forests

Article

Effects of Nitrogen Form on Root Activity and Nitrogen Uptake Kinetics in *Camellia oleifera* Seedlings

Rui Wang [1,2], Zhilong He [1,2], Zhen Zhang [1,2], Ting Xv [1,2], Xiangnan Wang [1,2], Caixia Liu [1,2] and Yongzhong Chen [1,2,*]

[1] Research Institute of Oil Tea Camellia, Hunan Academy of Forestry, Shaoshan South Road, No. 658, Changsha 410004, China
[2] National Engineering Research Center for Oil Tea Camellia, Changsha 410004, China
* Correspondence: chenyongzhong@hnlky.cn

Abstract: This study investigated the effects of nitrogen form on root activity and nitrogen uptake kinetics of *Camellia oleifera* Abel. seedlings, providing a scientific basis for improving nitrogen use efficiency and scientific fertilization in *C. oleifera* production. Taking one-year-old *C. oleifera* cultivar 'Xianglin 27' seedlings as subjects, 8 mmol·L^{-1} of nitrogen in varied forms (NO_3^-:NH_4^+ = 0:0, 10:0, 7:3, 5:5, 3:7, 0:10) was applied in this study as the treatment conditions to investigate the effects of different nitrogen forms on root activity and nitrogen uptake kinetics in *C. oleifera* seedlings. Comparing the performance of nutrient solutions with different NO_3^-:NH_4^+ ratios, the results showed that a mixed nitrogen source improved the root activity of *C. oleifera* seedlings based on total absorption area, active absorption area, active absorption area ratio, specific surface area, and active specific surface area. When NO_3^-:NH_4^+ = 5:5, the total absorption area and active absorption area of the seedling roots reached the maximum. The results of uptake kinetic parameters showed that Vmax NH_4^+ > Vmax NO_3^- and Km NO_3^- > Km NH_4^+, indicating that the uptake potential of ammonium–nitrogen by *C. oleifera* seedlings is greater than that of nitrate–nitrogen. The conclusion was that compared to either ammonium– or nitrate–nitrogen, the mixed nitrogen source was better for promoting the root activity of *C. oleifera* seedlings, and the best nitrate/ammonium ratio was 5:5.

Keywords: *Camellia oleifera*; root activity; nitrate–nitrogen; ammonium–nitrogen; uptake kinetics

Citation: Wang, R.; He, Z.; Zhang, Z.; Xv, T.; Wang, X.; Liu, C.; Chen, Y. Effects of Nitrogen Form on Root Activity and Nitrogen Uptake Kinetics in *Camellia oleifera* Seedlings. *Forests* **2023**, *14*, 161. https://doi.org/10.3390/f14010161

Academic Editor: Adele Muscolo

Received: 30 November 2022
Revised: 22 December 2022
Accepted: 12 January 2023
Published: 16 January 2023

1. Introduction

Camellia oleifera is a woody edible oil tree species in China and mainly distributed in red soil regions which are characterized by soil depletion and nutrient deficiency. It is widely cultivated in south–central and southern China since their growing commercial, medic, cosmetic and ornamental values in recent years provide an important guarantee for a targeted poverty alleviation strategy in China [1]. The genome of oiltea-camellia is very complex and not well explored. Recently, genomes of three oiltea-camellia species were sequenced and assembled [2–4]; multi-omic [1,5,6] studies of oiltea-camellia were carried out and provided a better understanding of this important woody oil crop.

Nitrogen is an essential macronutrient for plant growth and development and has an irreplaceable role in plant life [7]. Nitrogen deficiency is an important factor limiting the growth and fructification of *C. oleifera* [8], which can be resolved by the scientific application of nitrogen fertilizers. Plants have a higher demand for nitrogen than for other elements, and the two main forms of nitrogen that can be absorbed and used by plants are ammonium–nitrogen (NH_4^+-N) and nitrate–nitrogen (NO_3^--N). Although it is volatile, NH_4^+-N can be adsorbed and fixed by soil; thus, it is not easily lost via leaching. In comparison, NO_3^--N is non-volatile but is prone to leaching and denitrification losses, which affects the use efficiency of nitrogen fertilizers. Selective uptake characteristics for different nitrogen forms and the kinetic characteristics of nitrogen uptake by roots are important factors affecting nitrogen use efficiency in plants [9,10]. The kinetic approach is an effective method to study the nutrient uptake characteristics of plant roots and to identify the differences between different plant species [11]. In the early 1950s, Epstein et al. first applied the kinetic equation of enzymatic reactions to the study of nutrient ion uptake by plant roots [12], and in the mid-to-late 1970s, Barber et al. further modified the uptake kinetic equation and proposed the concept of critical concentration or minimum equilibrium concentration [13]. The Michaelis kinetic equation is a kinetic equation that can quantitatively characterize plant roots' nutrient uptake. This equation was used to investigate the effects of environmental conditions on nutrient uptake by plants [14]. The characteristic parameters obtained from this equation have important applications in comparing the barren resistance of different plant species (or varieties) [15,16]. To date, kinetic studies of nutrient ion uptake have been largely limited to crops and fruit trees [12,14,17,18]. There are few studies on nutrient ion uptake kinetics by roots of woody plants [19], and no relevant studies on *C. oleifera* have been reported.

In this study, different ratios of nitrate–/ammonium–nitrogen were applied to *C. oleifera* seedlings to investigate the root activity and root uptake kinetics under different nitrogen forms; these results would provide a theoretical basis for the fertilization of *C. oleifera* seedlings.

2. Materials and Methods

2.1. Overview of the Experimental Field

The experimental field is located at the experimental station of the National Oil-tea Camellia Engineering and Technology Research Center, at 113°01′ E, 28°06′ N, and 80–100 m above sea level. It belongs to a subtropical monsoon climate, with an annual average temperature of 16.8–17.3 °C, annual average rainfall of 1422 mm, a frost-free period of 275 days, and annual average relative humidity of 80%. The soil classifies as Quaternary red soil with a pH between 4.5 and 5.5, an organic matter content of 41.01 $g{\cdot}kg^{-1}$, a total nitrogen content of 2.68 $g{\cdot}kg^{-1}$, a total phosphorus content of 0.61 $g{\cdot}kg^{-1}$, and a total potassium content of 4.53 $g{\cdot}kg^{-1}$.

2.2. Experimental Materials

The *C. oleifera* cultivar 'Xianglin 27' one-year-old seedlings were used for the experiment. *C. oleifera* seeds were collected in October 2018, and uniform, full-grained, and disease-free seeds were selected for stratification. In March 2019, seeds with consistent germination states were selected and sowed in containers with a height of 12 cm and an upper diameter of 8 cm. The cultivation substrate consisted of yellow subsoil, perlite, and peat at a volume ratio of 3:1:1. The substrate had a pH of 5.88, and the concentrations of NH_4^+-N and NO_3^--N were 0.92 and 2.34 $mg{\cdot}L^{-1}$, respectively. Slow seedling was performed for 3 months after planting seeds in nutrition cups, and seedlings with consistent growth conditions and no pests or diseases were selected for fertilization in June 2019.

2.3. Experimental Design

Fertilizers were applied via liquid irrigation, and a modified Hoalgland nutrient solution (nitrogen-free) was added to ensure that the seedlings grew under normal nutritional conditions. The formulation of the nutrient solution is: K_2SO_4 261.39 $mg{\cdot}L^{-1}$, KH_2PO_4 136.09 $mg{\cdot}L^{-1}$, $CaCl_2$ 221.98 $mg{\cdot}L^{-1}$, $MgSO_4{\cdot}7H_2O$ 246.47 $mg{\cdot}L^{-1}$, $MnSO_4{\cdot}H_2O$ 1.54 $mg{\cdot}L^{-1}$, H_3BO_3 2.86 $mg{\cdot}L^{-1}$, $ZnSO_4{\cdot}7H_2O$ 0.22 $mg{\cdot}L^{-1}$, $CuSO_4{\cdot}5H_2O$ 0.08 $mg{\cdot}L^{-1}$, $Na_2MoO_4{\cdot}2H_2O$ 0.02 $mg{\cdot}L^{-1}$, and $FeSO_4{\cdot}7\ H_2O$ 20 $mg{\cdot}L^{-1}$. In all nutrient solutions, 7 $\mu mol{\cdot}L^{-1}$ of the nitrification inhibitor dicyandiamide ($C_2H_4N_4$) was added for nitrification inhibition.

The experiment employed a completely randomized block design. According to a previous study [20], six treatments (Table 1), including five experimental groups at a nitrogen level of 8.0 $mmol{\cdot}L^{-1}$ with different ratios of nitrogen forms ($[m(NO_3^-\text{-N})/m(NH_4^+\text{-N})]$ = 10:0, 7:3, 5:5, 3:7, 0:10), and a control group (no nitrogen fertilization) were set up.

For each treatment, three replicates, each containing 200 seedlings, were set up, as shown in Table 1. The first fertilization was carried out in mid-June, followed by 10 fertilizer applications at one-week intervals. Each seedling was thoroughly irrigated with 300 mL solution each time. The solution collected in trays was used for re-irrigation to ensure no nitrogen loss. The experiments were conducted in a greenhouse with a luminance of 6000–8000 lux, a temperature of 20.0–25.0 °C, and a humidity of 80%–85%. In addition to fertilization, other care and maintenance measures, such as normal watering and weed clearing, were performed.

Table 1. Nitrogen ratio under different treatments used in this study.

No.	$m(NO_3^-$-N)/$m(NH_4^+$-N)	Total Nitrogen/mmol·L^{-1}	Nitrogen Sources/mmol·L^{-1} $NaNO_3$	$(NH_4)_2SO_4$
A0(CK)	0:0	0	0	0
A1	10:0	8	8	0
A2	7:3	8	5.6	1.2
A3	5:5	8	4	2
A4	3:7	8	2.4	2.8
A5	0:10	8	0	4

2.4. Experimental Methods

The methylene blue method was used to determine root absorption activity [21]. The kinetic characteristics of the uptake of NO_3^--N and NH_4^+-N solutions by seedlings were determined by the conventional depletion method from late July to the middle of August, which was the most active stage of seedlings' growth. The content of NO_3^--N was determined using the colorimetric method [22], while that of NH_4^+-N was determined using the indophenol blue method [23]. Immediately after the uptake kinetics test, the *C. oleifera* seedlings were taken out and weighed after removing water from the root surface with absorbent paper.

2.5. Experimental Materials

The roots' kinetic uptake parameters were calculated using the method described by Hua Haixia and Zhai Mingpu. The maximum uptake rate V_{max} and the Michaelis constant K_m were calculated, and $\alpha = V_{max}/K_m$ [24,25]. Data were processed and statistically analyzed using Excel 2007 and SPSS25.0 software, and significant differences between treatments were compared using one-way analysis of variance (ANOVA) and the least significant difference (LSD) test ($p < 0.05$). Histograms were plotted with GraphPad Prism 8.0 and line graphs were plotted with OriginPro 8.5.1.

3. Results

3.1. Effects of Nitrogen Form on the Root Activity of C. oleifera Seedlings

3.1.1. Effect of Nitrogen Form on the Total Absorption Area of *C. oleifera* Seedling Roots

For simplicity, the nitrogen source treatments used in this study [$m(NO_3^-$-N)/$m(NH_4^+$-N)] = 10:0, 7:3, 5:5, 3:7, 0:10 will be referred to as A1–A5, respectively, and the control treatment will be referred to as A0. With the increasing proportion of NH_4^+-N, the total absorption area of the roots first increased and then decreased. This shows that the mixed-nitrogen-source treatment (A2–A4) had greater root absorption than the control (A0), which had a greater root absorption than the single-nitrogen-source treatment (A1, A5). For the single-nitrogen-source treatments, the total-nitrate treatment (A1) had a greater absorption than the total-ammonium treatment (A5). The total root absorption area of NO_3^-:NH+ 4 = 5:5, A3 treatment reached a maximum of 1.48 cm^2, which was 43.69% higher than the control, while the total absorption area of the total-ammonium (A5) treatment was the smallest at 0.85 cm^2, which was 17.48% lower than the control.

3.1.2. Effect of Nitrogen Form on the Active Absorption Area of *C. oleifera* Seedling Roots

As presented in Figure 1, the active absorption area of the roots also showed an increase, followed by a decreasing trend with increasing proportions of NH_4^+-N. Mixed-nitrogen-source treatment (A2–A4) had a greater active absorption area compared with the control (A0), which had a greater active absorption area than the single-nitrogen-source treatment (A1, A5). For the single-nitrogen-source treatments, A1 showed a greater active absorption area than A5. The active absorption area reached a maximum of 1.00 m^2 when using the A3 treatment, which was 78.57% higher than that of the control. The total-ammonium treatment resulted in the smallest active absorption area at 0.45 cm^2, which was 19.64% lower than the control. These results indicate that mixed nitrogen sources can significantly increase the active absorption area, and both total-nitrate and total-ammonium treatments will lower the active absorption area.

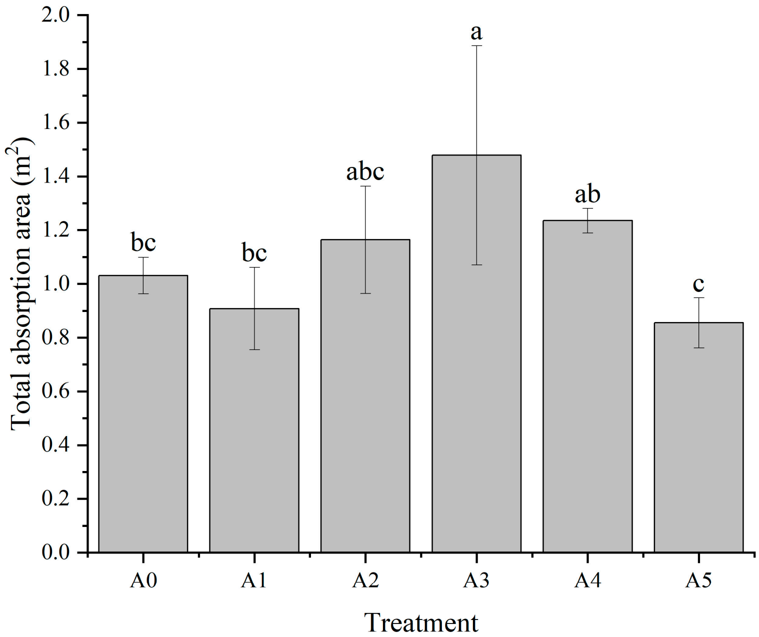

Figure 1. The effect of nitrogen form on the total absorption area of the roots of *C. oleifera* seedlings. Different lowercase letters indicate significant differences at $p < 0.05$.

3.1.3. Effect of Nitrogen Form on the Active Absorption Area Ratio of *C. oleifera* Seedling Roots

As shown in Figure 2, the active absorption area ratio tended to decrease, then increase, and then decrease with treatments A0–A5. Mixed-nitrogen-source treatments (A3, A4) had greater active absorption than the control (A0), which had greater active absorption than the single-nitrogen-source treatments (A1, A5). For the single-nitrogen-source treatments, A5 had greater active absorption than A1. The active absorption area ratio reached a maximum of 79.72% at NO_3^-:NH+ 4 = 3:7, which was 46.36% higher than that of the control, followed by that of A3 treatment with an active absorption area ratio that was 34.07% higher than the control's. There was no significant difference between A4 and A3 treatments, and both resulted in a significantly higher active absorption area ratio than the other treatments. The smallest active absorption area ratio, at 53.00%, was associated with the A5 treatment, which was 2.70% lower than that of the control. These results indicate that mixed nitrogen sources (A3 and A4) can significantly increase the active absorption area ratio.

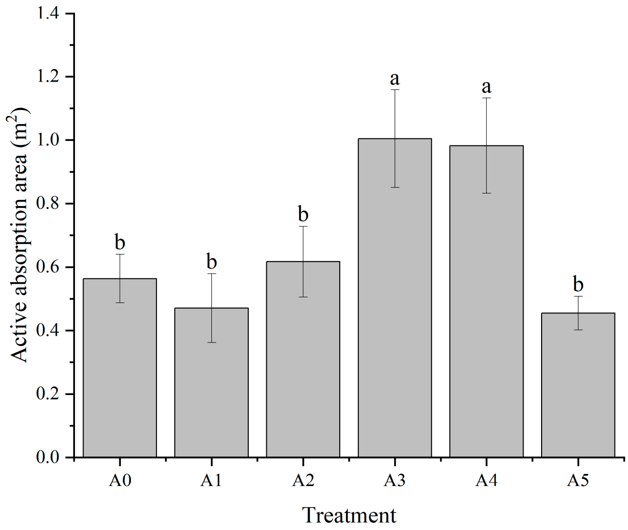

Figure 2. The effect of nitrogen forms on the active absorption area of the roots of *C. oleifera* seedlings. Different lowercase letters indicate significant differences at $p < 0.05$.

3.1.4. Effect of Nitrogen Form on the Specific Surface Area of *C. oleifera* Seedling Roots

The effect of nitrogen form on the specific surface area of *C. oleifera* seedling roots is shown in Figure 3. After treatments with nitrogen sources of different forms and ratios, the specific surface area of the roots showed a first increasing, then decreasing, and then increasing trend with an increasing proportion of NH_4^+-N. Specifically, seedlings had a greater root surface area after mixed-nitrogen-source treatment (A2, A4) compared with control (A0), which had a greater root surface area compared with seedlings after single-nitrogen-source treatment (A1, A5). Regarding the single-nitrogen-source treatments, A5 showed greater root surface area than A1. The root-specific surface area reached a maximum of 0.98 $cm^2 \cdot cm^{-3}$ in the A2 treatment, which was 30.67% higher than that of the control, followed closely by the A4 treatment. The root-specific surface area after the A2 treatment was significantly higher than those with single-nitrogen-source treatments (A1 and A5), but showed no significant differences compared to the other treatments. The A1 treatment led to the smallest root-specific surface area of 0.53 $cm^2 \cdot cm^{-3}$, which was 29.33% lower than that of the control. These results indicate that both total-nitrate and total-ammonium treatments will significantly reduce the specific surface area of *C. oleifera* seedlings.

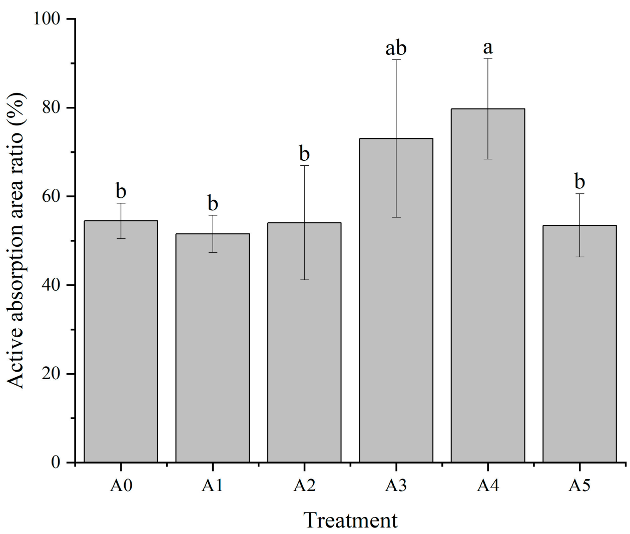

Figure 3. The effect of nitrogen forms on the active absorption area ratio of the roots of *C. oleifera* seedlings. Different lowercase letters indicate significant differences at $p < 0.05$.

3.1.5. Effect of Nitrogen Form on the Active Specific Surface Area of *C. oleifera* Seedling Roots

As shown in Figure 4, with the increased ratio of NH_4^+-N, the active specific surface area of the roots showed a first increasing and then decreasing trend. The active specific surface area of the roots reached a maximum value of 0.66 $cm^2 \cdot cm^{-3}$ under A4 treatment, which was 65.00% higher than the control. There was no significant difference between A4 and the other mixed-nitrogen-source treatments (A2 and A3), but the active specific surface area achieved by the A4 treatment was significantly higher than those by the single-nitrogen-source treatments (A1 and A5), and the control (A0). The active specific surface area was the smallest, at 0.27 $cm^2 \cdot cm^{-3}$, when A1 treatment was applied, which was 32.50% lower than the control. The results indicate that mixed nitrogen sources can significantly increase the proportion of active specific surface area, and both total-nitrate and total-ammonium treatments will reduce the active specific surface area of *C. oleifera* seedling roots.

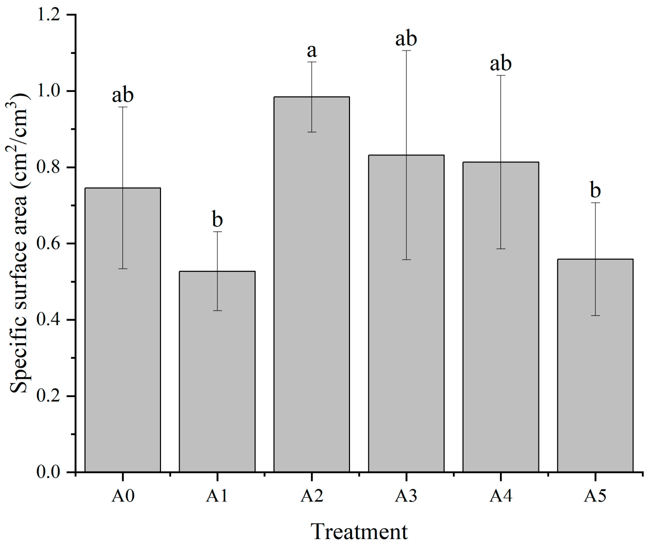

Figure 4. The effect of nitrogen forms on the specific surface area of roots of *C. oleifera* seedlings. Different lowercase letters indicate significant differences at $p < 0.05$.

3.2. Effects of Nitrogen Form on the Nitrogen Uptake Kinetics of C. oleifera Seedling Roots

3.2.1. Effect of Nitrogen Form on NO_3^- Uptake Kinetics of *C. oleifera* Seedling Roots

Figure 5 shows the concentration curves of NO_3^- at different uptake times in the NO_3^- depletion test of *C. oleifera* seedlings treated with different nitrogen forms. The kinetic response patterns of NO_3^--N uptake by seedlings subjected to different nitrogen treatments showed no significant difference. With the extension of the absorption time, the NO_3^- concentration in the fertilizer solution gradually decreased. Specifically, the NO_3^- concentration decreased rapidly within the first nine hours, with the most dramatic drop observed within the first two hours. From the ninth hour onwards, the NO_3^- concentration remained almost constant. Via fitting, the NO_3^- depletion equations for treatments with different nitrogen forms and ratios were listed in Table 2. The kinetic parameters of NO_3^- uptake by the roots were calculated according to the fresh weights of the roots of *C. oleifera* seedlings in different treatments.

As shown in Table 2, the *Vmax* values of different treatments in descending order, are A3 > A1 > A4 > A2 > A0 > A5. The *Vmax* of the A3 treatment was the highest, at 17.57μmol$\cdot g^{-1} \cdot h^{-1}$, indicating that the A3 treatment had the highest intrinsic potential for NO_3^- uptake. The A1 treatment came in second place, with a *Vmax* of 17.52 μmol$\cdot g^{-1} \cdot h^{-1}$, followed by the other mixed-nitrogen-source treatments (A4 and A2). The A5 treatment resulted in the lowest *Vmax*, which was lower than that of the control. These results indicate that the presence of NH_4^+ accelerates the uptake of NO_3^-; however, when NH_4^+ exceeds a certain percentage, the NO_3^- uptake rate decreases.

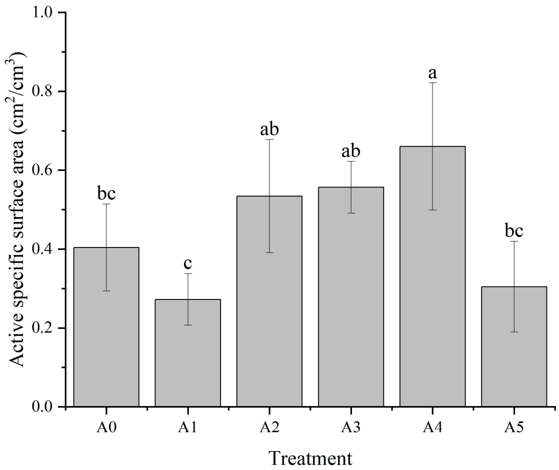

Figure 5. The effect of nitrogen forms on the active specific surface area of roots of *C. oleifera* seedlings. Different lowercase letters indicate significant differences at $p < 0.05$.

Table 2. Kinetic parameters of NO_3^- uptake by roots of *C. oleifera* seedlings.

Treatment	Equation	R^2	*Vmax* ($umol \cdot g^{-1} \cdot h^{-1}$)	*Km* ($mmol \cdot L^{-1}$)	α
A0	$Y = 0.0013x^2 - 0.044x + 2.0386$	0.9917	16.10	1.76	0.009
A1	$Y = 0.0014x^2 - 0.0462x + 2.0373$	0.9911	17.52	1.75	0.010
A2	$Y = 0.0009x^2 - 0.0432x + 2.0290$	0.9847	16.24	1.54	0.011
A3	$Y = 0.0014x^2 - 0.0531x + 2.0414$	0.9947	17.57	1.64	0.011
A4	$Y = 0.0009x^2 - 0.0441x + 2.0424$	0.9858	16.29	1.56	0.011
A5	$Y = 0.0013x^2 - 0.0436x + 2.0525$	0.9950	15.62	1.79	0.009

The *Km* values of different treatments followed a descending order, with A5 > A0 > A1 > A3 > A4 > A2, i.e., seedlings treated with mixed nitrogen sources (A2–A4) had a lower *Km* compared with seedlings treated with a total-nitrate source (A1), which had a lower *Km* than the control seedlings (A0), which, in turn, had a lower *Km* than total-ammonium-treated seedlings (A5). This indicates that adding a certain proportion of NH_4^+ can increase the affinity of *C. oleifera* roots with NO_3^-.

The α values of different treatments followed the order A3 = A4 = A2 > A1 > A0 = A5, i.e., mixed-nitrogen-source treatments (A2–A4) produced greater α values in the *C. oleifera* seedlings compared to the total-nitrate treatment (A1), which were greater than those in the control treatment (A0), which, in turn, were greater than the ones resulting from the total-ammonium treatment (A5). The mixed-nitrogen-source treatments (A2–A4) had the largest α value of 0.011, while the single-nitrogen-source treatments (A1, A5) had the smallest α value of 0.009, indicating that the presence of a certain percentage of NH_4^+ ions can increase the uptake rate of NO_3^- by the root.

3.2.2. Effect of Nitrogen Form on NH_4^+ Uptake Kinetics of *C. oleifera* Seedling Roots

Figures 6 and 7 show the curves of NH_4^+ concentration at different uptake times in the NH_4^+ depletion test of *C. oleifera* seedlings treated with different nitrogen forms. The kinetic response patterns of NH_4^+-N uptake by seedlings subjected to different treatments showed no statistical difference. In all samples, the NH_4^+ concentration decreased rapidly in the first 8 h, with the most obvious change observed within the first hour. The NH_4^+ concentration remained almost unchanged after 8 h. Via fitting, the NH_4^+ depletion equations for treatments with different nitrogen forms and ratios were listed in Table 3. The kinetic parameters of NH_4^+ uptake by the roots were calculated according to the fresh weights of the roots of *C. oleifera* seedlings in different treatments.

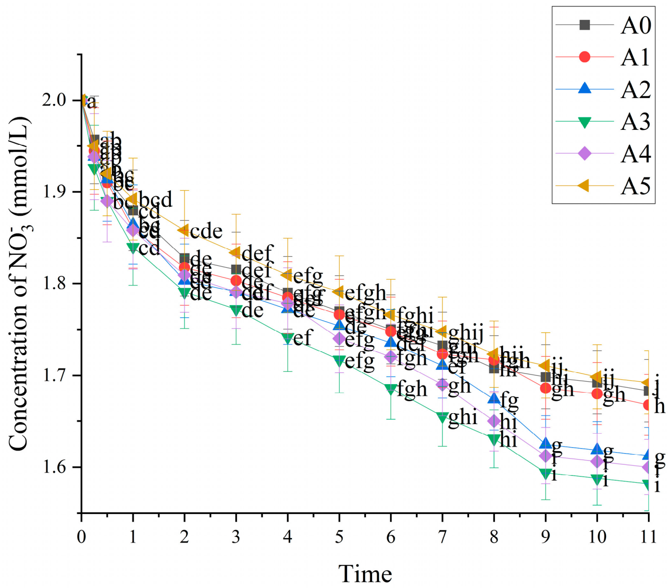

Figure 6. Absorption kinetics curve of nitrate in roots of *C. oleifera* seedlings.

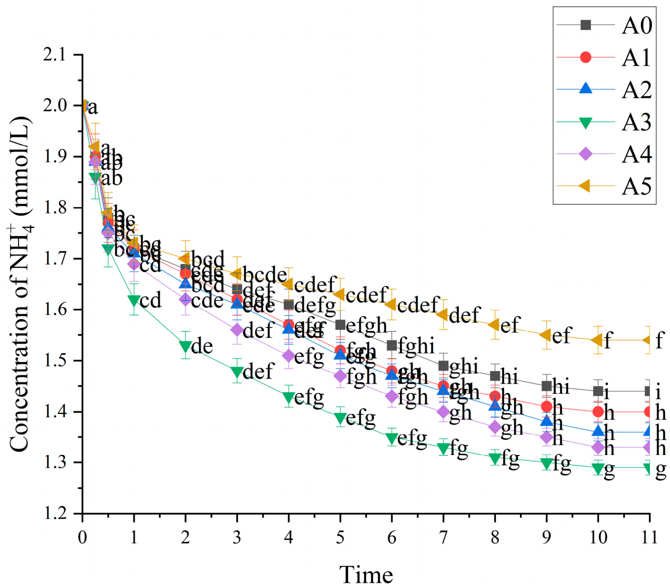

Figure 7. Absorption kinetics curve of ammonium in roots of *C. oleifera* seedlings.

Table 3. Kinetic parameters of NH_4^+ uptake by roots of *C. oleifera* seedlings.

Treatment	Equation	R^2	*Vmax* ($umol \cdot g^{-1} \cdot h^{-1}$)	*Km* ($mmol \cdot L^{-1}$)	α
A0	$Y = 0.0032\ x^2 - 0.0888x + 2.0549$	0.9889	32.49	1.59	0.020
A1	$Y = 0.0037x^2 - 0.0993x + 2.0744$	0.9946	35.01	1.57	0.022
A2	$Y = 0.0034x^2 - 0.0971x + 2.0632$	0.9927	33.95	1.54	0.022
A3	$Y = 0.0057x^2 - 0.1361x + 2.0988$	0.9924	45.07	1.49	0.030
A4	$Y = 0.0041x^2 - 0.1111x + 2.0825$	0.9947	39.35	1.52	0.026
A5	$Y = 0.0032x^2 - 0.0793x + 2.0401$	0.9676	29.62	1.67	0.018

As shown in Table 3, the *Vmax* values of different treatments followed a descending order, with A3 > A4 > A1 > A2 > A0 > A5. The A3 treatment has the highest *Vmax* of 45.07 μmol·g^{-1}·h^{-1}, indicating that the A3 treatment had the highest intrinsic potential for NH_4^+ uptake. The A3 treatment was followed by the A4 treatment with a Vmax of 39.35 μmol·g^{-1}·h^{-1}, and then by the A1 treatment. This indicates that the addition of NO_3^- accelerated NH_4^+ uptake. As for single-nitrogen-source treatments, the total-nitrate treatment (A1) had a higher *Vmax* than the total-ammonium treatment (A5), i.e., the NH_4^+ uptake rate was higher in the total-nitrate treatment.

The *Km* values of different treatments were in the descending order of A5 > A0 > A1 > A2 > A4 > A3, showing that mixed-nitrogen-source treatments (A2–A4) had a lower *Km* value than the total-nitrate treatment (A1), which had a lower *Km* value than control (A0) treatment, which, in turn, had a lower *Km* than the total-ammonium treatment (A5). This indicates that the involvement of a certain percentage of NO_3^- can enhance the affinity of *C. oleifera* roots with NH_4^+.

The α values of NH_4^+ uptake by the seedlings were ranked as A3 > A4 > A2 = A1 > A0 > A5, with the mixed-nitrogen-sources (A2–A4) and total-nitrate treatments (A1) producing greater α values of NH_4^+ uptake compared with control (A0), which had a greater α value of NH_4^+ uptake than the total-ammonium treatment (A5). This indicates that involving a certain percentage of NO_3^- can accelerate NH_4^+ uptake by the root.

4. Discussion

4.1. Effects of Nitrogen Form on the Root Activity of C. oleifera Seedlings

Root activity is an important indicator reflecting the nutrient uptake capacity. The total and active absorption area of the root can reflect the strength of root activity and, to some extent, the thickness, branching, and root hair volume of the root [26]. Study of nitrogen form supply on root respiration of walnut seedlings showed that the root respiration rate of walnut seedlings, as well as soluble sugar and starch content, significantly higher than other nitrogen forms treatment when the ratio of ammonium nitrogen to nitrate nitrogen was 50:50 [27]. A study on the effects of exogenous nitrogen forms on cucumber growth showed that the equal amount of ammonium–nitrogen and nitrate–nitrogen promoted the growth of branches, leaves and roots of cucumber seedlings, as well as having enhanced tolerance to sub-low temperatures [28]. In this study, when the ratio of ammonium–nitrogen to nitrate–nitrogen was 50:50, the roots of *C. oleifera* seedlings showed the strongest uptake ability of water and nutrient elements, i.e., the highest root activity, which was consistent with the findings in the other species mentioned above. The root activity was the lowest in the total-ammonium treatment, indicating that the *C. oleifera* root has poor adaptability to a pure NH_4^+-N environment. Therefore, mixed nitrogen forms can improve root activity, thus enhancing nitrogen nutrient uptake by the plants.

4.2. Effects of Nitrogen Form on NO_3^- and NH_4^+ Uptake by Roots of C. oleifera Seedlings

Plants have different uptake and transport pathways for NH_4^+-N and NO_3^--N. Extensive studies have shown that high concentrations of NH_4^+ inhibit NO_3^- uptake, which may be due to the inhibition of gene expression for NO_3^- carrier protein synthesis, as the presence of NH_4^+ affects the environment in which NO_3^- carriers are located on the cell membrane [29]. In this study, low concentrations of NH_4^+ accelerated the uptake of NO_3^--N by the roots of *C. oleifera* seedlings. This is likely because a certain concentration of NH_4^+ induces H^+ secretion by roots, promoting NO_3^-/H^+ cotransport, thus accelerating NO_3^- uptake [30]. However, as the NH_4^+ concentration gradually increased, the Vmax for NO_3^--N uptake decreased rapidly, which was confirmed by the NO_3^--N uptake characteristics of *C. oleifera* seedlings in this study. This conclusion is consistent with findings in other plants, such as citrange and banana plants [31,32].

The NO_3^--N concentration in the environment can also affect the uptake capacity of plant roots. The presence of NO_3^--N promotes root growth, which, in turn, promotes the uptake and utilization of NH_4^+-N by the underground part of the plant [33]. In this study, the *Vmax* of the total-nitrate treatment (A1) was lower than that of the A3 treatment but higher than all the other treatments, which may be because the root has sufficient NO_3^--N uptake carriers under pure NO_3^- supply [34]. In contrast, the *Vmax* of the total-ammonium treatment (A5) was lower than that of all the other treatments, which is consistent with the findings of Du Xvhua et al. on tea trees and Sun Minhong et al. on citrange [31,35].

The *C. oleifera* seedlings subjected to A3 treatment had higher Vmax and α values for the uptake rates of both NO_3^- and NH_4^+ than those in the other treatments, indicating that *C. oleifera* seedlings have a strong preference and competitiveness for both NH_4^+-N and NO_3^--N when applied in equal ratios.

By comparing the characteristic parameters of NH_4^+-N and NO_3^--N uptake kinetics by *C. oleifera* seedlings treated with different nitrogen forms and ratios, it was found that *Vmax* NH_4^+ > *Vmax* NO_3^-, i.e., the uptake potential of *C. oleifera* seedlings for NH_4^+-N, is greater than that for NO_3^--N. In addition, *Km* NO_3^- > *Km* NH_4^+, indicating that *C. oleifera* seedlings have a higher affinity with NH_4^+-N than with NO_3^--N. The results of the study show that *C. oleifera* seedlings prefer NH_4^+-N, which is likely due to the long-term adaptation of *C. oleifera* to acidic soils. In acidic soils, NO_3^- tends to be rapidly reduced to NH_4^+, and NH_4^+ becomes the main nitrogen source [36]. However, upon NH_4^+ uptake, the plants release H^+ into the soil, which will increase the acidity of the soil. Favorable growth of *C. oleifera* seedlings requires soil with a suitable acidity (pH 4.5–6.0), as soil that is too acidic will inhibit seedling growth. During NO_3^- uptake, *C. oleifera* releases HCO_3^- into the soil and raises the pH of the soil. Therefore, applying mixed NH_4^+-N and NO_3^--N at an appropriate ratio can stabilize the soil pH and promote the growth of *C. oleifera* seedlings. In addition, NO_3^- is easily mobile and has a high diffusion efficiency, as well as being prone to leaching loss, which reduces the uptake and utilization of NO_3^- by plants.5.

5. Conclusions

By comparing the characteristic parameters of different ratios of ammonium– and nitrate–nitrogen with either single ammonium– or nitrate–nitrogen, the mixed nitrogen source was better for promoting the root activity of *C. oleifera* seedlings, while both the total absorption area and active absorption area of the seedling roots were highest with the nitrogen source form of NO_3^-:NH_4^+ = 5:5. Moreover, *C. oleifera* seedlings have a higher uptake potential and affinity with NH_4^+-N than with NO_3^--N, indicating that *C. oleifera* prefers NH_4^+-N.

Author Contributions: Conceptualization, R.W. and Y.C.; methodology, R.W. and Z.Z.; software, R.W. and T.X.; validation, R.W., X.W. and Y.C.; formal analysis, R.W. and Z.H.; investigation, R.W. and Z.Z.; writing—review and editing, R.W. and C.L.; funding acquisition, R.W. and Y.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Major Special Project of Changsha Science and Technology Bureau (KQ2102007), and the Natural Science Foundation of Hunan Province (Grant No. 2022JJ30325 and No. 2021JJ40283).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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

References

1. He, Z.; Liu, C.; Zhang, Z.; Wang, R.; Chen, Y. Intergration of mRNA and miRNA analysis reveals the differentially regulatory network in two different *Camellia oleifera* cultivars under drought stress. *Front. Plant Sci.* **2022**, *13*, 1001357. [CrossRef] [PubMed]
2. Lin, P.; Wang, K.; Wang, Y.; Hu, Z.; Yan, C.; Huang, H.; Ma, X.; Cao, Y.; Long, W.; Liu, W.; et al. The genome of oil-Camellia and population genomics analysis provide insights into seed oil domestication. *Genome Biol.* **2022**, *23*, 1–21. [CrossRef] [PubMed]
3. Gong, W.; Xiao, S.; Wang, L.; Liao, Z.; Chang, Y.; Mo, W.; Hu, G.; Li, W.; Zhao, G.; Zhu, H.; et al. Chromosome-level genome of *Camellia lanceoleosa* provides a valuable resource for understanding genome evolution and self-incompatibility. *Plant J.* **2022**, *110*, 881–898. [CrossRef] [PubMed]

4. Shen, T.F.; Huang, B.; Xu, M.; Zhou, P.Y.; Ni, Z.X.; Gong, C.; Wen, Q.; Cao, F.L.; Xu, L.A. The reference genome of camellia chekiangoleosa provides insights into camellia evolution and tea oil biosynthesis. *Hortic. Res.* **2022**, *9*, uhab083. [CrossRef] [PubMed]
5. He, Z.; Liu, C.; Wang, X.; Wang, R.; Tian, Y.; Chen, Y. Leaf Transcriptome and Weight Gene Co-expression Network Analysis Uncovers Genes Associated with Photosynthetic Efficiency in *Camellia oleifera*. *Biochem. Genet.* **2021**, *59*, 398–421. [CrossRef]
6. Wu, B.; Ruan, C.; Shah, A.H.; Li, D.; Li, H.; Ding, J.; Li, J.; Du, W. Identification of miRNA-mRNA Regulatory Modules Involved in Lipid Metabolism and Seed Development in a Woody Oil Tree (*Camellia oleifera*). *Cells* **2021**, *11*, 71. [CrossRef]
7. Zhang, X.Y.; Ding, X.G.; Zhang, Y.Z.; Liu, Y.J.; Cai, J.; Li, Y.Q. Effects of nutrient deficiencies on root system in *Camellia gauchowensis* seedlings. *Nonwood For. Res.* **2014**, *32*, 170–174.
8. Inokuchi, R.; Kuma, K.I.; Miyata, T.; Okada, M. Nitrogen-assimilating enzymes in land plants and algae: Phylogenic and physiological perspectives. *Physiol. Plant.* **2002**, *116*, 1–11. [CrossRef]
9. Christian, S.; Wood, C.C.; Roeb, G.W. Characterization of Arabidopsis AtAMT2, a high-affinity ammonium transporter of the plasma membrane. *Plant Physiol.* **2002**, *130*, 1788–1796.
10. Sun, M.; Guo, W.S.; Zhu, X.K.; Feng, C.N.; Guo, K.Q.; Peng, Y.X. Kinetics of Nitrate and Ammonium Uptake by Different Wheat Genotypes at Seedling Stage. *J. Triticeae Crops* **2006**, *26*, 84–87.
11. Nishikawa, T.; Tarutani, K.; Yamamoto, T. Nitrate and phosphate uptake kinetics of the harmful diatom Coscinodiscus wailesii, a causative organism in the bleaching of aquacultured Porphyra thalli. *Harmful Algae* **2010**, *9*, 563–567. [CrossRef]
12. Epstein, E.; Hagen, C.E. A kinetic study of the absorption of alkali cations by barley roots. *Plant Physiol.* **1952**, *27*, 457–474. [CrossRef]
13. Zhang, G.P.; Zhang, G.H. Studies on variation among wheat genotypes in Nutilization. *J. Plant Nutr. Fertil. Sci.* **1996**, *2*, 331–336.
14. Huang, J.G.; Yang, B.J.; Yuan, L. Study on kinetics of absorption by various white variety. *J. Plant Nutr. Fertil. Sci.* **1995**, *1*, 38–43.
15. Shi, R.H. *The Principles of Plants Nutrients*; Jiangsu Science and Technology Publishing House: Nanjing, China, 1989.
16. Yao, Q.H.; Liu, W.D.; Chen, M.L.; Xie, Z.C. Studies on the verification of mechanistic models of nutrient(P) uptake by plant root system. *J. Plant Nutr. Fertil. Sci.* **1999**, *5*, 263–272.
17. Ni, J.S.; Jiang, X.C.; Feng, X.X.; Huang, M.Q. Study on various variety of maize seedling in absorption, exudates and nitrate reductase activity. *Acta Phytophysiol. Sin.* **1988**, *14*, 188–195.
18. Guo, B.H. Study on rice absorption dynamics of Si, K, Ca, and Mg. *J. China West Norm. Univ. Nat. Sci.* **2003**, *24*, 396–401.
19. Jiang, T.H.; Zheng, S.J.; Shi, J.Q.; Hu, A.T.; Shi, R.H.; Xv, M. Several considerations in kinetics research on nutrients uptake by plants. *J. Plant Nutr. Fertil. Sci.* **1995**, *1*, 11–17.
20. Wang, R.; Chen, L.; Wang, X.; Tang, W.; Peng, Y.; Zhang, Z.; Li, A.; Chen, Y. Effects of different proportion of nitrogen forms on the growth and physiological characteristics of Camellia oleifera seedlings. *J. Nanjing For. Univ. Nat. Sci. Ed.* **2019**, *43*, 26–32. (In Chinese)
21. Lu, W.J.; Li, Y.S. *Plant Physiology Experiment Tutorial*; China Forestry Publishing House: Beijing, China, 2017.
22. Lü, W.X.; Ge, Y.; Wu, J.Z. and Chang, J. Study on the method for the determination of nitric nitrogen, ammoniacal nitrogen and total nitrogen in plant. *Spectrosc. Spectr. Anal.* **2004**, *24*, 204–206.
23. Bao, S.D. *Soil Agrochemical Analysis*; China Agricultural Publishing House: Beijing, China, 2000; pp. 49–55.
24. Hua, H.X.; Liang, Y.C.; Lou, Y.S.; Zhang, J. Comparison of research methods for silicon uptake kinetics of rice. *Plant Nutr. Fertil. Sci.* **2006**, *12*, 358–362.
25. Zhai, M.P.; Jiang, S.N. Dynamics of nutrient absorption in root systems of Populus×xiao zhuanica and *Robinia pseudoacacia*. *J. Beijing For. Univ.* **2006**, *28*, 29–33.
26. Li, D.Y.; Zhou, Y.C. Responses of seedlings growth of Pinus massoniana to calcium concentration. *J. Cent. South Univ. For. Technol.* **2017**, *27*, 39–45.
27. Ma, B. Physiological and Biochemical Effects of Nitrogen Form Supply on Root Respiration of Walnut Seedlings. Master's Thesis, Xinjiang Agriculture University, Urumqi, China, 2021.
28. Ma, C. Study on Regulation of Exogenous Nitrogen Forms on Growth, Nitrogen Uptake and Metabolism of Cucumber. Ph.D. Thesis, Gansu Agriculture University, Lanzhou, China, 2020.
29. Britto, D.T.; Kronzucker, H.J. NH_4^+ toxicity in higher plants: A critical review. *J. Plant Physiol.* **2002**, *159*, 567–584. [CrossRef]
30. Kronzuker, H.J.; Glass, A.D.M.; Siddiqi, M.Y. Inhibition of nitrate uptake by ammonium in barley. *Plant Physiol.* **1999**, *120*, 283–291. [CrossRef]
31. Sun, M.; Lu, X.; Cao, X.; Li, J.; Xiong, J.; Xie, S. Effect of Different Nitrogen Forms on Root Growth and Dynamic Kinetics Characteristics for *Citrus sinensis* × *Poncirus trifoliata*. *Sci. Silvar Sin.* **2015**, *51*, 114–120.
32. Wang, L.; Wang, W.; Huang, C.H.; Chang, C.R. Effect of Nitrate Uptake Kinetic with Different Ratios of Ammonium and Nitrate in Banana Seedlings. *Chin. J. Trop. Crops* **2012**, *33*, 988–992.
33. Ren, J.; Xv, C.Y.; Lin, Y.M. Kinetic Characteristics of Different Forms of Absorbing Nitrogen in Root System of *Fraxinus mandshurica* Rupr. Seedling. *Plant Physiol. Commun.* **2008**, *44*, 919–922.
34. Qin, S.; Li, Z.; Ma, H.; Liu, L.; Liu, G. Effects of Different Nitrogen Forms on Root Respiratory Metabolism and on Biomass in Seedlings of *Cerasus sachalinensis*. *Acta Hortic. Sin.* **2011**, *38*, 1021–1028.

35. Du, X.H.; Peng, F.R. Effect of Inorganic Nitrogen Forms on Growth and Kinetics of Ammonium and Nitrate Uptake in *Camellia sinensis* L. *Acta Agron. Sin.* **2010**, *36*, 327–334. [CrossRef]
36. Rennenberg, H.; Wildhagen, H.; Ehlting, B. Nitrogen nutrition of poplar trees. *Plant Biol.* **2010**, *12*, 275–291. [CrossRef]