilizer includes potassium sulfate and potassium chloride, which are physiologically acidic fertilizers. The application of potassium chloride is more economical than potassium sulfate [34], but chloride ions can inhibit the activity of *Nitrosomonas* in soil and reduce the N loss due to removal by nitrification or nitrosification of ammonium N fertilizer [35]. Potassium sulfate is suitable for soil lacking sulfur (S), and is gentler on seedlings than potassium chloride, because the effect of sulfate on seedlings is more likely to be osmotic stress, whereas chloride ions have a high specific ion toxicity [36–38]. Potassium sulfate should not be applied to soil with strong reducing properties [39]. Soaking root fertilizer application can alleviate the problem of nutrient uptake of seedlings [40,41], but large-scale fertilization will reduce the economic benefits and increase the cultivation time. Additionally, the traditional application of soaking root fertilizer for seedlings has a low utilization rate, an unreasonable formula, a low K content and a low proportion of organic fertilizer, which can easily cause soil and water pollution [42,43]. In conclusion, a method of soaking root fertilizer application for *E. urograndis* is urgently needed to reduce the cultivation costs in plantations and optimize the cultivation process.

In this study, two complex fertilizers were used as two separate formulas, in addition to ammonium bicarbonate and urea as the N source, and superphosphate and *potassium chloride* as the P and K sources, respectively. A total of eight treatments with seven fertilizer formulas, and a control treatment with only water, were established based on previous experiments. The roots of *E. urograndis* seedlings at the same growth and development levels were soaked in eight different water-soluble fertilizer treatments. The effects of the eight treatments on the physiological and morphological characteristics of seedlings were

investigated, and the relationship between the effects on the roots and other traits was analyzed. The quality of the seedlings under the different treatments in different periods was comprehensively evaluated. The feasibility and effectiveness of this dipping method were evaluated by comparing the difference between the best formula and the control.

2. Materials and Methods

2.1. Plant Materials and Environmental Conditions

The experiment was conducted from 8 November 2022 to 14 January 2023 near a shaded shed (110°6′ E, 21°15′ N) at the Southern National Forest Seedling Demonstration Base in China. The test site has an average annual temperature of 23.1 °C and receives 1567 mm of rainfall per year, mainly during the rainy season from May to September. The annual relative humidity of the test site is 80.4% and the site's altitude measures 95.55 m. The soil types are mainly shallow marine sediment lateritic soil, basalt lateritic soil and sand shale lateritic soil, with a pH value ranging from 4.5 to 5.3. The initial concentrations of N, P and K concentrations were 1.20 g/kg, 0.87 g/kg and 2.61 g/kg, respectively. The initial experiment used a growing medium composed of 50% soil, 25% peat soil, and 25% vermiculite. The study consisted of eight treatments, each with 20 pots of first-rate *E. urograndis* seedlings in mesh bags. In total, there were 160 plants. Each seedling with a mesh bag was 30 cm tall, the height of each mesh bag after loading soil was 7 cm, and the bottom diameter was 4 cm. All seedlings were purchased from Longge Nursery, Suixi County, Zhanjiang City, Guangdong Province on 7 November 2022. Following purchase, they were placed in a sheltered area away from direct sunlight.

2.2. Fertilizer Preparation and Handling

Two types of complex fertilizers were purchased from Yara Trading (Shanghai) and Hubei Sanning Chemical (HSC); nitrate N accounted for 6.5%, and ammonium N accounted for 8.5%. Potassium fertilizer (potassium chloride) was purchased from Sinochem Fertilizer. Organic N fertilizer (urea) was purchased from Jiangsu Jinmei Hengsheng Chemicals(Xuzhou, China). Inorganic N fertilizer (ammonium bicarbonate) was purchased from ChengDu YuLong Chemicals (Chengdu, China). Phosphate fertilizer (calcium superphosphate) was purchased from Hubei Huangmailing Phosphate Chemicals (Xiaogan, China), as shown in Table 1.

Table 1. The soaking root fertilizer formulas.

Treatment	N Content [1]	P Content	K Content	Nutrient Mass Concentration	Product Content	Product and Ingredient Concentration
F1	0	0	0	0	0	Water
F2	22.5	22.5	22.5	1.35%	150	Complex fertilizer [2]-(Yara Trading)
F3	22.5	22.5	22.5	1.35%	150	Complex fertilizers [2]-(HSC)
F4	15	40	20	1.5%	370.5	Potassium chloride (60%), ammonium bicarbonate (17.2%) and calcium superphosphate (16%)
F5	15	40	0	1.1%	337.2	
F6	20	40	0	1.2%	366.3	
F7	25	30	0	1.1%	332.8	
F8	10	20	0	0.6%	146.7	Urea (46%), and calcium superphosphate (16%)

[1] The N, P, and K contents are the dissolved nutrient mass per 5 L of clear water, and product content is the applied fertilizer mass (g). [2] The mass concentrations of the N, P, and K components in the F2 and F3 treatments were 15%.

2.3. Experimental Design

After watering the seedlings and letting them sit overnight, we completely dissolved the seven types of fertilizer in 5 L of water on the morning of November 8. We mixed the growing medium and put it into pots. Next, we soaked 20 seedlings in each fertilizer treatment (including the control treatment) for 5 min, and then immediately transplanted them into planting pots with a capacity of 15 L and a top diameter of 28 cm. One plant was

planted in each pot and a total of 160 potted plants were placed outside the shaded shed. Each week, the pot positions treated with the same formula were randomly adjusted to avoid marginal effects that could affect the test results. The seedlings were watered sufficiently to maintain normal growth during the experiment, and no topdressing was added.

2.4. The Determination of Growth Characteristics, Root Parameters, and Biomass Per Organ

The height (H) of each seedling was measured from the base of the stem to the apex using a tape measure (accurate to 0.1 cm), and the crown width was also measured using a tape measure. A vernier caliper (accurate to 0.01 mm) was used to measure the ground diameter (D) of the thickest point at the base of the stem. The first measurement was taken on November 8. As initial growth indicators, growth traits were measured every 14 days for all seedlings for a total of five measurements. The height increase (Hi) was defined as the difference in height between the current measurement and the last measurement. The cumulative height increase (CHI) was the difference between the current measurement and the initial seedling height. The crown width was the average distance between the two leaves furthest apart in the north–south and east–west directions of the seedling. After the third measurement (day 28) and the fifth measurement (day 56), five seedlings were randomly selected from each treatment for destructive sampling. The whole plant was cleaned, the root, stem and leaf parts were separated, the root soil was washed, and the fresh root weight (FRW), fresh stem weight (FBW) and fresh leaf weight (FLW) were measured on a balance (accurate to 0.01 g), and the aboveground fresh weight (AFW) was the sum of FLW and FBW. The vernier caliper was used to measure root neck diameter (RND) and axial root diameter (ARD) at 1cm below the neck.

After determining the ARD, the root system to be tested was scanned with the Expression 12000XL image scanner to obtain images. The WinRHIZO root analysis system (WinRHIZO Pro 2007D, Regent Instruments Inc., Québec City, QC, Canada) was used to determine the morphological characteristic of the roots, and the total root length (TL) and total root surface area (TSA) were quantitatively analyzed. After the root parameters were determined, the samples were placed in the oven, dried at 60°C to a constant weight, and the root dry weight (RDW), stem dry weight (DBW), leaf dry weight (LDW), aboveground part dry weight (ADW) and seedling dry weight (SDW) were determined. The ADW was the sum of DBW and LDW. The root–stem ratio (R/S) and seedling index (SI) were calculated according to Formulas (1) and (2) [44], respectively:

$$\text{Root-stem ratio} = \text{RDW}/\text{ADW} \quad (1)$$

$$\text{Seedling index (\%)} = (\text{D}/\text{H} + \text{RDW}/\text{ADW}) \times \text{SDW} \quad (2)$$

2.5. The Determination of Leaf and Photosynthetic Characteristics

On days 28 and 56, vernier calipers were used to measure the leaf thickness of each seedling from the top to the 3–5 pairs, and the mean value was taken as the mean leaf thickness (MBT) of the seedling. On day 56, five seedlings were randomly selected from each treatment and three leaves (large, medium and small) were cut. The grid method (calculated the number of unit grids occupied by a leaf) was used to calculate the mean leaf area (MLA) of each seedling. Subsequently, five seedlings were selected from each treatment, and seedling photosynthetic parameters from the top to the fourth leaf pair of each seedling were measured under natural light between 9:00 and 11:00 using an Li-6400 photosynthetic measurement system (Li-COR, Lincoln, NE, USA). During the determination of photosynthetic characteristics, the light intensity set by the photosynthesizer measurement system was maintained at 1200 $\mu mol \cdot m^{-2} s^{-1}$. The net photosynthetic rate (*Pn*, $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), transpiration rate (*Tr*, $mmol\ H_2O \cdot m^{-2} \cdot s^{-1}$), intercellular carbon dioxide concentration (*Ci*, $\mu mol \cdot CO_2\ m^{-2} \cdot s^{-1}$), and stomatal conductance (*Gs*, $mmol\ H_2O \cdot m^{-2} \cdot s^{-1}$) of the seedlings, and the atmospheric CO_2 concentration (*Ca*, $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$) were recorded, and water-use efficiency (WUE) and stomatal limitation (*Ls*) were calculated according to Formulas (3) and (4) [45]. The same leaf photosynthetic parameters were mea-

sured continuously for 5 days before and after days 28 and 56, and the average value was calculated as the seedling photosynthetic value. Each set of measurements was performed in reverse order from the last measurement to avoid the influence of the leaf measurement order on the result:

$$\text{Water-use efficiency } (\mu\text{mol}CO_2 \cdot \text{mmol}^{-1}H_2O) = Pn/Tr \quad (3)$$

$$\text{Stomatal limitation } (\%) = (Ca - Ci)/Ca \times 100\% \quad (4)$$

2.6. The Determination of Total Nutrient Uptake

After measuring the biomass of each organ on day 56, we crushed the dried roots, stems, leaves and soil samples and passed them through a standard soil sieve. We determined the total amounts of N, P, and K in the soil by using the Kjeldahl digestion, molybdenum blue colorimetric analysis and flame photometry, respectively [46]. For each plant organ, we used the Kjeldahl Method to determine the total N and used acidic calcination to determine the total P and K [47]. The total N, P, and K absorption and the mass concentrations of seedlings, roots, stems, and leaves were expressed as xs, xr, xp, and xl, where x represents N, P or K.

2.7. Comprehensive Evaluation of the Soaking Root Fertilizer Treatments

A principal component analysis (PCA) was applied to analyze all measurement indices of the F1–F8 soaking root fertilizer treatments after the third and fifth measurements, respectively. A mathematical formula for the principal component score was constructed with the goal of achieving 85% of the cumulative variance contribution rate of the principal components. The score of each principal component of the formula was the average of the corresponding principal component score of seedlings, and the comprehensive score of the formula was the weighted cumulative value of each principal component score of the formula. Formulas with high synthesis scores in both measures were screened.

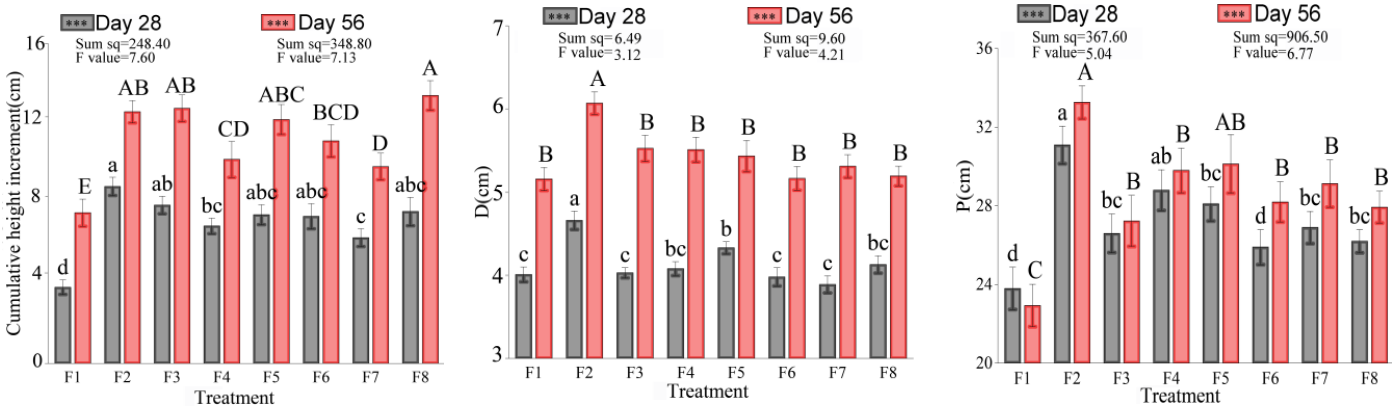
2.8. Data Processing and Statistical Analysis

Data were collated using WPS Office 2019, and R-4.2.2 (R Foundation for Statistical Computing, Vienna, Austria) was used for one-way analysis of variance (ANOVA). PCA and Duncan's test were used for multiple comparisons. Origin 2022 was used for constructing figures.

3. Results

3.1. The Effects of Different Soaking Root Fertilizer Treatments on Seedling Growth Properties

There were significant differences in the CHI, ground diameter, and average crown width (Figure 1) of *E. urograndis* seedlings among the different treatments and time periods. Multiple comparative analyses of the third measurement results showed that the CHI of F2 was the greatest (8.30 cm), which was 132.16% higher than F1, followed by F3, F5, F6, and F8, with F1 being the lowest (Figure 1a). The highest ground diameter (4.66 mm) was recorded for F2, which was 16.21% higher than F1, and was subsequently followed by F5, F8, F4, and F7 (Figure 1b). The largest average crown width (31.13 cm) was recorded for F2, which was 30.67% higher than F1, followed by F4, while F1 had the smallest (Figure 1c). The results for the fifth measurement were different. The CHI of F8 was the greatest (12.57 cm), which was 77.83% higher than F1, followed by F3, F2, and F5, while the lowest increase recorded for was F1 (Figure 1a). The largest ground diameter (6.07 cm) was recorded for F2, which was 17.69% higher than F1 and was significantly different from the other treatments. Conversely, the seedling ground diameter in the other treatments did not exhibit any significant differences (Figure 1b). The highest average crown width (33.27 cm) was recorded for F2, which was 45.06% higher than F1, followed by F5 and F1 (Figure 1c).



(**a**) (**b**) (**c**)

Figure 1. The results of the variance analysis on the growth traits of *E. urograndis* seedlings under different soaking root fertilizer treatments: (**a**) cumulative height increase (CHI, cm), (**b**) ground diameter (D, cm), (**c**) crown width (P, cm). Both the sum of squares (Sum sq) and the F value were rounded to the second decimal place. Significant differences in the third and fifth measurements for each treatment are indicated by lower and upper case letters, respectively. (Duncan's test; "***": $p < 0.001$; mean ± SE).

Height increment at the same measurement time is an essential indicator of seedling growth status. Four sets of high growth data were obtained from the five sets of growth trait measurements. The seedling height increments with the different formulas for distinct periods exhibited significantly varied seedling growth rates (Figure 2). On day 14, F5, F4, and F2 had the highest seedling height increments, which were 253.65%, 249.59%, and 235.77% higher than F1, respectively, followed by F3, F8 and F6. The lowest was F1. On day 28, F2 had the highest seedling height increment (4.19 cm) and was 77.87% higher than F1, which F4 had the lowest. On day 42, there were no significant differences in seedling height increments among all treatments. However, on day 56, the F2 seedling had the lowest height increment and was 19.71% lower than F1. The F1 seedling height increment increased moderately. F3 and F8 seedlings both had higher height increments, 97.81% and 89.78% greater than F1, respectively. In summary, the seedling height increment of F1 seedlings increased after 14 days, while that of other treatments showed a general trend of height increment decrease. The seedling height increments of F2 and F7 decreased rapidly after day 28, while the seedling height increments of F3 and F8 increased slightly after 42 days.

3.2. The Effects of Different Soaking Root Fertilizer Treatments on the Root Parameters of Seedlings

At the third measurement (Day 28), the different formulas of soaking root fertilizer treatments produced significant differences in the TL, TSA, and RND of seedlings (Figure 3a). However, by the fifth measurement, these differences had become insignificant (Figure 3b). The results of multiple comparisons in the third measurement showed that F8 had the highest TL (853.27 cm) and TSA (34.99 cm^2), which were 6.31% and 8.44% higher than F1, respectively, with F5 and F2 having the next highest values (Figure 4a,b). There was no significant difference between the root projection area and ARD.

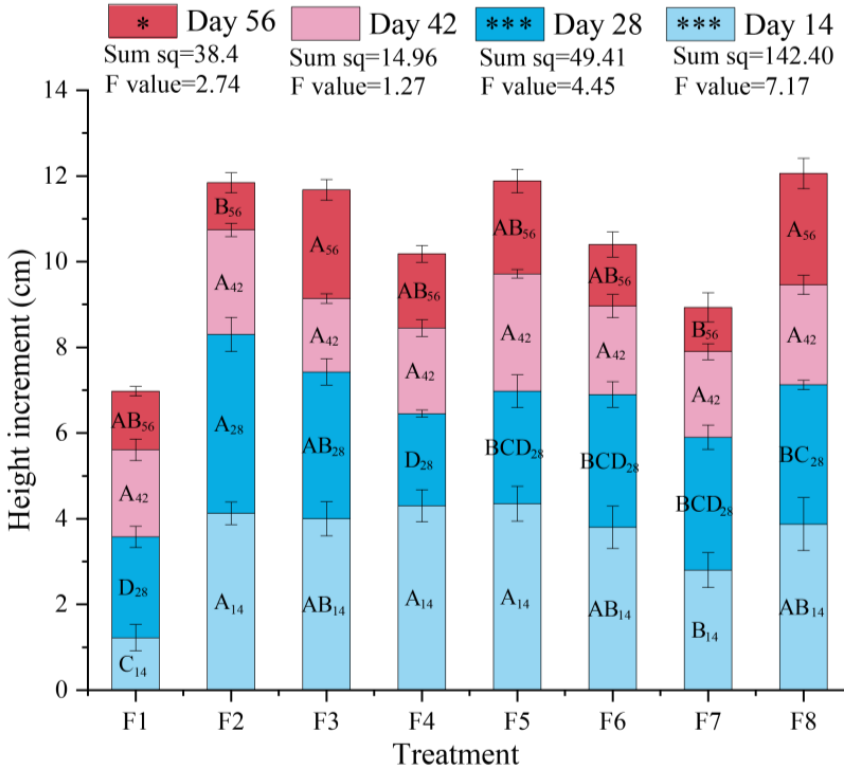
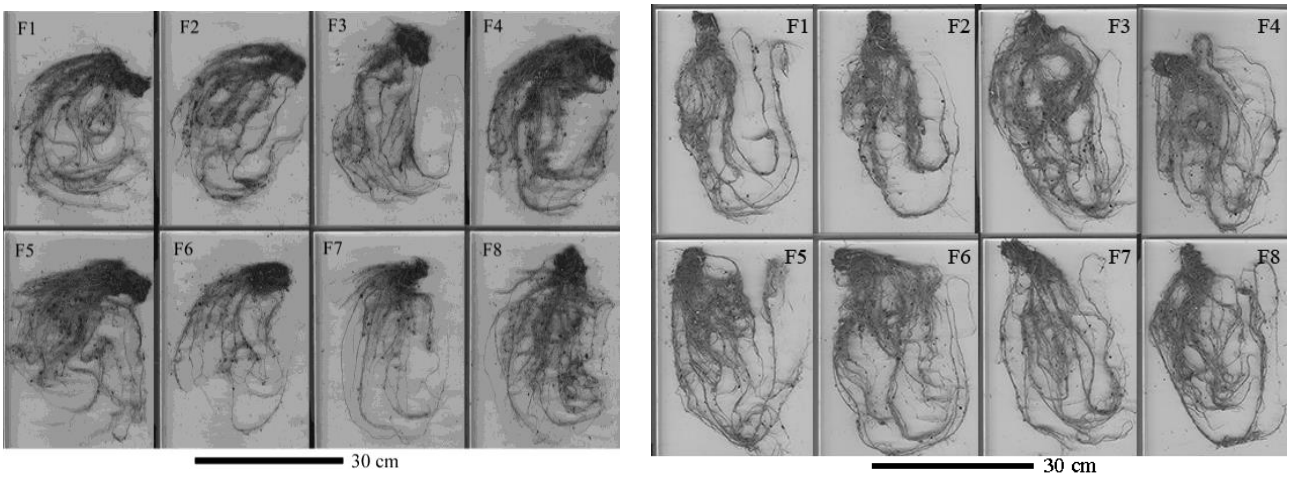


Figure 2. The results of the variance analysis of height increment data of *E. urograndis* seedlings with different soaking root fertilizer treatments. The same color is the calculated result for the same time measurement, and a total of four calculations were conducted. Significant differences in seedling growth height (Hi, cm) for each calculation are indicated by capital letters. (Duncan's test; "***": $p < 0.001$; "*": $p < 0.05$; mean ± SE).



(**a**) (**b**)

Figure 3. Images of roots under different soaking root fertilizer treatments during two measurements: (**a**) the third measurement and (**b**) the fifth measurement. F1–F8 refer to the various fertilization treatments applied to *E. urograndis* seedlings (see Table 1).

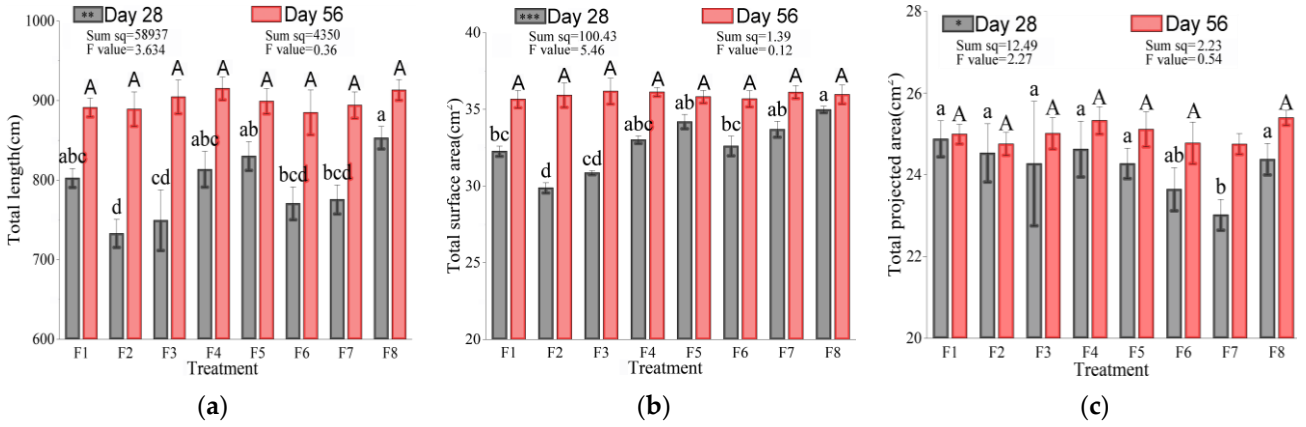


Figure 4. The results of the variance analysis of root parameters of *E. urograndis* seedlings under different soaking root fertilizer treatments: (**a**) total root length (TL, cm), (**b**) total root surface area (TSA, cm^2), (**c**) total projected root area (TPA, cm^2). Significant differences in the third and fifth measurements for each treatment are indicated by lower and upper case letters, respectively. (Duncan's test; "***": $p < 0.001$; "**": $p < 0.01$; "*": $p < 0.05$; mean ± SE).

3.3. The Effects of Different Soaking Root Fertilizer Treatments on the Biomass of Seedling Organs

Biomass is an important parameter for measuring the organic matter content of seedlings. There were significant differences in the biomass of seedlings' organs under different soaking root fertilizer treatments (Figure 5). At the third measurement, F2 and F8 had the highest RDWs (Figure 5a), which were 105.34% (5.61 g) and 104.03% (5.57 g) higher than F1, respectively. The ADW (Figure 5b) of F2 was also the highest, which was 147.46% (6.04 g) higher than F1. The application of soaking root fertilizer significantly increased the dry weight of seedlings, but there were no significant differences in the dry weight of seedlings under the different soaking root fertilizer treatments (Figure 5c). The highest R/S (Figure 5d) and SI (Figure 5e) were recorded for F8, but the differences among treatments were not significant. At the fifth measurement, the RDW, ADW, and SDW were the highest for F2, and were 49.63% (8.52 g), 68.28% (9.42 g) and 58.88% (17.95 g) higher than F1, respectively, with F4 having the next highest values. The highest R/S and SI were recorded for F4. A comparison between the two measurements showed that F4 was the only treatment in which the R/S increased, and F8 was the only treatment in which the SI decreased. The F1 dry weight index was the lowest in both measurements. In conclusion, F2 was the treatment most conducive to seedling material accumulation, F8 had a strong effect within 28 days, and F4 had an excellent performance after 28 days.

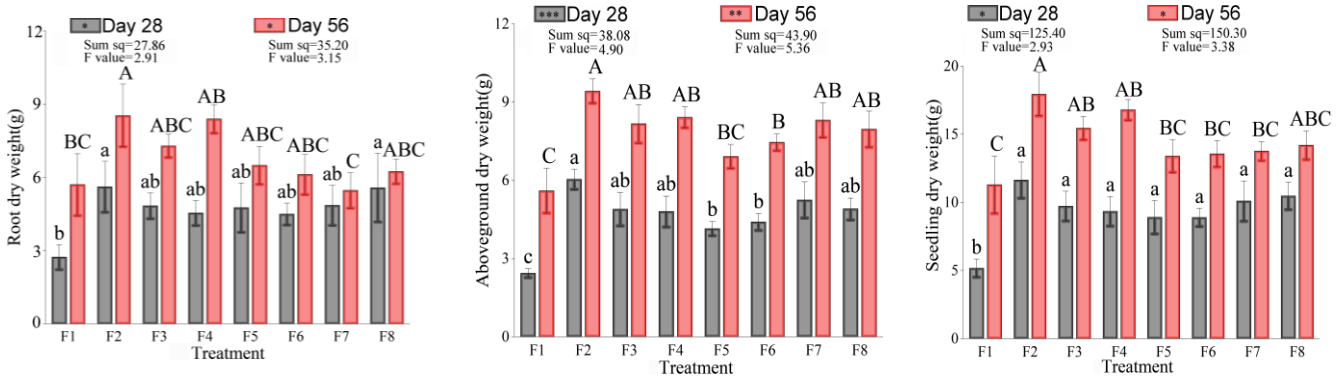
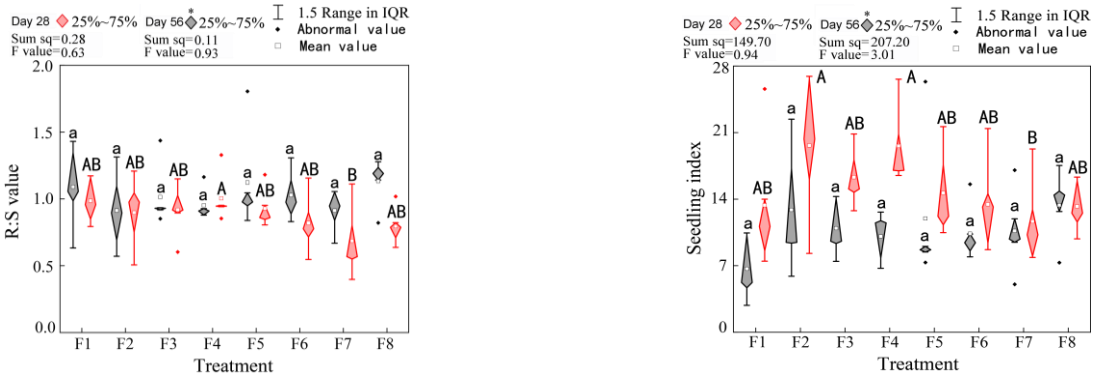


Figure 5. *Cont.*

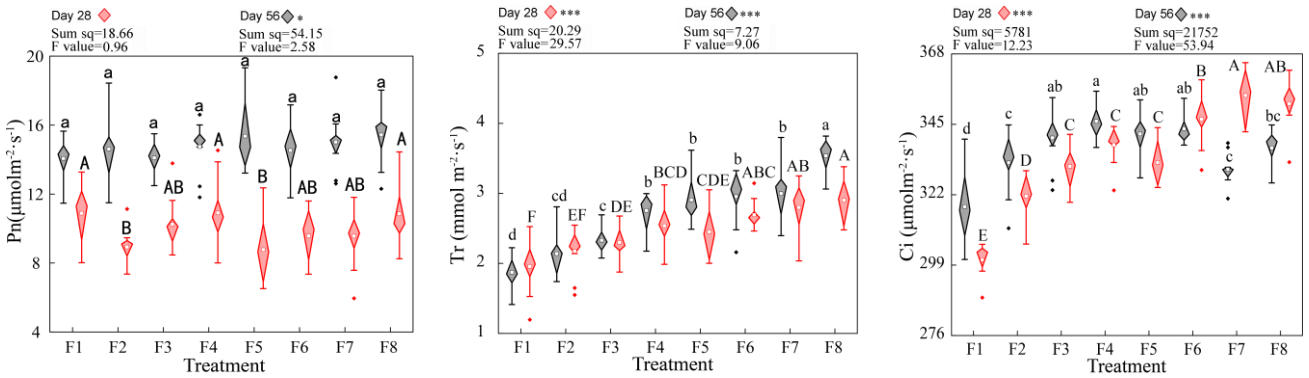


(**d**) (**e**)

Figure 5. The results of the variance analysis of seedling biomass of *E. urograndis* under different soaking root fertilizer treatments: (**a**) root dry weight (RDW, g), (**b**) aboveground dry weight (ADW, g), (**c**) seedling dry weight (SDW, g), (**d**) root stem ratio (R/S), (**e**) seedling index (SI)' Significant differences in the third and fifth measurements for each treatment are indicated by lower and upper case letters, respectively. (Duncan's test; "***": $p < 0.001$; "**": $p < 0.01$; "*": $p < 0.05$; mean ± SE).

3.4. The Effects of Different Soaking Root Fertilizer Treatments on Seedling Photosynthesis

The different soaking root fertilizer treatments had significant effects on the photosynthesis of *E. urograndis* seedlings. At the third measurement, there were no significant differences in the *Pn* among all the treatments (Figure 6a). The highest Tr (Figure 6b) was recorded for F8, which was 89.30% (3.54 mmol $m^{-2}{\cdot}s^{-1}$) higher than F1. F1 had the lowest *Tr*. The *Ci* (Figure 6c) and *Gs* (Figure 6d) of F4 were the highest, and were 8.76% (345.99 μmol $m^{-2}{\cdot}s^{-1}$) and 57.14% (0.44mmol $m^{-2}{\cdot}s^{-1}$) higher than F1, respectively. The *Ci* and *Gs* of F1 were the lowest. The F1 had the highest WUE (Figure 6e), followed by F2, while F7 and F8 had the lowest. The F1 also had the highest *Ls* (Figure 6f), followed by F7 and F2, and F4, F5, F6 and F8 had the lowest WUE. At the fifth measurement, the *Pn* was the highest for F4, and was 0.17% (10.90 μmol $m^{-2}{\cdot}s^{-1}$) higher than F1, followed by F1 and F8. Both F5 and F2 had the lowest *Pn*. The highest *Tr* (2.90 mmol $m^{-2}{\cdot}s^{-1}$) was recorded for F8, which was 48.24% higher than F1, and F1 had the lowest *Tr*. F8 had a 16.94% (351.73 μmol $m^{-2}{\cdot}s^{-1}$) higher *Ci* than F1, and F1 had the lowest *Ci*. F8 had the highest *Gs* (0.59 mmol $m^{-2}{\cdot}s^{-1}$), which was 200.70% higher than F1, followed by F7. Both F1 and F2 had the lowest *Gs*. The WUE of F1 was the highest, followed by F2 and F3, with F8 being the lowest. F1 had the highest *Ls*, followed by F2, F7 and F8. In conclusion, the photosynthetic capacities of F8 and F4 were the strongest, while those of F2 and F1 were the weakest.



(**a**) (**b**) (**c**)

Figure 6. *Cont.*

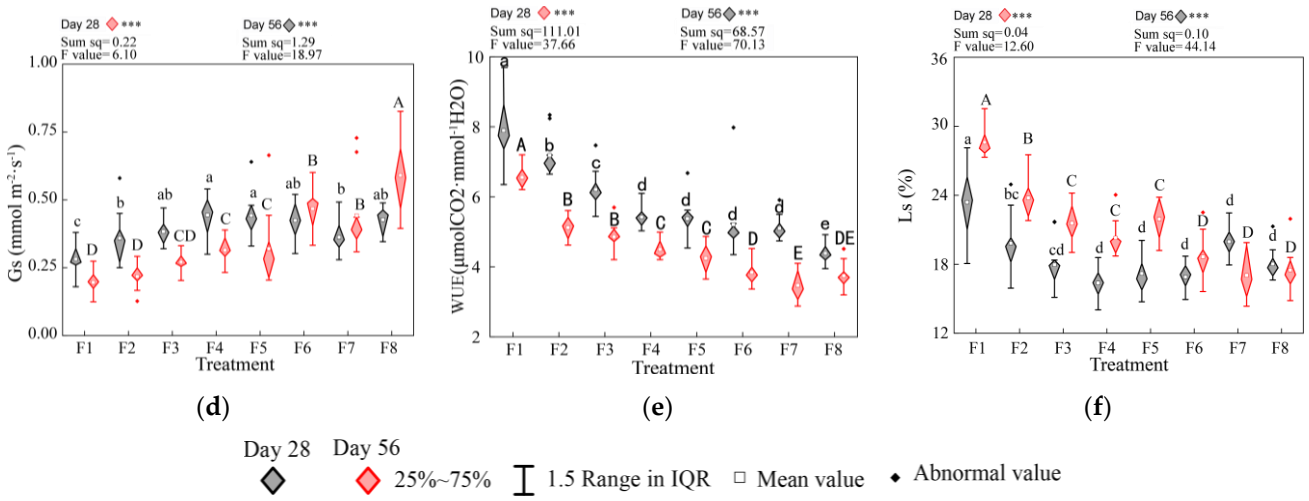


Figure 6. The photosynthetic parameters of *E. urograndis* seedlings under different soaking root fertilizer treatments: (**a**) net photosynthetic rate (*Pn*, μmol m^{-2}·s^{-1}), (**b**) transpiration rate (*Tr*, mmol m^{-2}·s^{-1}), (**c**) intercellular carbon dioxide concentration (*Ci*, μmol m^{-2}·s^{-1}), (**d**) stomatal conductivity (*Gs*, mmol m^{-2}·s^{-1}), (**e**) water use efficiency (WUE, μmol·CO_2 $mmol^{-1}$ H_2O), (**f**) stomatal limitation (*Ls*, %). Significant differences in the third and fifth measurements for each treatment are indicated by lower and upper case letters, respectively. (Duncan test; "***": $p < 0.001$; "*": $p < 0.05$; mean ± SE).

3.5. The Effects of Different Soaking Root Fertilizer Treatments on Total Nutrient Uptake and Concentrations in Seedlings and Organs

The nutrient accumulation of *E. urograndis* seedlings and organs was significantly affected by the different soaking root fertilizer treatments. In terms of total nutrient uptake, the soaking root fertilizer application generally increased the N, P, and K contents in seedlings and all organs, although there was no difference in the N content in leaves among the different treatments (Figure 7a). The highest N contents in seedlings, roots, stems and leaves were recorded in F2, which were 35.26% (114.75 mg), 51.96% (53.83 mg), 46.88% (11.48 mg) and 22.51% (49.43 mg) higher than F1, respectively. The N contents of F4 seedlings and roots were second to those of F2, and were 21.44% (101.51 mg) and 40.77% (49.87 mg) higher than F1, respectively. In seedlings and stems, the highest P content was recorded in F4 (12.3 and 3.51 mg, respectively), which was significantly different from the other treatments and was 161.18% and 94.49% higher than F1, respectively. The highest P content in roots was recorded in F2 (5.14 mg), which was 111.72% higher than F1, followed by F4 (4.72 mg), and the highest P content in leaves was recorded in F8 (4.38 mg), which was 173.38% higher than F1, followed by F4 (4.06 mg). In seedlings and leaves, the highest K contents were recorded in F8 (134.99 and 63.89 mg), which were 71.84% and 116.76% higher than F1, followed by F2. In roots, the highest K content was recorded in F4 (62.06 mg, 96.14% higher than F1), and in stems, it was recorded in F2 (29.11 mg, 66.89% higher than F1). From the perspective of nutrient concentrations (Figure 7b), the N concentrations of seedlings, stems, and leaves were the highest in F1 (7.61, 3.46 and 12.61 g·kg^{-1}), and the N concentrations of F4 seedlings and organs were the lowest. The highest P concentration in seedlings and stems was recorded for F4 (0.73 and 1.02 g·kg^{-1}, respectively), which were 78.04% and 256.99% higher than F1, respectively. In leaves, the highest P concentration was recorded in F8 (0.94 g·kg^{-1}, 96.75% higher than F1), followed by F4. In roots, the highest P concentration was recorded in F5 (0.61 g·kg^{-1} 42.79% higher than F1), followed by F2 and F8, with F1 being the lowest. The K concentration was the highest in F8 seedlings and leaves (9.47 and 13.56 g·kg^{-1}, respectively), which were 35.00% and 51.09% higher than F1, respectively. In roots, the K concentrations in F8 and F4 were 30.84% (7.41 g·kg^{-1}) and 29.90% (7.36 g·kg^{-1}) higher than F1. In conclusion, F2, F4 and F8 achieved an excellent performance in terms of growth indices and had the highest total nutrient uptakes; F2 and F4 had the highest root total nutrient uptake, and F8 had the highest total nutrient uptake

in the leaves. F1 plants with poor growth absorbed more N than P and K. The soaking root fertilizer application increased the absorption ratio of P and K, especially in F4 and F8. The F4 treatment achieved the greatest P absorption, while K absorption was the greatest for F8. The nutrient distribution of F2 and F4 was more uniform, and the nutrient concentration of F8 leaves was higher.

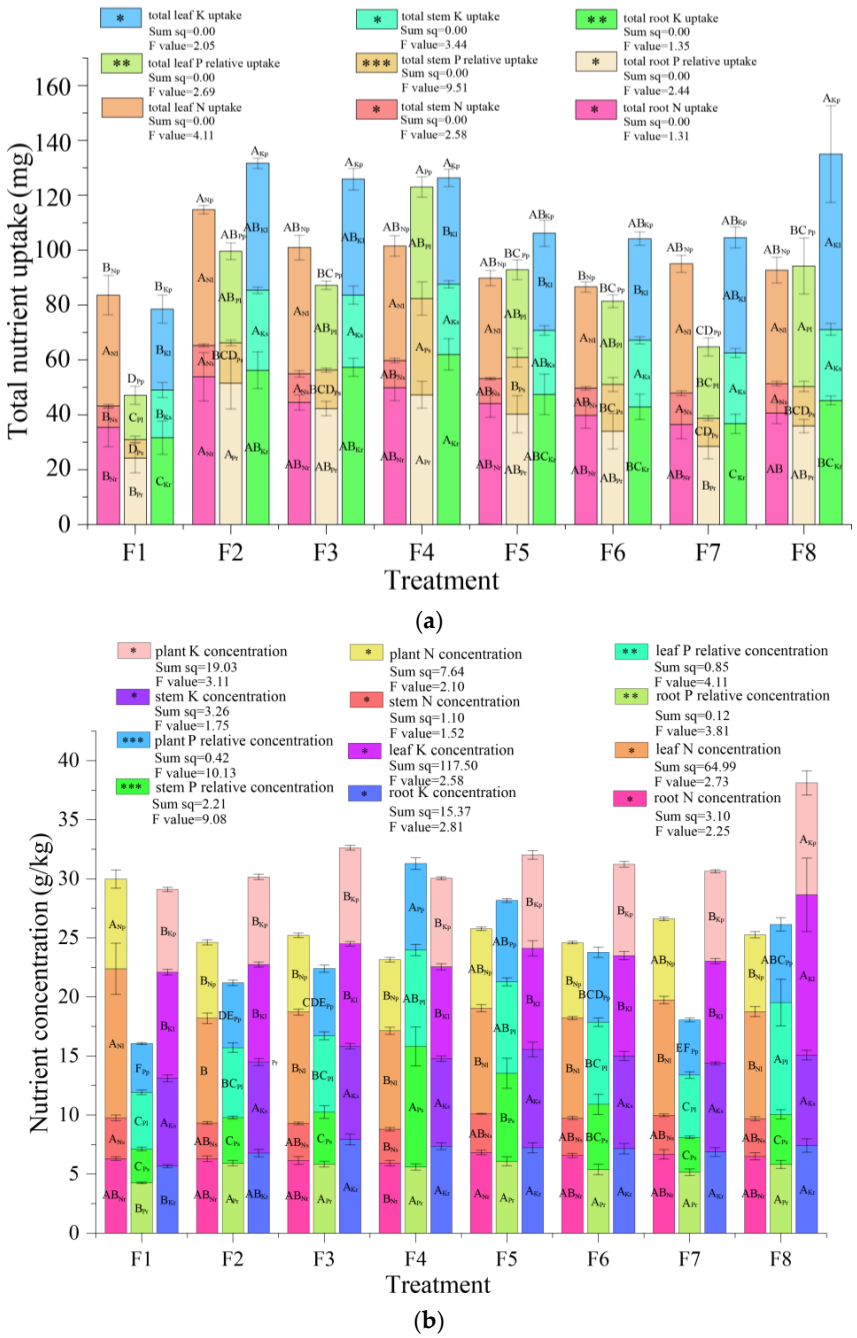


Figure 7. The N, P, and K contents and concentrations of organs and whole seedlings of *E. urophylla* under different soaking root fertilizer formulas: (**a**) total nutrient uptake (g) and (**b**) nutrient mass concentration (g/kg). Multiple comparisons were made between the same letter subscripts, and the results are represented by capital letters. The relative content/concentration of P is 10 times the real content/concentration of P. Both the sum of squares (Sum sq) and the F value were rounded to the second decimal place. The subscripts Np, Pp, and Kp represent the content or concentration of N, P, and K in the whole plant, respectively. (Duncan's test; "***": $p < 0.001$; "**": $p < 0.01$; "*": $p < 0.05$; mean ± SE).

3.6. The Comprehensive Evaluation of E. urograndis Seedlings with Different Soaking Root Fertilizer Formulas

A correlation analysis was conducted to determine the relationships between the root indices measured on day 56 and the other growth indices. As shown in Figure 8, growth traits and biomass indices were closely related to RDW and the total nutrient absorption of roots. The photosynthesis of seedlings was strongly and significantly correlated with the K concentration in the roots (positively correlated with *Ci*, and negatively correlated with WUE and *Ls*). The strong positive correlation of the SI with S/R was extremely significant. Generally, the nutrient concentration in roots was related to the aboveground nutrient concentrations in the seedlings, among which the total amount of P and K in the roots was significantly negatively correlated with the N concentration, and significantly positively correlated with the P concentration. In summary, the changes in root morphology or physiological traits were related to the changes in seedling growth, photosynthetic function, biomass and aboveground nutrients, which need to be considered in combination with the changes in the specific traits of roots after root dipping.

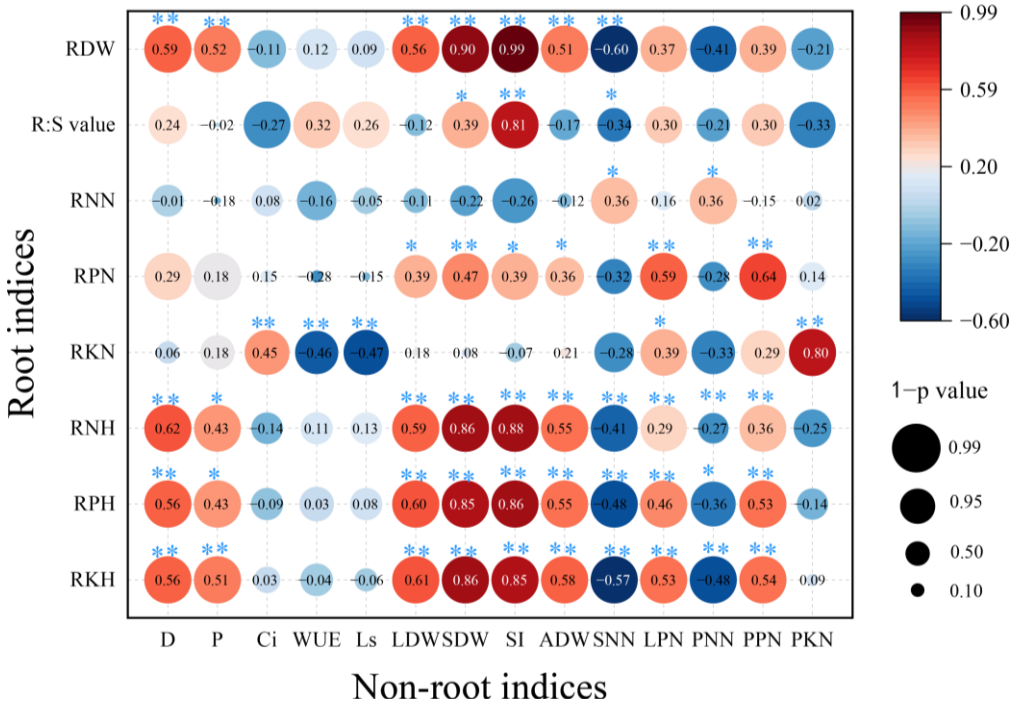


Figure 8. A correlation analysis between root indices and the other growth indices at day 56. "*" indicates a significant correlation, $p < 0.05$, and "**" indicates an extremely significant correlation, $p < 0.01$. The darker the blue, the more negative the correlation, and the darker the red, the more positive the correlation. The larger the circle, the more prominent it is.

3.7. Comprehensive Evaluation of E. urograndis Seedlings with Different Soaking Root Fertilizer Formulas

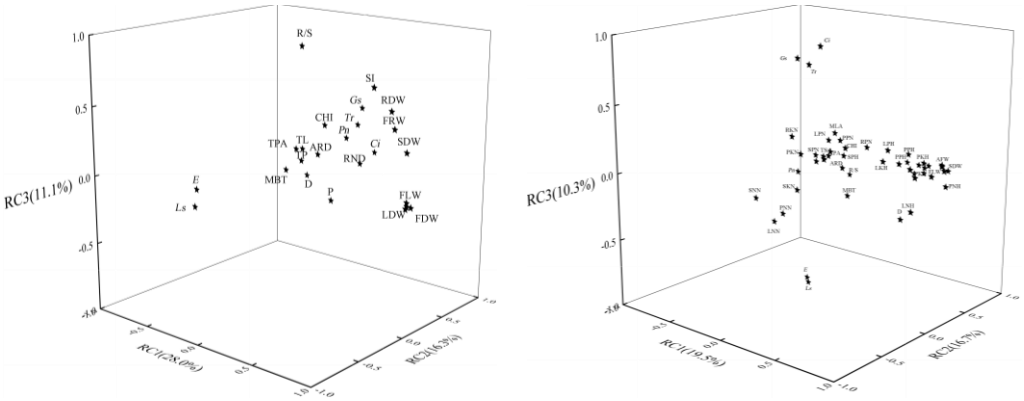
Taking a cumulative contribution rate of 80% as a criterion, the 24 indicators measured on day 28 and the 49 indicators measured on day 56 were subjected to a PCA after denoising. The first PCA showed that seven principal components were retained, while eight were retained in the second PCA. The cumulative contribution rate of the principal components measured on day 28 was 85.1%, among which the principal component with the largest contribution rate (Figure 9a, RC1) accounted for 28.0%, corresponding to the biomass of various organs. The second principal component (Figure 9a, RC2) accounted for 16.3%, corresponding to photosynthetic functions; and the third (Figure 9a, RC3) accounted for 11.1%, and corresponded to the root traits. The cumulative contribution rate of the principal components measured on day 56 was 79.9%, and the principal component with the largest

contribution rate accounted for 19.5% (Figure 9b, RC1), corresponding to the aboveground growth and N index of the plant, the second principal component accounted for 16.7% (Figure 9b, RC2), corresponding to the root traits and SI; and the third accounted for 10.3% (Figure 9b, RC3), corresponding to the photosynthesis parameters. Based on the PCA results, the variance of each component was taken as the weight of the weighted summation, and the comprehensive evaluation formulas P_1 and P_2 of *E. urograndis* seedlings with different soaking root fertilizer treatments at days 28 and 56 were constructed as Formulas (5) and (6):

$$P_1 = 0.28\ RC1 + 0.163\ RC2 + 0.111\ RC3 + 0.091\ RC4 + 0.082\ RC5 + 0.073\ RC6 + 0.051\ RC7 \quad (5)$$

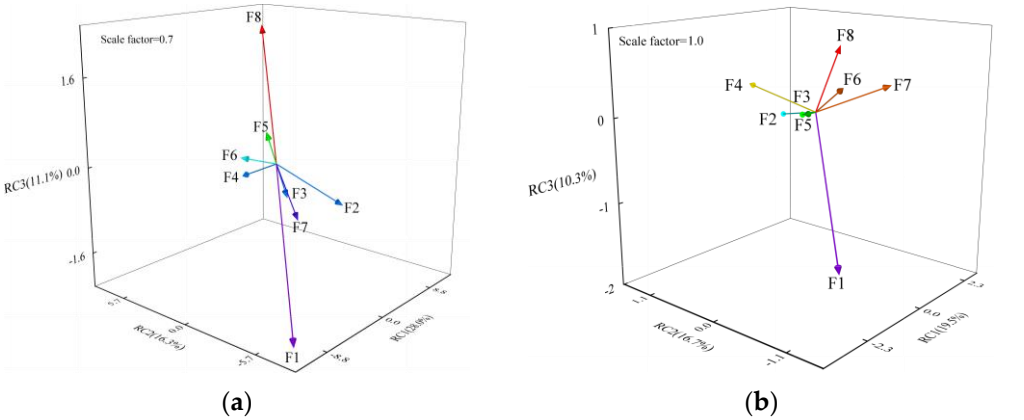
$$P_2 = 0.195\ RC1 + 0.167\ RC2 + 0.103\ RC3 + 0.098\ RC4 + 0.075\ RC5 + 0.062\ RC6 + 0.051\ RC7 + 0.048\ RC8 \quad (6)$$

According to the formula's calculation, treatment F8 had the highest seedling score within one month, followed by F4 (Table 2). The RC2 of F8 was 2.06 times higher than that of F2, and the RC3 of F8 was 3.62 times higher than that of F2. On day 56, treatment F4 had the highest seedling score, followed by F2 (Table 2). The RC2 of F4 was 5.9% higher than that of F2, and the RC3 of F4 was 126.6% higher than that of F2. This indicates that the photosynthetic function and root traits of F8 seedlings within one month and F4 seedlings within two months were better than F2 (Figure 10). The treatment with the highest sum of two scores was F4, while the lowest was F1 (control), suggesting that root dipping improved seedling quality and the performance of the F4 treatment was the best (Table 2).



(**a**) (**b**)

Figure 9. PCA loading plots under two measurements: (**a**) the third measurement and (**b**) the fifth measurement.



(**a**) (**b**)

Figure 10. RC1, RC2 and RC3 scores of seedlings under different soaking root fertilizer treatments during two measurements: (**a**) the third measurement and (**b**) the fifth measurement.

Table 2. The comprehensive scores of the main components of *E. urograndis* at days 28 and 56.

Time	F1	F2	F3	F4	F5	F6	F7	F8
Day 28	−4.637	0.945	0.052	1.17	−0.013	0.076	0.115	1.873
Day 56	−7.920	3.515	1.157	3.597	−0.385	−0.863	−1.161	2.060

4. Discussion

The nutrients required for the growth and development of seedlings are mainly derived from their photosynthesis and the absorption of soil mineral nutrients through the roots. In general, the levels of N, P, K, and other nutrients that can be used by plants in the soil are not sufficient to maintain the normal growth of seedlings, and the transplanting of seedlings will separate plants from the soil and take away some nutrients, thus causing nutrient loss. Therefore, fertilization should be carried out during the growth of seedlings [48]. Soaking root fertilizer application is an important component of plantation cultivation technology. Soaking root fertilizer is currently applied to *Eucalyptus* seedlings by traditional methods of base fertilization such as hole, strip, and spreading applications, which cannot maintain fertilizer efficiency for a long time. The fertilizer utilization rate is low, the amount of fertilizer applied is large, and the labor cost is high [49]. In this study, for the first time, the dipping roots method was used to apply soaking root fertilizer. Five raw materials, complex fertilizer, potassium chloride, ammonium bicarbonate, calcium superphosphate and urea, were used to develop seven soaking root fertilizer treatments. The differences in growth, root system, biomass, photosynthesis, and total nutrient uptakes were analyzed.

4.1. The Effects of Different Soaking Root Fertilizer Treatments on Seedling Growth

The uptake of water and total nutrients available for plants to absorb near their roots directly affects their survival, growth and development [50,51]. The strong *Pn* and high *Gs* of *Eucalyptus* give it the potential for rapid carbon assimilation [52,53], but the rapid growth of *Eucalyptus* in the early stages is needed to fully reach this potential. A soaking root fertilizer rich in P can directly provide the nutrients required by the roots and promote the rapid growth of *Eucalyptus* in the early stages of development [54]. The increase in height and crown width of *Eucalyptus* in the early growth stage can also improve the utilization rate of light energy and promote root development [55,56]. Therefore, excellent soaking root fertilizer formulas and science-based fertilization methods are important ways to promote seedling growth and improve seedling quality. In this study, a total of eight treatments (including a water control) were established through preliminary experiments. The results of a variance analysis showed that the eight treatments had significant effects on the CHI, ground diameter, and crown width of *E. urograndis* seedlings at different periods. Therefore, it was necessary to select the best soaking root fertilizer treatments at different periods. The highest growth indices on day 28 were recorded for F2, while the greatest CHI on day 56 was recorded for F8. Because F2 received a granular complex fertilizer, it had the advantages of low moisture absorption, no caking, and good physical properties [57], and could be rapidly absorbed by plants. The performance of the fast-acting complex fertilizer in F2 was confirmed by the successive decrease in the high growth rate in the five growth trait measurements and the ranking of the CHI of F2 on day 56. This may be related to the fact that F2 received potassium sulfate compound fertilizer, which is a physiological acid fertilizer. When applied to the acidic soil in the experiment, the accumulation of sulfate ions (SO_4^{2-}) combined with excessive watering accelerated the soil acidification [58] and promoted the release of active aluminum in the soil. The aluminum ion (Al^{3+}) and phosphate ion (PO_4^{3-}) will form insoluble salts to fix P. The base ions needed by plants will also be replaced by H^+ and Al^{3+} and leach into the soil solution, while Al^{3+} also has a toxic effect on seedling growth [59,60]. It can form hydrogen sulfide to inhibit the growth and development of seedling roots [61], which may also be the reason for the poor root performance of F2 and F3 (Figure 4). The F8 treatment had a higher P/N ratio and the

N source was urea, while P can promote the N absorption of *Eucalyptus* seedlings [62]. F1 performed significantly worse than the fertilized seedlings. The poor performance of F7 may be because: (1) it has the lowest N/P ratio, and (2) the seedlings absorbed the lowest actual nutrient concentrations. The nutrient concentration of F7 was only sightly higher than that of F8 (Table 1). Ammonium bicarbonate was more likely to decompose under moist conditions than urea, resulting in F7 seedlings absorbing fewer nutrients during root dipping. In conclusion, the F2 treatment had the best effect on the growth of *E. urograndis* seedlings, although this gradually weakened after one month, while the F8 treatment had a slight advantage over other formulas in promoting growth within two months.

4.2. Effects of Different Soaking Root Fertilizer Treatments on Root of Seedlings

Root morphology can determine the utilization efficiency of the N and P of seedlings [63], which may reflect the growth potential of seedlings better than growth traits [11]. The different treatments had significant effects on the root system of *E. urograndis* seedlings. In the first 28 days, the root system of F8 developed better than that of F4–F7, which may be because urea was more stable than ammonium bicarbonate under a humid environment and acidic soil. The root growth of F2 and F3 was not as good as that of other treatments. In addition to being affected by the SO_4^{2-} in the compound fertilizer, the poor root growth was also related to the higher proportion of K. Plants are generally subjected to low K stress at a certain stage of growth, and different plants have different responses to K deficiency [64–66]. Plant root morphology can be regulated by phytohormones [67]. A decrease in the K content promotes root growth, adjusts the root morphological structure and increases root surface area to improve K^+ uptake [68–70]. After 28 days, there were no significant differences in seedling root characters, which indicated that the *Eucalyptus* seedlings themselves had a strong rooting ability. The biomass of seedlings determines the performance of fixing plants and the spatial accumulation of resources.

A correlation analysis was conducted to determine the degree of connection between factors, but it could not identify the causal relationship, and it was therefore necessary to determine the internal mechanism through in-depth research. The SI directly reflects the quality of seedlings, while the R/S value focuses on the balance between roots and stems. The two parameters were significantly positively correlated, indicating that for *E. urograndis*, seedlings with well-developed roots usually have a higher quality [71]. Excellent growth traits were identified in F4, F2, and F8 because they had the highest RDW and the highest root nutrient concentrations. These treatments were the best for absorbing water and minerals, and promoting seedling growth. The K concentration in roots was significantly positively correlated with seedling photosynthesis, which proved that the improvement of the K absorption ability of roots is one of the reasons for the enhancement of photosynthesis in *E. urograndis* seedlings. F4 and F2 had both higher root P and K contents, as well as higher aboveground NPK contents and P concentrations, indicating that these two treatments promoted the absorption of root nutrients, especially P. This agreed with the results of a previous study of the P fertilization of maize [72]. In summary, root-dipping fertilization improved morphological traits, such as RDW and TL, and physiological traits, such as root nutrient content and concentrations, and also promoted photosynthesis and root nutrient absorption, ultimately improving the quality of the seedlings.

4.3. The Effects of Different Soaking Root Fertilizer Treatments on the Biomass of Seedlings

The biomass allocation of the various organs of *Eucalyptus* seedlings varies depending on the different environments to which they are adapted [73–75]. The biomass allocation of each organ in the aboveground parts reflects the adaptability of seedlings to the environment, while the biomass in the root parts reflects the uptake of nutrients by seedlings [76,77]. In this study, soaking root fertilizer significantly increased the biomass of seedling organs. In the first 28 days, the ADW and LDW of F2 were higher than in the other fertilization treatments, and significantly higher than in F5 and F6. However, there were no significant differences in the RDW and SDW of F2 compared to the other fertilization treatments.

This was no longer apparent on day 56, at which point the dry weight of all organs was significantly higher in F2. This indicated that F2 delayed the low K stress period, and the potassium ion (K^+) that was abundant in the early stage was rapidly absorbed, which promoted the growth of aboveground parts. The F2 treatment did not need the rapid growth of roots to absorb soil nutrients until all the K^+ in the fertilizer was absorbed. The only treatment in which the SI decreased with time was F8, which may be consumed at the earliest due to the low total nutrient uptake [78]. After 56 days, the R/S value was the highest for F4, which was the only treatment in which it increased over time. These results indicate that a compound fertilizer with reasonable proportions of nutrients is more suitable for the root growth of *E. urograndis* seedlings than a complex fertilizer [79,80].

4.4. The Effects of Different Soaking Root Fertilizer Formulas on Photosynthetic Characteristics and Nutrient Accumulation in Seedlings

Photosynthesis represents another part of the nutrient intake mechanism for seedlings and is the basis of energy metabolism [81]. The photosynthetic properties of seedlings under the different treatments were significantly different. Due to the elimination of the effects of water stress and environmental changes on leaves over time in the experiment, the *Tr* of the treatment without SO_4^{2-} was higher than that of F2 and F3, resulting in a lower WUE. However, there was no significant difference in the *Pn* of the seedlings under the different treatments within 28 days, which may be because the toxic effect of SO_4^{2-} was greater than that of low K stress. In the experiment, the short-term K deficiency of seedlings without the K treatment promoted the accumulation of abscisic acid (ABA) in roots, induced the generation of reactive oxygen species (ROS) [82] and reduced photosynthetic efficiency [83]. The enrichment of SO_4^{2-} in the soil of the F2 and F3 treatments led to an increase in the Al^{3+} concentration, and Al^{3+} has an influence on leaf photosynthesis [84,85]. This adverse effect is not as serious as that of Al^{3+} on roots by comparing root and photosynthetic indices in this experiment [86]. On the other hand, phosphorus fixation reduces the electron transfer ability of leaves [87] and impairs photosynthesis [88], both of which reduce the photosynthetic performance of F2 and F3 leaves. These deficiencies in the F2–F8 treatments resulted in varying degrees of reduced photosynthesis, resulting in there being no significant difference between them and F1 within 28 days. However, K is not indispensable for the early growth of *Eucalyptus* seedlings [64], and the low K stress gradually disappeared over time, whereas the SO_4^{2-} toxicity persisted. The F4 seedlings did not have low K stress and SO_4^{2-} enrichment, while the F8 N fertilizer was urea, which may be more suitable for the N uptake of *Eucalyptus* seedlings than ammonium bicarbonate. Therefore, the excellent performance of F8 and F4 resulted in a difference in photosynthetic capacity compared to F2 after 56 days. F1 had the lowest LDW, *Gs*, *Tr*, and *Ci*, which was sufficient to demonstrate seedling leaf dysplasia, while the unusual *Pn* value of F1 in the last measurement was even higher than that of F2 and F5. This may be related to the characteristics of the tree species, with *Eucalyptus* having a relatively high *Pn* under natural growth conditions [89].

The ratio of fertilizer elements depends on the initial soil-available total nutrient uptake, nutrient utilization rate, fertilizer content, and target yield [48]. The total nutrient uptake and their concentrations in seedlings and organs reflect the nutritional status of plants. Phosphorous usually contributes to the growth and development of seedling roots [90], while K promotes photosynthesis [91]. The F8 and F4 treatments were rich in P and K, and the nutrients in F8 are concentrated in the leaves, focusing on K uptake. This partly explains the high growth rate of F8 in the early stage, because the high nutrient proportion of leaves promotes the photosynthesis and synthesis of organic matter [83,88]. The root total nutrient uptake of F4 is higher and focused on the absorption of P, and the root status reflects the quality and growth potential of the seedlings at that time [90]. Therefore, the root system of F4 seedlings was more developed, which was conducive to the continuous absorption of nutrients by the seedlings and their subsequent growth. The F2 treatment had the highest N content, but this was because it had the highest biomass

(Table 3). Therefore, the dilution effect of the increased biomass after fertilization was greater than the promotional effect of the fertilizer on the N absorption capacity [92]. Combined with the characteristics of the F2 complex fertilizer, this indicated that F2, as a quick-release fertilizer (QRF), may cause seedling overgrowth in the short term and fail to improve the growth potential and development quality, such as the photosynthetic capacity and root development degree (Figures 4–6). This results in the low quality of seedlings, which is also one of the disadvantages of QRFs [93]. F1 had the highest N concentration, which was a consequence of the low biomass (Table 3). Meanwhile, F1 lacked P and K nutrients, and exhibited poor transpiration efficiency and root traits, indicating poor growth and quality of the F1 seedlings. The F1 seedling quality was not as good as seedlings that had their roots dipped.

Table 3. The analysis results of the N content correlation between F1 and F2.

Variate-x	Variate-y	Correlation Coefficient	*p*-Value [1]
N content of F1	Biomass	0.899	0.000 ***
	concentration	0.369	0.108
N content of F2	Biomass	0.965	0.000 ***
	concentration	0.414	0.069

[1] The N content of F1 and F2 was significantly correlated with biomass, "***" $p < 0.001$.

4.5. Comprehensive Evaluation of Different Fertilization Methods and the Influence of the Basal Treatment on E. urograndis Seedlings

The method of fertilization affects the efficiency of fertilization and also affects the growth and development of seedlings [94,95]. Compared with no fertilization, the application of soaking root fertilizer by dipping roots into a fertilizer solution promoted seedling growth. In this study, individual differences were found in different traits of the seedlings under different treatments. A single index variance analysis was not sufficient to reflect the comprehensive growth and development parameters of the seedlings. After obtaining the complete experimental data set, 24 (day 28) and 49 (day 56) indicators were simplified into seven and eight principal components, respectively, and the comprehensive evaluation formulas for days 28 and 56 were calculated to evaluate the comprehensive quality of seedlings. The results showed that F4, F8, and F2 were the best treatments for promoting seedling growth, while F5 and F6 were ineffective treatments. The F8 and F2 treatments best met the rapid growth requirements of seedlings within 28 consecutive days, while F4 was the best treatment for meeting the requirement of continuous growth promotion within 56 days and onward. The results of PCA indirectly proved that the F2 formula may only promote seedling overgrowth without focusing on improving seedling quality (Figure 10). The fact that F4 and F8 achieved good performance with the highest and lowest nutrient mass concentrations, respectively, indicates that the fertilizer for *Eucalyptus* seedling growth should not only maintain nutrient balance, but also consider the control of the appropriate nutrient concentration. The soaking root fertilizer of the F4 formula could not only ensure the early growth of seedlings, but also compensate for the deficiency of the subsequent fertility of the QRF. The results were basically consistent with the results of the one-way analysis of variance.

5. Conclusions

The following conclusions were derived from the results of this study. 1) In the future management of *E. urograndis* plantations, if topdressing is planned within one to two months, the fertilizer formula used for dipping roots should be urea (0.2% N) + calcium superphosphate (0.4% P). If the topdressing is planned after two months, the fertilizer formula should be ammonium bicarbonate (0.3% N) + calcium superphosphate (0.8% P) + potassium chloride (0.4% K). These two treatments would best enable seedlings to achieve the growth conditions required for topdressing in these two time periods. 2) A reasonable fertilizer formula for soaking root fertilizer would improve photosynthetic function, promote root

development, and ensure the growth and quality of seedlings by promoting the absorption of nutrients by roots, so achieving the extension of fertilizer efficiency and avoiding the problem of the short fertilizer efficiency that has been encountered with traditional QRFs. 3) It is feasible to apply root fertilizer to *E. urograndis* seedlings by dipping the roots. This method promotes the early growth of seedlings, improves the efficiency and benefits of planting root fertilizers, and saves time and labor. This study of the root dipping method provides a theoretical basis and a practical reference for the large-scale plantation cultivation of *E. urograndis* and other tree species.

Author Contributions: S.C. and J.Y. conceived of and designed the experiments; S.Z. and J.Y. performed the experiments; S.Z. analyzed data; S.Z. wrote the manuscript; S.C. and L.O. writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the China National Key R&D Program during the 13th Five-year Plan Period (Project Number: 2016YFD0600500) and the *Eucalyptus* high-yield and high-efficiency technology Qinzhou Demonstration Forest Base construction project (Project Number: Suheng [2022] No. 1).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We are grateful to Yangdong Zheng and Rongqiang Chen for biomass and photosynthesis measurements.

Conflicts of Interest: The authors declare no conflict of interest.

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