Article

High $\text{NH}_4^+/\text{NO}_3^-$ Ratio Inhibits the Growth and Nitrogen Uptake of Chinese Kale at the Late Growth Stage by Ammonia Toxicity

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Abstract: The aim of this study was to determine the effects of various $\text{NH}_4^+/\text{NO}_3^-$ ratios in a nutrient solution on the growth and nitrogen uptake of Chinese kale under hydroponic conditions. The four $\text{NH}_4^+/\text{NO}_3^-$ ratios in the nutrient solution were CK (0/100), T1 (10/90), T2 (25/75), and T3 (50/50). An appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratio (10/90, 25/75) promoted the growth of Chinese kale. T2 produced the highest fresh and dry weight among treatments, and all indices of seedling root growth were the highest under T2. A high $\text{NH}_4^+/\text{NO}_3^-$ ratio (50/50) promoted the growth of Chinese kale seedlings at the early stage but inhibited growth at the late growth stage. At harvest, the nutrient solution showed acidity. The pH value was the lowest in T3, whereas NH_4^+ and $\text{NH}_4^+/\text{NO}_3^-$ ratios were the highest, which caused ammonium toxicity. Total N accumulation and N use efficiency were the highest in T2, and total N accumulation was the lowest in T3. Principal component analysis showed that T2 considerably promoted growth and N absorption of Chinese kale, whereas T3 had a remarkable effect on the pH value. These findings suggest that an appropriate increase in NH_4^+ promotes the growth and nutrient uptake of Chinese kale by maintaining the pH value and $\text{NH}_4^+/\text{NO}_3^-$ ratios of the nutrient solution, whereas excessive addition of NH_4^+ may induce rhizosphere acidification and ammonia toxicity, inhibiting plant growth.

Keywords: growth rate; root morphology; ammonium to nitrate ratio; nutrient solution composition; nitrogen efficiency

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1. Introduction

Nitrogen is an essential nutrient in plant growth. It is the main component of many important organic compounds and participates in many physiological and biochemical processes in plants [1]. The forms of nitrogen that plants can absorb and utilize include ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), soluble protein, and free amino acids; higher plants mainly absorb NH_4^+ and NO_3^- [2]. However, the processes of absorption, storage, transportation, and assimilation of the two types of N in plants are very different. The different forms of N affect the growth and development of plants and ultimately affect their yield and quality.

Most crops prefer to absorb NH_4^+ rather than NO_3^- under the condition of hydroponics because a single NO_3^- nutrient consumes a large amount of energy during the reduction process. The absorption of NO_3^- increases the pH value of the solution, which leads to an insufficient supply of iron and other trace elements and decreases the content of chlorophyll, thus affecting the yield and quality [3]. The energy cost of NH_4^+ absorption and assimilation is lower than that of NO_3^- [4]. However, single NH_4^+ nutrition causes many problems, such as ammonium toxicity, blocked leaf expansion, reduced organic acid synthesis, and decreased osmotic regulation [5].

An increasing number of studies have shown that adding a mixture of NH_4^+ and NO_3^- in appropriate proportions is more advantageous for crop growth and development and that NH_4^+ and NO_3^- can interact when both N forms are provided together [4,6]. Compared with the addition of NH_4^+ or NO_3^- alone, appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratio nutrition can significantly promote plant growth, increase plant biomass, soluble sugar, soluble protein, and vitamin C content, and reduce nitrate content in strawberry [7], mini-Chinese cabbage [8], flowering Chinese cabbage [9], and Chinese kale [10]. In addition, it is well-known that the absorption of NH_4^+ can induce net release of H^+ and acidify the rhizosphere, whereas the absorption of NO_3^- can increase H^+ uptake through the H^+ cotransport system in PM and alkalize the rhizosphere [11,12]. A plant's uptake of different forms of N and its transport and assimilation mechanisms depend on its $\text{NH}_4^+/\text{NO}_3^-$ ratios. Our recent research further showed that an appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratio triggers plant growth and nutrient uptake of flowering Chinese cabbage by optimizing pH value in nutrient solution [13].

Chinese kale (*Brassica alboglabra* L. H. Bailey) is an important vegetable in south China. The flower stalk and leaves of Chinese kale are rich in anticarcinogenic compounds and antioxidants, including glucosinolates, carotenoids, vitamin C, and total phenolics [14,15]. Chinese kale best absorbs NO_3^- under hydroponic conditions and easily accumulates nitrate. In our previous study, an appropriate increase of NH_4^+ in nutrient solution enhancement improved the yield and quality of Chinese kale and significantly reduced the nitrate content in its product organs [16]; however, the physiological mechanism of this regulation is still unclear.

Based on previous research, this study further investigates the effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on plant growth, seedling root morphology, nutrient solution composition, and plant nutrient absorption to reveal the physiological mechanism by which different $\text{NH}_4^+/\text{NO}_3^-$ ratios regulate the growth and N uptake of Chinese kale.

2. Materials and Methods

2.1. Plant Material

The experiment was conducted in a greenhouse at the College of Horticulture, South China Agricultural University from March 2013 to January 2014. The average temperature from colonization to the end of the experiment was 24–30/20–24 °C (day/night). Chinese kale seeds 'Ivba' were provided by Guangzhou Academy of Agriculture Science. Chinese kale seeds were sown in a perlite medium. Seedlings with one developed true leaf and one core were watered with a 1/4 dose of Hoagland nutrient solution every 4 days. After 1 month, three consistent seedlings with three developed leaves and one core were selected and transplanted into hydroponic containers with 5.5 L of nutrient solution. There were 10 replications in each treatment arranged in a randomized complete block design.

2.2. Treatments

In this experiment, four different $\text{NH}_4^+/\text{NO}_3^-$ ratios were set based on 1/2 dose of Hoagland sloe NO_3^- nutrient solution formula (Table 1): CK, 0/100; T1, 10/90; T2, 25/75; and T3, 50/50. General formula for mineral elements: B, 0.5 mg L⁻¹; Mn, 0.5 mg L⁻¹; Zn, 0.05 mg L⁻¹; Cu, 0.02 mg L⁻¹; Mo, 0.01 mg L⁻¹. Fe was supplied by EDTANaFe at a concentration of 50 mg L⁻¹. Additionally, 0.2 g L⁻¹ ampicillin (excellent grade pure) was added to inhibit microbial activity. Chinese kale seedlings were transplanted and watered with a 1/4 dose of Hoagland nutrient solution (adjusted according to Table 1), adding 3/4 dose of mother liquor after 12 days, and adding pure water to the original volume of 5.5 L. Pure water was added to the original volume every 3 days. Electrical conductivity and pH were measured during the experiment. In nutrient solutions with different $\text{NH}_4^+/\text{NO}_3^-$ ratios, NH_4^+ was supplied by NH_4Cl . KCl or CaCl_2 was added to maintain a constant concentration of K^+ and Ca^{2+} among the treatments. All nutrient solutions were aerated for 15 min per hour using a controlled pump.

Table 1. Nutrient solution with different $\text{NH}_4^+/\text{NO}_3^-$ ratios (Unit: mM).

Treatments	$\text{NH}_4^+/\text{NO}_3^-$	KNO_3	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	KH_2PO_4	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	$(\text{NH}_4)_2\text{SO}_4$	K_2SO_4	CaCl_2
CK	0/100	2.5	2.5	0.5	1.4	-	-	-
T1	10/90	1.75	2.5	0.5	1.4	0.375	0.375	-
T2	25/75	0.625	2.5	0.5	1.4	0.9375	0.9375	-
T3	50/50	-	1.875	0.5	1.4	1.875	1.25	0.625

The seeds of Chinese kale were sown in a medium containing 0.5% agar. After 5 days, seedlings with a radicle length of 1.5 cm were transplanted into hydroponic containers with 1.1 L of nutrient solution for different $\text{NH}_4^+/\text{NO}_3^-$ ratio treatments.

2.3. Parameter Measurements

Chinese kale seedlings were harvested when they reached marketable maturity, plant height, and stem diameter, and their fresh and dry weight (determined after 1 h at 120 °C and 48 h at 75 °C in a drying oven) were measured (3 biological replicates per treatment, 12 plants per replicate). The fresh weight of the product organ (flower stalk above the 4th node) is called the economic yield. The fresh and dry weight were measured at 9, 15, 21, and 27 days after treatment to calculate the growth rate. The growth rate was measured by dividing the difference in fresh weight before and after sampling by the number of days.

The seeds of Chinese kale were accelerated to bud on a medium containing 0.5% agar. After 5 days, seedlings with a radicle length of 1.5 cm were selected and treated with different $\text{NH}_4^+/\text{NO}_3^-$ ratios. After 2 weeks, the root morphological indices were analyzed (3 biological replicates per treatment, 12 plants per replicate). The root samples were stained with 0.16% neutral red solution, scanned using an applied digital scanner (LA2400), and quantitatively analyzed using the WinRHIZO Pro LA2400 software (Regent Instruments, Quebec City, QC, Canada) for total root length, root surface area, root volume, and average root diameter.

Total N content was determined according to the method suggested by Avery and Rhodes [17], and total N content was multiplied by the dry weight of the whole plant to calculate N accumulation. N loss (NL), N loss rate (NLR), mean residence time of N (MRT), N productivity (NP), and N use efficiency (NUE) were calculated as described by Eckstein and Karlsson [18] using the following formulae: $\text{NL} = [(\text{NS}_{\text{applied}} - \text{NS}_{\text{remain}}) - (\text{N}_{\text{harvest}} - \text{N}_{\text{transplant}}) \times n]/n$; $\text{NLR} = \text{NL}/(\text{NS}_{\text{applied}} - \text{NS}_{\text{remain}})$; $\text{MRT} = (\text{N}_{\text{harvest}} - \text{N}_{\text{transplant}})/[(\ln \text{N}_{\text{harvest}} - \ln \text{N}_{\text{transplant}}) \times (\text{NL}/t)]$; $\text{NP} = [(W_{\text{harvest}} - W_{\text{transplant}})/(T_{\text{harvest}} - T_{\text{transplant}})] \times [(\ln \text{N}_{\text{harvest}} - \ln \text{N}_{\text{transplant}})/(\text{N}_{\text{harvest}} - \text{N}_{\text{transplant}})]$; $\text{NUE} = \text{NP} \times \text{MRT}$. In the above formulae, W represents the dry weight of the plant, T represents the sampling time, N represents the amount of N absorbed by seedlings, NS represents the amount of N in nutrient solution, n represents the number of seedlings in each hydroponic bucket, and t represents the number of days in the whole growth period. Total P and K were determined at 660 nm using a spectrophotometer and atomic absorption spectrophotometer [19].

2.4. Data Analysis

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 19.0. The differences between treatments were compared using the least significant difference (LSD) with a significance level of $p < 0.05$. The tables and figures were created using Excel 2013 and SigmaPlot 11.0, respectively. Multivariate principal component analysis (PCA) was performed using OriginPro 9.0.

3. Results

3.1. Growth and Biomass

The growth of Chinese kale was significantly affected by the different $\text{NH}_4^+/\text{NO}_3^-$ ratios (Figure 1 and Figure S1). Compared with CK, plant height increased by 18.89% and 24.70% in T1 and T2, respectively (Figure 1A). Similarly, the stem diameter was the highest

in T2, increasing by 13.89%, whereas there was no significant difference between CK and T3 (Figure 1B). The economic yield was the highest in T2 and lowest in T3 at the harvest stage of Chinese kale (Figure 1C). To further study the dynamic changes in Chinese kale growth under different $\text{NH}_4^+/\text{NO}_3^-$ ratios, fresh and dry weights were measured at 9, 15, 21, and 27 days after treatment. T1, T2, and T3 significantly increased the biomass of Chinese kale during the growth stage (0–21 d) compared to the control. However, in contrast to T1 and T2, the fresh weight and dry weight of T3 at harvest time decreased by 17.57% and 12.60%, respectively, compared with CK (Figure 1D,E). This result is consistent with economic yield. At the early stage of Chinese kale growth (0–15 d), the root/shoot ratio was the highest in CK, followed by T1, and the difference between T2 and T3 was not significant, indicating that the treatment of increasing NH_4^+ in the nutrient solution would reduce the dry matter distribution of the roots. At the harvest stage of Chinese kale (27d), the root/shoot ratio of T3 was 1.13, 1.24, and 1.12 times that of CK, T1, and T2, respectively, indicating that the effect of high $\text{NH}_4^+/\text{NO}_3^-$ ratio treatment on the shoot was greater than that on the root at the late growth stage of Chinese kale (Figure 1F).

Figure 1. Effect of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on the growth and biomass of Chinese kale. (A) plant height at the harvest stage. (B) stem diameter at the harvest stage. (C) economical plant yield at the harvest stage. (D) dynamic changes in fresh weight. (E) dynamic changes in dry weight. (F) dynamic changes in the ratio of root to shoot fresh weight. CK = 0/100, T1 = 10/90, T2 = 25/75, T3 = 50/50. DAT, days after treatment. The data represent mean $\pm$ SE ($n = 3$). Different letters in Figure 1C–F indicate significant differences at $p < 0.05$.

Furthermore, we analyzed the effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on the growth rate of Chinese kale seedlings and found that the growth of Chinese kale was slow in the early growth stage and fast in the late growth stage (Table 2). During the period of 0–9 d after treatment, seedling growth rates were highest in T2 and T3, with no significant difference between them, followed by T1, and were the lowest in the control. During the period of 9–15 d after treatment, the change trend of the four treatments was consistent with that of 0–9 days. During the period of 15–21 d after treating, the growth rates of seedlings reached the maximum in T2 and T3, which were $519.6 \text{ mg d}^{-1} \text{ plant}^{-1}$ and $388.4 \text{ mg d}^{-1} \text{ plant}^{-1}$, respectively. During the period of 21–27 d after treatment, the seedling growth rates reached the maximum in CK and T1, which were $656.1 \text{ mg d}^{-1} \text{ plant}^{-1}$ and $625.1 \text{ mg d}^{-1} \text{ plant}^{-1}$, respectively. In conclusion, at 0–21 d, the growth rate of seedlings was the highest in T2, followed by T3. However, the difference between them was not significant: at 0–27 d, the growth rate of seedlings was the lowest in T3, which was only 87.45%, 79.47%, and 73.08% that of the control, T1, and T2, respectively, indicating that the growth was significantly inhibited.

Table 2. Effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on the growth rate of Chinese kale (Unit: $\text{mg d}^{-1} \text{ plant}^{-1}$).

Treatments	$\text{NH}_4^+/\text{NO}_3^-$	0~9	9~15	15~21	21~27	0~21	0~27
CK	0/100	$27.7 \pm 2.8 \text{ b}$	$141.8 \pm 4.9 \text{ c}$	$237.5 \pm 47.5 \text{ c}$	$656.1 \pm 17.0 \text{ a}$	$120.2 \pm 15.3 \text{ c}$	$239.0 \pm 8.1 \text{ bc}$
T1	10/90	$33.7 \pm 2.0 \text{ ab}$	$167.1 \pm 17.5 \text{ b}$	$341.3 \pm 65.9 \text{ b}$	$625.1 \pm 27.0 \text{ a}$	$159.7 \pm 22.2 \text{ bc}$	$263.0 \pm 22.3 \text{ ab}$
T2	25/75	$40.5 \pm 3.9 \text{ a}$	$197.2 \pm 4.2 \text{ a}$	$519.6 \pm 156.0 \text{ a}$	$509.0 \pm 76.4 \text{ b}$	$222.2 \pm 47.7 \text{ a}$	$286.0 \pm 21.4 \text{ a}$
T3	50/50	$39.9 \pm 5.3 \text{ a}$	$186.8 \pm 9.3 \text{ a}$	$388.4 \pm 17.5 \text{ ab}$	$304.9 \pm 57.7 \text{ c}$	$181.4 \pm 9.7 \text{ ab}$	$209.0 \pm 20.4 \text{ c}$

Data are presented as mean $\pm$ SE ($n = 3$). Different letters indicate significant differences at $p < 0.05$.

To study the effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on the root growth of Chinese kale seedlings, we measured the main root length, root total length, root surface area, root volume, and average root diameter (Table 3, Figure S2). The main root length of Chinese kale seedlings in T1 and T2 was slightly higher than that in CK, and that of T3 was slightly lower than that of CK. Compared with CK, the total root lengths of Chinese kale seedlings in T1 and T2 were 1.38 and 1.91 times than those of CK, respectively. Root volumes of Chinese kale seedlings in T1 and T2 were 1.74 and 2.87 times that of CK, respectively. The root surface area and average root diameter of Chinese kale seedlings were maximum in T2. We concluded that the optimal root growth and root indices of Chinese kale seedlings occurred under T2 ($\text{NH}_4^+/\text{NO}_3^- = 25/75$). However, a high $\text{NH}_4^+/\text{NO}_3^-$ ratio (50/50) may inhibit the main root elongation of Chinese kale seedlings to a certain extent. In addition, root activity showed no significant difference between the control, T1, and T2; however, it decreased by 20.85% in T3 compared with the control. (Figure S3).

Table 3. Effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on root morphological indices of Chinese kale seedlings.

Treatments	$\text{NH}_4^+/\text{NO}_3^-$	Main Root Length (cm plant ⁻¹)	Root Total Length (cm plant ⁻¹)	Root Surface Area (cm ² plant ⁻¹)	Root Volume (cm ³ plant ⁻¹)	Average Root Diameter (mm)
CK	0/100	$6.91 \pm 0.77 \text{ ab}$	$65.8 \pm 11.5 \text{ c}$	$13.7 \pm 3.2 \text{ c}$	$0.23 \pm 0.07 \text{ cd}$	$0.66 \pm 0.04 \text{ b}$
T1	10/90	$7.23 \pm 0.38 \text{ ab}$	$90.8 \pm 2.7 \text{ b}$	$20.9 \pm 5.1 \text{ b}$	$0.40 \pm 0.19 \text{ b}$	$0.73 \pm 0.16 \text{ ab}$
T2	25/75	$7.72 \pm 0.84 \text{ a}$	$126.0 \pm 8.9 \text{ a}$	$31.9 \pm 4.2 \text{ a}$	$0.66 \pm 0.16 \text{ a}$	$0.81 \pm 0.09 \text{ a}$
T3	50/50	$6.63 \pm 0.49 \text{ b}$	$61.4 \pm 12.4 \text{ c}$	$14.5 \pm 3.7 \text{ c}$	$0.28 \pm 0.09 \text{ bc}$	$0.75 \pm 0.07 \text{ ab}$

Data are presented as mean $\pm$ SE ($n = 3$). Different letters indicate significant differences at $p < 0.05$.

3.2. Dynamic Changes in Nutrient Solution Composition

To elucidate the mechanism by which different $\text{NH}_4^+/\text{NO}_3^-$ ratios affect the growth of Chinese kale, we conducted dynamic monitoring of the physicochemical properties of the nutrient solution and N content (Figure 2). The initial electrical conductivity (EC) value of the nutrient solution increased as the NH_4^+ proportion increased. The EC values were

629, 673, 732, and 879 $\mu\text{S cm}^{-1}$ in the control, T1, T2, and T3, respectively (Figure 2A). With the growth of Chinese kale, both the EC value and ion concentration of the nutrient solution gradually decreased. During the entire growth period of the plant, the decrease in EC value in T2 was the largest, and the decrease in T1 and T3 was also greater than that of the control, but the decrease in EC value in T3 was the lowest during the harvest period. This indicates that an appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratio could promote the ion absorption of Chinese kale plants, and the $\text{NH}_4^+/\text{NO}_3^-$ ratio (50/50) could promote ion absorption of plants at the early and middle stages but significantly inhibit ion absorption of plants at the late growth stage.

Figure 2. Changes in electrical conductivity value (A), pH value (B), NO_3^- content (C), NH_4^+ content (D), total N content (E), and $\text{NH}_4^+/\text{NO}_3^-$ ratios (F) in nutrient solution under different $\text{NH}_4^+/\text{NO}_3^-$ ratios. CK = 0/100, T1 = 10/90, T2 = 25/75, T3 = 50/50. DAT, days after treatment. The data represent mean $\pm$ SE ($n = 3$).

The initial pH value of the nutrient solution decreased as the NH_4^+ proportion increased; however, the difference was not significant, both were at approximately 6.75 (Figure 2B). During the entire growth period, the pH value in the CK was mostly weakly alkaline. Before and after the replenishment of the nutrient solution, the average pH of the nutrient solution showed a gradual increase and reached 8.15 at harvest time. The nutrient solution in T1 was weakly acidic at the early stages, and the pH value was stable at

approximately 6.5. At 21 d, the pH value was greater than 7.0, the nutrient solution became weakly alkaline, and the pH of the nutrient solution further increased to 8.20 at harvest time. The nutrient solution in T2 was always weakly acidic, and the pH value decreased gradually at the early stage and reached the lowest value at 5.24 at 18 d. The pH value gradually increased and reached 6.73 at harvest time. The nutrient solution in T3 was acidic during the entire growth period, the pH of the nutrient solution decreased gradually, and the pH of the nutrient solution was 3.63 at the harvest stage. In combination with plant biomass, Chinese kale was found to be insensitive to environmental pH and could grow normally in a nutrient solution pH range of 4.73–8.15. An appropriate acidic environment was more conducive to the growth of Chinese kale; however, an over-acidic environment ($\text{pH} < 4$) could significantly inhibit the growth of Chinese kale and reduce the growth rate of plants.

During the plant growth period, the NO_3^- content in the four treatments decreased gradually as NH_4^+ proportion increased (Figure 2C). After 12 d of treatment, the NO_3^- content in the control, T1, T2, and T3 decreased by 45.74, 43.99, 37.38, and 25.12 mg L^{-1} , respectively, compared with that before treatment. After the replenishment of nutrient solution, the NO_3^- content in the control, T1, T2, and T3 was 164.26, 145.01, 120.12 and 79.88 mg L^{-1} , respectively. At harvest, the NO_3^- content in the control, T1, T2, and T3 decreased by 154.00, 145.00, 120.05, and 79.77 mg L^{-1} , respectively, compared with that after the replenishment of nutrient solution. The proportions of NO_3^- absorbed by the four treatments (CK, T1, T2, and T3) to the total NO_3^- were 95.11%, 99.99%, 99.96%, and 99.90%, respectively, indicating that the three treatments (T1, T2, and T3) all promoted NO_3^- uptake by Chinese kale plants, and there was no significant difference between the different treatments. Similarly, with an increase in NH_4^+ proportion, the NH_4^+ content in the three treatments also decreased gradually (Figure 2D). After 12 d of treatment, the NH_4^+ content in T1, T2, and T3 decreased by 4.94, 12.26, and 14.74 mg L^{-1} , respectively, compared with that before treatment. After nutrient solutions were added, the NH_4^+ content in T1, T2, and T3 were 16.06, 40.24, and 90.26 mg L^{-1} , respectively. After 3 d, they decreased by 9.53, 9.52, and 37.55 mg L^{-1} , respectively. Thereafter, they slowly decreased to 0.03, 0.01, and 34.77 mg L^{-1} , respectively, at harvest. The proportions of NH_4^+ absorbed by the three treatments to the total NH_4^+ were 99.86%, 99.98%, and 66.89%, respectively, indicating that low and medium $\text{NH}_4^+/\text{NO}_3^-$ ratios could significantly promote the absorption of NH_4^+ by Chinese kale compared with the high $\text{NH}_4^+/\text{NO}_3^-$ ratio.

With the growth of the plants, the total N content in the four treatments decreased gradually with an increase in NH_4^+ proportion (Figure 2E). At harvest, the total N content in the control, T1, T2, and T3 was decreased by 154.00, 161.03, 160.28, and 135.26 mg L^{-1} , respectively, compared with that after the replenishment of nutrient solution. The greatest reduction in total N content was observed in T1 and T2, and the final content at harvest was 0.04 and 0.08 mg L^{-1} , respectively. This indicates that a low $\text{NH}_4^+/\text{NO}_3^-$ ratio (10/90) significantly promoted the absorption of total N by Chinese kale, whereas a high $\text{NH}_4^+/\text{NO}_3^-$ ratio inhibited the absorption of total N. In addition, the three treatments (T1, T2, and T3) promoted the absorption of P by Chinese kale, whereas the high $\text{NH}_4^+/\text{NO}_3^-$ ratio significantly inhibited the absorption of K by Chinese kale at the late growth stage (Figure S4).

We further analyzed the $\text{NH}_4^+/\text{NO}_3^-$ ratio in the four treatments (Figure 2F). Before and after the replenishment of the nutrient solution, the $\text{NH}_4^+/\text{NO}_3^-$ ratio in T1 showed a decreasing and then an increasing trend. It decreased to a minimum of 0.001 at 21 d and increased to a maximum of 0.445 at the harvest stage. The $\text{NH}_4^+/\text{NO}_3^-$ ratio in T2 remained between 0.243 and 0.435, which decreased significantly to 0.003 at 24 d of treatment and then increased to 0.214 at the harvest stage. The $\text{NH}_4^+/\text{NO}_3^-$ ratio in T3 gradually increased during plant growth, and it increased rapidly after the replenishment of nutrient solution and reached a maximum of 95.144 at the harvest stage.

3.3. N Content and N Use Efficiency

The total N content of Chinese kale first increased and then decreased during the growth period (Figure 3A). The N content of Chinese kale increased rapidly from 0 d to 9 d of growth, and T2 and T3 were significantly higher than those of the control and T1. The N content of Chinese kale increased slowly from 9 d to 15 d of growth, and T3 was higher than that of the other three treatments. Except for the control, the N content of Chinese kale plants decreased significantly from 15 to 21 d of growth. The N content in T3 increased by 5.40%, 5.40%, and 6.93% compared with CK, T1, and T2, respectively, at 27 d. In addition to total N content, seedling dry weight also had a significant effect on total N accumulation. Total N accumulation in Chinese kale increased continuously during the growth period (Figure 3B). In the middle and late periods of plant growth, the total N accumulation was the highest in T2, and the total N accumulation of control plants was the lowest in the period from 0 d to 25 d of growth, whereas the total N accumulation of T3 plants was the lowest at 27 d, which was reduced by 7.88%, 16.13%, and 21.62% compared with CK, T1, and T2, respectively.

Figure 3. The content (A) and accumulation (B) of total N affected by different $\text{NH}_4^+/\text{NO}_3^-$ ratios during the growth period of Chinese kale. CK = 0/100, T1 = 10/90, T2 = 25/75, T3 = 50/50. DW, dry weight; DAT, days after treatment. The data represent mean $\pm$ SE ($n = 3$).

To further study the N utilization of Chinese kale plants in response to different $\text{NH}_4^+/\text{NO}_3^-$ ratios, we analyzed the N loss, N productivity, N residence time, and N use efficiency (Table 4). We found that there was a certain amount of N loss in Chinese kale production under hydroponic conditions. Compared with the control, N loss in T1, T2, and T3 was reduced by 7.37%, 27.40%, and 23.71%, respectively, and the N loss rate was reduced by 11.83%, 30.90%, and 12.97%, respectively, with significant differences between treatments. N retention time was prolonged in all three treatments; however, it was most significant in T2. N productivity was the highest in T2, followed by T1, and lowest in T3; however, there was no significant difference between the different treatments. Owing to the difference in N loss rate, N productivity, and N retention time, N use efficiency was significantly different between the four treatments. N use efficiency was the highest in T2, followed by T1; however, there was no significant difference between T1 and T3. The results showed that an appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratio (25/75) significantly reduced N loss, prolonged N retention time, and improved N productivity and N use efficiency compared with the control.

Table 4. Rate of N loss, N residence time, N productivity, and N use efficiency in nutrient solutions with different $\text{NH}_4^+/\text{NO}_3^-$ ratios during the growth period of Chinese kale.

Treatments	$\text{NH}_4^+/\text{NO}_3^-$	N Loss (mg Plant ⁻¹)	Rate of N Loss (%)	N Residence Time (d)	N Productivity (mg mg ⁻¹ d ⁻¹)	N Use Efficiency (mg mg ⁻¹)
CK	0/100	102.1 ± 5.1 a	27.9 ± 1.2 a	13.9 ± 0.1 c	4.6 ± 0.6 a	63.3 ± 1.7 c
T1	10/90	94.6 ± 1.5 b	24.6 ± 0.4 b	16.1 ± 0.4 b	4.7 ± 0.2 a	75.1 ± 5.4 b
T2	25/75	74.1 ± 3.2 c	19.3 ± 0.8 c	21.8 ± 0.3 a	4.8 ± 0.7 a	104.1 ± 4.1 a
T3	50/50	77.9 ± 3.3 c	24.3 ± 0.9 b	17.0 ± 0.4 b	4.3 ± 0.6 a	72.3 ± 4.2 b

Data are presented as mean ± SE ($n = 3$). Different letters indicate significant differences at $p < 0.05$. Significant differences among the treatments were determined using SPSS 17.0.

3.4. Principal Component Analysis

Principal component analysis (PCA) was used to visualize the effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on the growth and N uptake of Chinese kale (Figure 4). The computed model captured 84.5% of the total observed variance with the first two principal components (PCs). Four treatments (CK, T1, T2, and T3) were distributed in distinct quadrants in the PCA scatter plot. All indexes of plant growth and N uptake were the best in the T2 treatment, and biomass and root morphology were strongly correlated with N use efficiency. In addition, the $\text{NH}_4^+/\text{NO}_3^-$ ratio in the nutrient solution and root/shoot ratio were the highest in T3 and were significantly negatively correlated with the pH value.

Figure 4. Principal component analysis showing differences and correlations in the investigated parameters of Chinese kale under different $\text{NH}_4^+/\text{NO}_3^-$ ratio.

4. Discussion

The main forms of inorganic N, NH_4^+ and NO_3^- can be absorbed and utilized by plants [20,21]. To date, there have been many reports on the effects of different N forms and ratios on crop biomass [22]. Our previous study showed that the biomass of Chinese kale was largest when the nutrient solution increased NH_4^+ by 25% to 30%; when the nutrient solution increased NH_4^+ by 45% to 50%, the biomass of kale was significantly lower than that of the control treatment with only NO_3^- [9]. In this study, the high $\text{NH}_4^+/\text{NO}_3^-$ ratio (50/50) significantly increased the biomass of Chinese kale and promoted plant growth at the early stage; however, it showed a strong inhibitory effect at the late growth stage

(21–27 d). During this period, the plant growth rate was $304.9 \text{ mg d}^{-1} \text{ plant}^{-1}$, which was only 46.5% of plant growth in the control with only NO_3^- (Figure 1, Table 2). This showed that the effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on plant growth could also be closely related to the duration of treatment and growth stage of plants, and that a high concentration of NH_4^+ applied for a short time would not cause ammonium toxicity [23,24]. In this study, 2.25, 5.60, and 11.23 mmol L⁻¹ Cl^- was added to the nutrient solution of T1, T2, and T3, respectively, being less than the salt-osmotic stress threshold level of approximately 40 mmol L⁻¹ NaCl for most plants [25]. Therefore, the amount of Cl^- in the nutrient solution used in this study was insufficient for salt stress.

Plants mainly absorb nutrients through their roots. Of all mineral nutrients, nitrogen has the greatest influence on root morphology, growth, and distribution in the medium [26]. The absorption of NO_3^- by plants results in rhizosphere alkalization, while the absorption of NH_4^+ results in rhizosphere acidification [27]. In the present study, the nutrient solution was weakly acidic under a medium $\text{NH}_4^+/\text{NO}_3^-$ ratio (25/75), and the root morphology index was the highest (Table 3). Root morphology and biomass were significantly positively correlated with pH under a medium $\text{NH}_4^+/\text{NO}_3^-$ ratio (Figure 4). Appropriate ratios of $\text{NH}_4^+/\text{NO}_3^-$ promote root growth and increase root dry weight [28,29]. Pepper treated with a 25:75 ratio increased root length, surface area, and root volume and tips [30]. Therefore, an appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratio (25/75) may promote root growth by maintaining the pH value, thereby increasing biomass. The pH of the rhizosphere also affects the absorption of NH_4^+ and NO_3^- by plants [31]. With the growth and development of plants, the pH value of the nutrient solution gradually decreased under a medium $\text{NH}_4^+/\text{NO}_3^-$ ratio (25/75). The NO_3^- was quickly and completely absorbed, and only NH_4^+ remained in the nutrient solution at the late growth stage of Chinese kale (Figure 2). When the proportion of NH_4^+ in the nutrient solution was more than 75%, cabbage growth was reduced by 87% due to the accumulation of a large amount of free ammonia in the leaves [32]. The pH value and NH_4^+ proportion showed a significant negative correlation under a high $\text{NH}_4^+/\text{NO}_3^-$ ratio (50/50) (Figure 4). However, the pH value was acidic, which was not conducive to the root development of Chinese kale seedlings. In addition, the accumulation of large amounts of NH_4^+ in Chinese kale leaves resulted in ammonium toxicity, which inhibits plant growth.

The change in pH value in the rhizosphere results in different absorption of NH_4^+ and NO_3^- , which affects the content and accumulation of N in plants [9]. In this study, the total N content of the three treatments (T1, T2, and T3) was significantly increased at 0–15 d after treatment compared to that of the control treatment with NO_3^- alone (Figure 3A). This is consistent with previous studies on Chinese flowering cabbage [13]. Total N accumulation increased continuously throughout the growth period of the Chinese kale. At the late plant growth stage, the total N accumulation of the high $\text{NH}_4^+/\text{NO}_3^-$ ratio (50/50) was the lowest (Figure 3B). This is similar to the results of previous studies [33]. There was a negative correlation between N accumulation and biomass under high $\text{NH}_4^+/\text{NO}_3^-$ ratios (Figure 4). NH_4^+ at higher concentrations caused toxicity in bamboo, as it inhibits root growth and N accumulation [34]. Therefore, a high $\text{NH}_4^+/\text{NO}_3^-$ ratio reduced the uptake and accumulation of N in plants at the late growth stage of Chinese kale, thus inhibiting plant growth. Different $\text{NH}_4^+/\text{NO}_3^-$ ratios also significantly affect the absorption of other nutrients [35]. In this study, a high concentration of NH_4^+ promoted the absorption of P but inhibited the absorption of K by Chinese kale at the late stage (Figure S4). There is a strong correlation between pH change in a solution and net cation or anion uptake in geranium and petunia [36]. The elevation of the NH_4^+ ratio in the fertilizer solution decreased the soil solution pH at 35 days after sowing, resulting in an increase in tissue P and a decrease in K content in French marigold “Orange Boy” [37]. This may be caused by the interaction between ions and the pH change in the rhizosphere.

This study adopts the definition of nutrient use efficiency by Berendse and Aerts [38], and divides nitrogen use efficiency into N productivity and average retention time of N. N productivity reflects a plant’s rapid growth strategy. The average retention time

of nitrogen reflects a plant's nutrient retention strategy [39]. In this study, N loss in the nutrient solution increased by NH_4^+ treatment was significantly lower than that of the control with NO_3^- alone (Table 4). In our recent research, the contents of NH_4^+ , NO_3^- , and total N in the nutrient solution without plant culture were almost constant during the growth period [13]. Therefore, in a closed hydroponic system, the loss of N may be related to the N metabolism of plants or caused by changes in the nutrient solution composition during plant cultivation. Combined with the experimental conditions, this part of the N loss in this experiment should be attributed to gaseous loss. The volatile forms of gaseous nitrides include NH_3 , N_2O , and NO. Previous studies have found that, compared with NH_4^+ , when NO_3^- is used as the nitrogen source, the volatilization of N_2O in wheat and of NO in rice is enhanced [40–42]. However, the main loss forms of nitrides during kale production are still unclear, and further experiments are needed. In this study, the medium $\text{NH}_4^+/\text{NO}_3^-$ ratio (25/75) had the highest N use efficiency, unlike *Brassica napus*, which had the highest N use efficiency with a high $\text{NH}_4^+/\text{NO}_3^-$ ratio (75/25) [43]. We observed that the $\text{NH}_4^+/\text{NO}_3^-$ ratio in T2 was maintained at approximately 0.15 (Figure 2D), which may have contributed to balanced N absorption. The $\text{NH}_4^+/\text{NO}_3^-$ ratio in T3 increased rapidly at the late stage of plant growth (Figure 2D), which may have caused insufficient assimilation of NH_4^+ after its absorption or protein degradation owing to NH_4^+ toxicity, thereby reducing N productivity. Thus, applying appropriate ratios of $\text{NH}_4^+/\text{NO}_3^-$ is an important way to improve plant N use efficiency [44].

5. Conclusions

An appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratio (25/75) increased biomass and promoted the growth of Chinese kale, whereas high $\text{NH}_4^+/\text{NO}_3^-$ (50/50) promoted the growth of Chinese kale seedlings at the early growth stage but inhibited growth at the late growth stage. With the prolongation of treatment time, the pH value of T3 nutrient solution decreased continuously, whereas the $\text{NH}_4^+/\text{NO}_3^-$ ratio was the highest in T3 at the harvest stage. In addition, T2 had the highest total N accumulation and N use efficiency, whereas total N accumulation was lowest in T3. Thus, an appropriate $\text{NH}_4^+/\text{NO}_3^-$ ratio (25/75) promotes the growth and N uptake of Chinese kale by maintaining the pH value of the nutrient solution, whereas excessive addition of NH_4^+ may induce rhizosphere acidification and ammonia toxicity, thereby inhibiting plant growth. This study provides a theoretical basis for the effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on plant growth and N absorption and utilization.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae8010008/s1>, Figure S1: Plant morphology of Chinese kale treated with different $\text{NH}_4^+/\text{NO}_3^-$ ratios; Figure S2: The root morphology of Chinese kale seedlings treated with different $\text{NH}_4^+/\text{NO}_3^-$ ratios; Figure S3: The root activity of Chinese kale under different $\text{NH}_4^+/\text{NO}_3^-$ ratios; Figure S4: Effect of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on the content and accumulation of total K (A,B) and P (C,D) in the growth period of Chinese kale.

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