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Increasing the nitrate-to-ammonium ratio improved plant growth and nitrogen uptake in pineapple seedlings

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Introduction: Previous studies indicate that the combined supply of both nitrate nitrogen (NO_3^-) and ammonium nitrogen (NH_4^+) is more beneficial for pineapple growth than the exclusive supply of either NO_3^- or NH_4^+ alone. However, the optimal ratio of $\text{NO}_3^-:\text{NH}_4^+$ that maximizes pineapple growth remains to be fully elucidated, which limits the optimization of nitrogen fertilizer management in pineapple cultivation.

Methods: Uniform plantlets of *Ananas comosus* L. cv. Comte de Paris were subjected to hydroponic experiments with five $\text{NO}_3^-:\text{NH}_4^+$ ratios (0:100, 30:70, 50:50, 70:30, and 100:0) and a fixed total nitrogen concentration of 4 mM. The shoot and root growth characteristics, as well as carbon (C) and nitrogen (N) accumulation traits of pineapple plants, were systematically investigated. Additionally, the NO_3^- and NH_4^+ uptake rates of roots were determined.

Results: Compared with other treatments, the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 significantly increased total root length and surface area, and exhibited the highest NO_3^- uptake rate. With increasing nitrate proportions in the nitrogen supply, plant N uptake and the contents of chlorophyll a (Chl a) and chlorophyll b (Chl b) were significantly increased. Specifically, relative to the sole NH_4^+ treatment (0:100), the 70:30 ratio treatment increased leaf area by 61.10%, leaf dry matter content by 24.40%, plant N accumulation by 19.49%, and plant C accumulation by 21.62%. Enhanced N uptake and photosynthetic pigment synthesis facilitated leaf area expansion, thereby promoting overall plant growth.

Discussion: These results indicate that a $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 is optimal for pineapple growth, as it effectively enhances both root and shoot development and improves C and N accumulation. This finding provides valuable insights for optimizing nitrogen fertilizer management strategies, which is crucial for promoting pineapple productivity in practical cultivation.

KEYWORDS

growth, nitrogen absorption, $\text{NO}_3^-:\text{NH}_4^+$ ratio, pineapple, root morphology

1 Introduction

Nitrogen (N) is the nutrient most required by plants and the main component of amino acids, proteins, nucleic acids, chlorophyll, and coenzymes (Marschner, 2012). Ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) are the most available N sources in soil solution to be absorbed by plant roots (Marschner, 2012). Appropriate N form and adequate N supply are the essential basis for optimal plant growth, maximum N use efficiently, and crop yield (Yang et al., 2025). However, the lack of expertise in N management and the unscientific N fertilization practices adopted by farmers resulted in inadequate or excessive amounts of N supply that fail to meet the needs of plant growth. This problem is widespread in agricultural production of crops such as grains, vegetables, and fruits, causing a series of environmental problems (Li et al., 2025). The N absorbed by plants is supplied in organic and inorganic forms, of which ammonium and nitrate are the two major inorganic N forms that can be absorbed and utilized directly from the rhizosphere (Xu et al., 2012; Pereira and Cushman, 2019).

In higher plants, nitrate is delivered to the shoot via xylem after being absorbed into the root and is then converted to ammonium by nitrate reductase (NR) in the cytoplasm and nitrate reductase (NiR) in plastids before being assimilated to synthesize amino acids (Xu et al., 2012). Nitrate reduction is a highly energy-consuming physiological activity, relying on photosynthesis (Pereira and Cushman, 2019; Ortigosa et al., 2020). Direct ammonium absorption would save photosynthetic products and energy, thereby facilitating plant growth (Hachiya and Sakakibara, 2017). Crassulacean acid metabolism (CAM) is characterized by nocturnal CO_2 uptake and concentration, reduced photorespiration, and increased water-use efficiency when compared to C_3 and C_4 plants (Borland and Griffiths, 1989; Yamori et al., 2014; Pereira et al., 2017). Despite the toxicity effects that ammonium showed for most CAM plants, NH_4^+ can be advantageous for plant growth due to the low energy requirement needed for its assimilation, when compared to NO_3^- and urea (Pereira et al., 2018; De Andrade-Santos et al., 2020). In contrast, nitrate nitrogen can exist in cells at a high concentration and is quickly transported to other tissues for protein synthesis without damaging plant metabolism; however, the absorption of nitrate nitrogen by roots requires more photosynthetic energy than that of ammonium nitrogen (Coskun et al., 2013; Bittsánszky et al., 2015). The preference and availability for a particular form may vary depending on the form and plant's ability to assimilate the element into organic compounds (Da Cunha et al., 2024). Previous studies have demonstrated that plant species exhibit distinct preferences for inorganic nitrogen (N) forms. When both ammonium (NH_4^+) and nitrate (NO_3^-) coexist in the growth environment, most plants display a clear preference for one form over the other. For instance, blueberry and pecan show a tendency to favor NH_4^+ (Zhang et al., 2021; Chen et al., 2023), whereas strawberry, Labiate species, and maize are inclined toward NO_3^- (Tabatabaei et al., 2006; Zhu et al., 2014; Wang et al., 2019). Beyond species-specific preferences, the combined application of NH_4^+ and NO_3^- has also been reported to facilitate N uptake and enhance N use efficiency in cherry rootstocks (Xu et al., 2025).

Pineapple (*Ananas comosus* [L.] Merr. var. *comosus*) is among the five most important tropical fruits in the world. About 90 countries have areas of pineapple cultivation; however, the 10 largest producers account for 70% of total world production (Food and Agriculture Organization of the United Nations, 2023). As a key CAM plant, pineapple growth and productivity heavily depend on nitrogen as an essential macronutrient that directly influences fruit development and yield (Rao et al., 1977; Rodrigues et al., 2014; Darnaudery et al., 2018; Maia et al., 2020; Ma et al., 2022). Previous studies have shown that pineapple probably uses nitrogen in the NH_4^+ , NO_3^- , urea, and glycine forms (Stewart et al., 1925; Sideris et al., 1938; Chen et al., 2018; Chen et al., 2023). Depending on the N form and concentration, the use of sole NH_4^+ or NO_3^- supply with NH_4^+ solution in 2.86 mmol L^{-1} or NO_3^- solution in $11.43 \text{ mmol L}^{-1}$ enhanced the pineapple plant growth; however, other concentrations inhibited the growth of pineapples (Stewart et al., 1925). The growth promotion of pineapple seedlings was also found by foliar application of NH_4^+ or amide nitrogen, relative to the foliar application of NO_3^- (Chen et al., 2018). Studies have shown that pineapple (cultivar Perola) is better adapted to NH_4^+ than organic nitrogen (Endres and Mercier, 2001). In addition, pineapple absorbs ammonium nitrogen at a significantly higher rate than nitrate with nutrient solutions containing either NH_4^+ or NO_3^- as the sole nitrogen source (Sideris et al., 1938). In field-grown pineapple plants, pineapple cultivars preferred to acquire NH_4^+ , followed by glycine, and the uptake rate of NO_3^- was the lowest, using the stable isotope ^{15}N tracer technique (Chen et al., 2023). However, Ravoo (1973) reported that the absorption of NO_3^- from ammonium nitrate culture was higher (60%) than that of NH_4^+ (40%), possibly indicating that pineapple plants prefer NO_3^- to NH_4^+ . Nevertheless, other studies found that mixtures of NH_4^+ and NO_3^- were beneficial for plant growth compared to NH_4^+ or NO_3^- alone (Roosta and Schjoerring, 2008; Hachiya and Sakakibara, 2017; Wang et al., 2019). Therefore, the specific application ratio of NH_4^+ to NO_3^- for pineapple plant growth remains elusive. The present study aimed to investigate the growth and nitrogen assimilation in the pineapple with different NH_4^+ and NO_3^- ratios to identify the nitrogen management capable of increasing the growth response. We hypothesize that low ammonium with high nitrate concentration supply promotes nitrogen absorption and stimulates pineapple growth. Specifically, we investigated the effects of different NH_4^+ and NO_3^- ratios in constant levels of N on plant growth, photosynthetic pigments, root morphology, and carbon and nitrogen accumulation. The findings will provide valuable insights into potential strategies of N management for sustainable pineapple production.

2 Materials and methods

2.1 Plant growth conditions

Plantlets of *Ananas comosus* L. cv. Comte de Paris with uniform weight and appearance (approximately 100 g in weight and 15 cm in height) were selected. Subsequently, the selected plantlets were transferred to 1/2-strength modified Hoagland solution (pH 5.5)

for a 45-day hydroponic pre-culture, with the solution renewed weekly. After pre-culture, plantlets with uniform growth were selected for the nutrient uptake assay. The plantlets were subjected to different $\text{NO}_3^-:\text{NH}_4^+$ ratio treatments under hydroponic conditions. The nutrient solutions were composed of 4 mM nitrogen (with different $\text{NH}_4^+:\text{NO}_3^-$ ratio), 1.0 mM KH_2PO_4 , 2.0 mM K_2SO_4 , 2.0 mM $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 0.1 mM $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ for macro-elements, and 10 μM $\text{MnSO}_4\cdot 4\text{H}_2\text{O}$, 10 μM $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$, 1.0 μM $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, 50 μM H_3BO_3 , 0.5 μM $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$, 0.2 μM $\text{CoSO}_4\cdot 7\text{H}_2\text{O}$, 0.1 mM NaCl and 50 μM Fe-Na-EDTA- $2\text{H}_2\text{O}$ for micro-elements and $\text{Ca}(\text{NO}_3)_2$ for the nitrate source or NH_4Cl for the ammonium source (Sideris et al., 1938). The $\text{NO}_3^-:\text{NH}_4^+$ ratio treatments were 0:100, 30:70, 50:50, 70:30, and 100:0. Each treatment contained 4 replicates. Each replicate included one plant grown in 700 mL of nutrient solution. The pH of the nutrient solution was maintained at $\text{pH } 5.5 \pm 0.1$ with 1 $\text{mmol}\cdot\text{L}^{-1}$ HCl or NaOH. The nutrient solution was renewed every three days. The room temperature was 28°C, the relative humidity was 80%, the photosynthetic photon flux density was 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and the photoperiod was 8h/16h (day/night). The plants were harvested after three months of treatment.

2.2 Ammonium and nitrate uptake rates

The pre-cultured pineapple plantlets as detailed in Section 2.1 were subjected to a 3-day nitrogen starvation treatment, with the culture medium substituted with pure water. Following nitrogen starvation, the roots were soaked in 0.1 mM CaSO_4 for 5 minutes to exchange the ions adhering to them. Following rinsing three times with reverse osmosis water ($\text{NO}_3^- \leq 0.1 \text{ mg}\cdot\text{L}^{-1}$), the roots were immersed in the nutrient solutions corresponding to the aforementioned hydroponic treatments, which contained a fixed total nitrogen concentration of 4 mM with varying $\text{NO}_3^-:\text{NH}_4^+$ ratios (0:100, 30:70, 50:50, 70:30, 100:0) and were supplemented with nitrate as $\text{Ca}(\text{NO}_3)_2$ and ammonium as NH_4Cl . Each pineapple seedling was carefully placed in a 600 mL absorption bottle for an hour from 9:00 to 10:00, with its roots submerged in either 500 mL of nutrient solution. The exterior of the bottle was covered with tin foil to prevent light from affecting the roots. After one hour of reaction, the residual fluid was collected, and the fresh weight of the corresponding roots was measured. The concentrations of ammonium and nitrate retained in the uptake solution were determined using Nessler's reagent spectrophotometry and the sulfonamide colorimetric method, according to the manufacturer's instructions, respectively. The ammonium absorption rate was calculated as,

Nitrogen absorption rate = (initial concentration - sample concentration) $\times$ volume/(absorption time $\times$ root weight).

2.3 Leaf growth, photosynthetic pigment, root morphology, and activity

The D leaf is defined as the youngest physiologically mature leaf on the plant and is also the tallest leaf on the plant. The D leaf width

was measured in the median region of the leaf. The leaf area was measured with a laser area meter (model CI-203, CID Bio-Science, WA, USA). Determination of the photosynthetic pigments content followed the method described by Li et al. (2000).

The root morphology was determined by WinRHIZO Pro (Regent Instruments, QC, Canada) after the root images were scanned with a scanner (Epson Expression 12000XL, Seiko Epson Corporation, Japan). The total root length, root surface area, root volume, and average root diameter were obtained. Fresh and white root samples of each plantlet were collected at the end of the treatment. The root activity was determined through the 2, 3, 5-triphenyltetrazolium chloride method (TTC) using detection kits (Suzhou Comin Biotechnology Co. Ltd., Suzhou, China) following the manufacturer's protocols.

2.4 Carbon and nitrogen concentration

At the end of the treatment, the root was soaked in a 0.1 mM CaSO_4 solution for 5 minutes to exchange the ions adsorbed on the roots. Then, the roots and shoots were harvested. After drying at 105°C for 30 minutes, the shoot and root were dried at 80°C to constant weight. The dry biomass was ground using a ball mill and then passed through a 0.425 mm sieve. The carbon and nitrogen concentrations were determined by an element analyzer (vario PYRO cube, Elementar Analysensysteme GmbH, Germany).

2.5 Statistical analysis

Statistical analyses were performed using SPSS 25.0, and the differences among different treatments were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's *post hoc* multiple comparisons tests ($P < 0.05$). Figures were drawn by Origin 2021.

3 Results

3.1 Leaf growth

The $\text{NO}_3^-:\text{NH}_4^+$ ratios significantly affected the total leaf area of pineapple (Figure 1D). With the increase of nitrate ratio in the nutrition solution culture, the leaf area increased initially and then decreased. The maximum leaf area was found in the treatment with $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 (Figure 1D). Compared with the supply of ammonium nitrogen alone, the leaf area increased by 22.36%, 19.81%, 61.10% and 46.52% (Figure 1D) in the 30:70, 50:50, 70:30, and 100:0, respectively. In addition, the $\text{NO}_3^-:\text{NH}_4^+$ ratio had a significant influence on the leaf number (Figure 1C) and the specific leaf area (Figure 1E), but it showed no significant effect on the D leaf length and width of pineapple (Figures 1A, B). Compared with the supply of sole ammonium nitrogen, the leaf number increased by 15.69%, 7.84%, 17.65% and 17.65% (Figure 1C), and the specific leaf area increased by 15.63%, 22.49%, 29.29% and 29.53% (Figure 1E) in the 30:70, 50:50, 70:30, and 100:0, respectively.

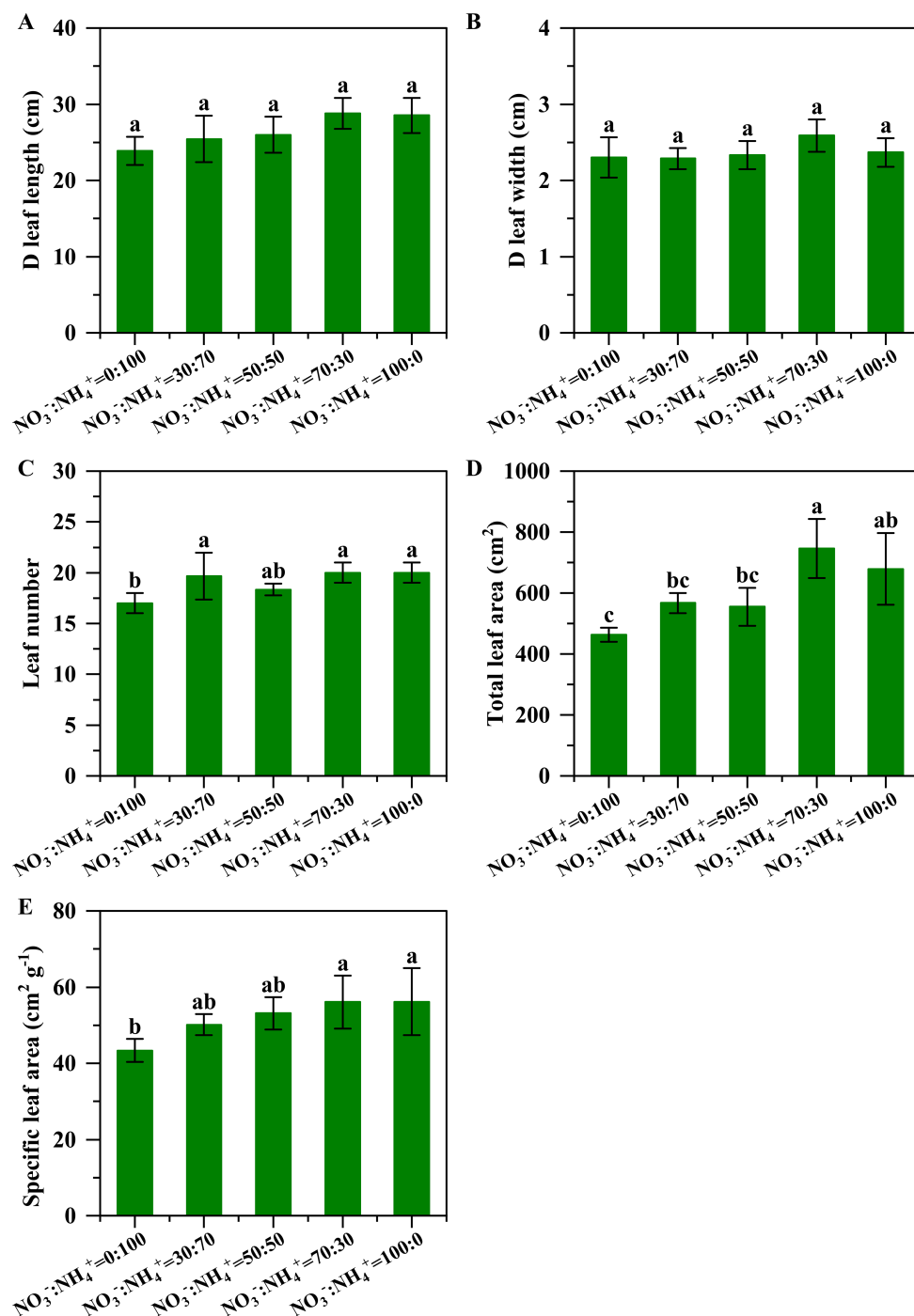


FIGURE 1

Leaf growth of pineapple under different NO₃⁻: NH₄⁺ ratio treatments. The D leaf length (A), D leaf width (B), leaf number (C), total leaf area (D), and specific leaf area (E). The different letters above the columns represent significant differences between treatments ($P < 0.05$).

3.2 Leaf photosynthetic pigments

The contents of chlorophylls a and b showed an upward trend with increasing nitrate proportions (Figure 2A). The NO₃⁻: NH₄⁺ ratios of

50:50 and 70:30 significantly increased the contents of chlorophyll a and b. The content of carotenoids was the highest under the sole ammonium treatment, followed by the treatment with NO₃⁻: NH₄⁺ ratio of 70:30, compared with other treatments (Figure 2B).

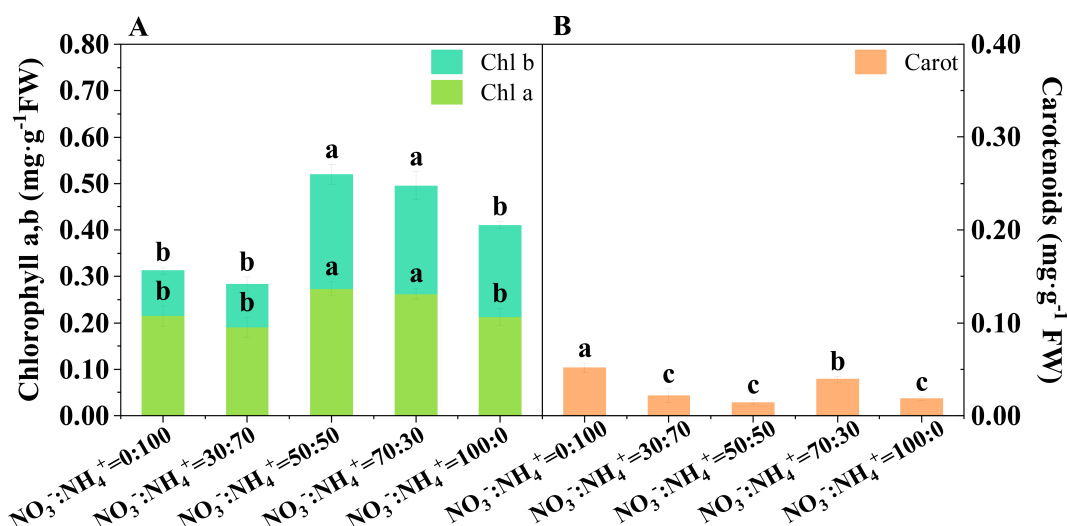


FIGURE 2

Photosynthetic pigments concentration in the D leaves of *Ananas comosus* var. comte de Paris for three months with different NO₃⁻: NH₄⁺ ratio treatments. The chlorophyll a and b (A) and carotenoids (B). Different letters indicate significant statistical differences (P<0.05).

3.3 Root morphology and activity

With the increase of nitrate proportions, the length, surface area, volume, and average diameter of the root firstly decreased and then increased (Figure 3). The maximum root volume and average diameter were observed under the NO₃⁻: NH₄⁺ ratio of 70:30. Compared to the treatment with the supply of only ammonium nitrogen, the root length changed by -4.85%, -19.68%, 15.32% and 25.58% (Figure 3A), the total root surface area varied by -6.47%, -14.56%, 12.35% and 20.73% (Figure 3B), the total root volume altered by -21.80%, -12.81%, 25.83% and 20.76% (Figure 3C), and the total root average diameter modified by -12.06%, -6.05%, 1.59% and -3.14% (Figure 3D) with the increase of nitrate proportion to 30%, 50%, 70%, and 100%, respectively. With the decrease of the ammonium ratio, the root activity of pineapple was obviously decreased and then increased, and the root activity was highest when the ammonium was supplied alone (Figure 4).

3.4 Ammonium and nitrate uptake rates

With the decreasing proportion of ammonium, the absorption rates of ammonium decreased. However, the absorption rates of nitrate first increased and then decreased. The maximum absorption rate of nitrate was observed under the NO₃⁻: NH₄⁺ ratio of 70:30 (Figure 5A), while the highest absorption rate of ammonium was found in the only ammonium supplied treatment (Figure 5B).

3.5 Biomass accumulation

With the increase of nitrate proportion, the biomass firstly decreased and then increased. The highest dry weights of pineapple

leaf and stem were noted in the NO₃⁻: NH₄⁺ ratio of 70:30 treatment (Figure 6A). Pineapple in the treatment with the NO₃⁻: NH₄⁺ ratio of 50:50 showed the lowest dry weight of leaf and stem. Significantly more dry matter was partitioned into the shoots with the increase of nitrate proportion (Figure 6B).

3.6 Plant C and N concentration and accumulation

The leaf N concentration was similar among all the treatments (Figure 7A). However, the specific leaf N content of pineapple plants decreased with the increasing nitrate proportion, with the lowest specific leaf N content found in the only nitrate treatment (Figure 7B). The carbon and nitrogen accumulation firstly decreased and then increased with increasing nitrate proportions (Figures 7C, D). The treatment with NO₃⁻: NH₄⁺ ratio of 70:30 had the maximum carbon and nitrogen accumulation. Compared with only ammonium treatment, increasing the nitrate proportions improved the nitrogen accumulation by 9.93%, 3.06%, 19.49% and 5.68% (Figure 7C), and the carbon accumulation by 3.77%, -6.26%, 21.62% and 8.63% (Figure 7D), respectively. There was a significant positive linear relationship between the nitrogen and carbon accumulation (Figure 8).

4 Discussion

Appropriate NO₃⁻: NH₄⁺ ratios promoting plant growth has been reported for various plant species such as the optimal NO₃⁻: NH₄⁺ ratio of 17: 83 for blueberry (Zhang et al., 2021); 25: 75 for pecan (Chen et al., 2023); 50:50 for coffee (Carr et al., 2020) and yellow passion fruit (Da Cunha et al., 2024) as well as 75:25 for strawberry, Labiatae and maize (Tabatabaei et al., 2006; Zhu et al.,

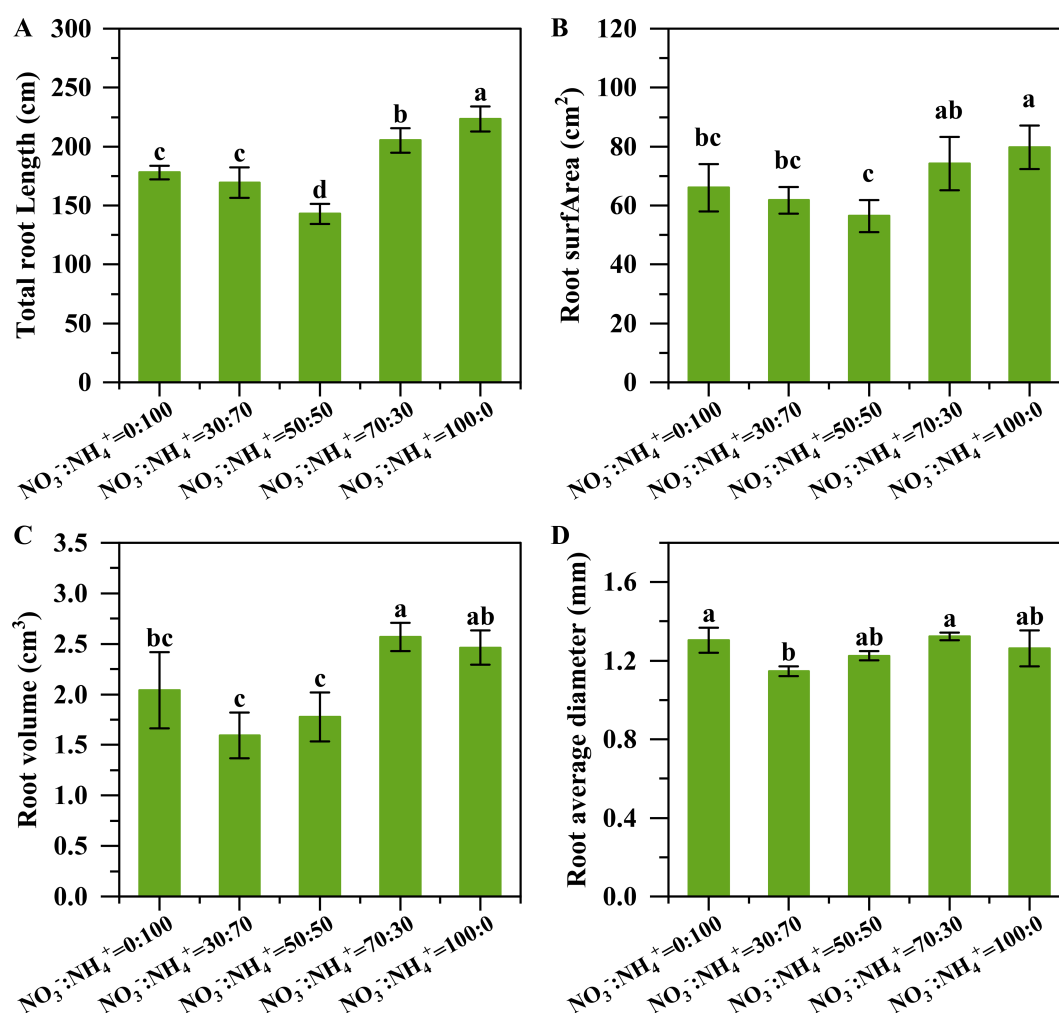


FIGURE 3

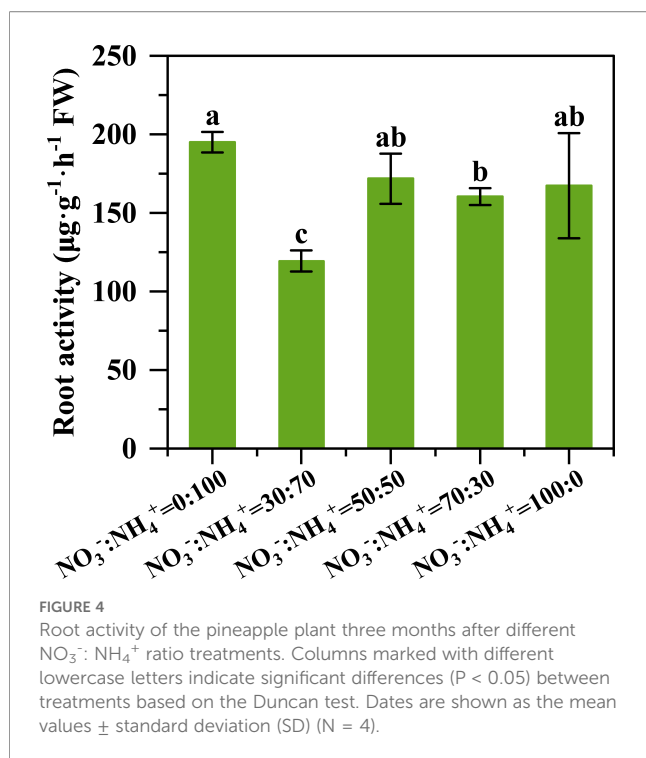
Root morphology of pineapple under different $\text{NO}_3^-:\text{NH}_4^+$ ratio treatments. The root total length (A), root total surface area (B), root volume (C), and root average diameter (D). Different lowercase letters above the columns represent significant differences between treatments ($P < 0.05$).

2014; Wang et al., 2019). In this study, the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 treatment achieved the highest biomass, carbon, and nitrogen accumulation, showing a significant improvement compared with other ratios involving the sole application of either ammonium or nitrate (Figure 6).

The $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 treatment improved the root growth and nitrate uptake ability, contributing to enhancing nitrogen assimilation. The root morphology plays an essential role in nitrogen uptake (Meister et al., 2014). Numerous studies have shown that an appropriate $\text{NO}_3^-:\text{NH}_4^+$ ratio can promote the formation of better root morphology (Tabatabaei et al., 2006; Hu et al., 2017; Liu et al., 2017; Wang et al., 2019; Carr et al., 2020; Zhang et al., 2021). Our results revealed that when the proportion of nitrate nitrogen exceeded 50%, the total root length and surface area of pineapple plants increased significantly (Figure 3), leading to a larger absorption area, thereby enhancing nitrogen uptake. Such responses were confirmed again by the leaf N concentration that was not reduced by the dilution effect, but significantly higher plant

N and C accumulation (Figures 7C, D). Nutrient uptake of plants is determined by both the root system that can contact the nutrients and the root assimilation ability. In the current study, we found that pineapple roots had the strongest NO_3^- absorption rate when supplied with the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30, which consequently promoted nitrogen uptake under this treatment. It was noteworthy that the NH_4^+ absorption rate decreased significantly along with the decreasing NH_4^+ ratios (Figure 5). A previous study with maize noted the maximum nitrate absorption rate under the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 75:25 (Wang et al., 2019; Yang et al., 2025).

The improved plant nitrogen uptake due to increased root growth and enhanced root NO_3^- absorption rate of pineapple can enhance the synthesis of chlorophylls and carotenoids (Bassi et al., 2018; Wen et al., 2019). In the current study, the content of chlorophyll a and b in the leaves of the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 supply treatment was significantly higher than that of the ammonium and nitrate supply treatments alone. Thus, the photosynthetic capacity of leaves is expected to be



significantly enhanced, enabling the synthesis of more sugars (Bassi et al., 2018).

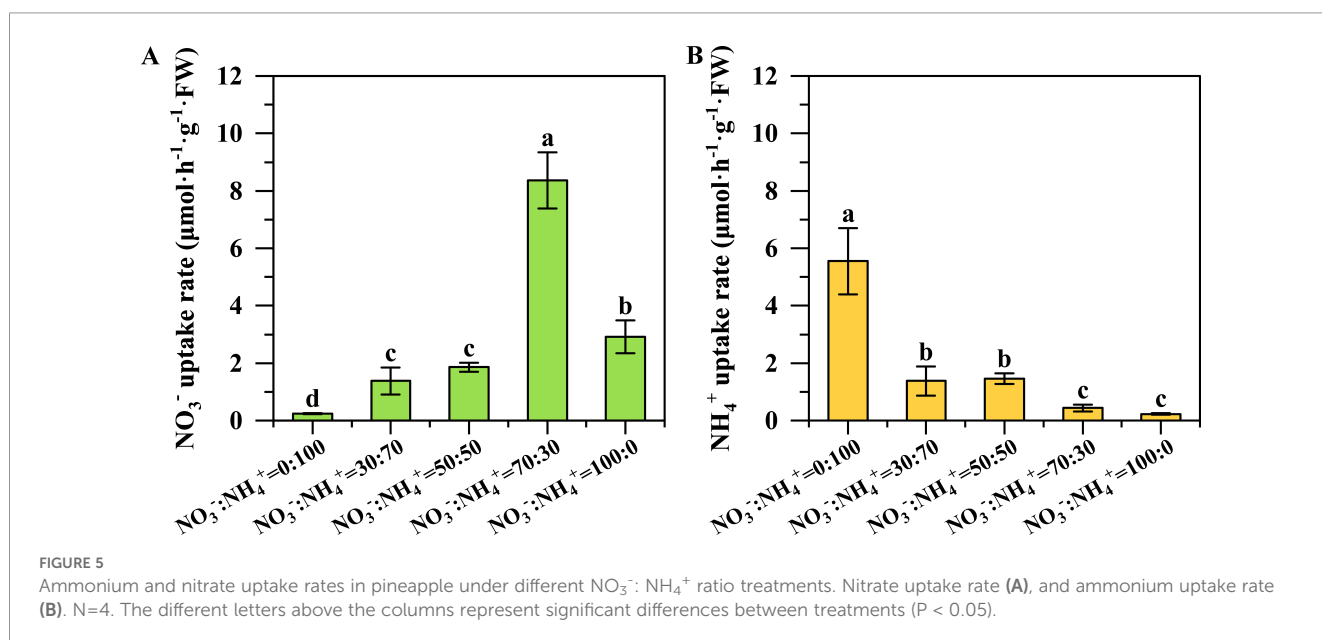
The increased root growth and N uptake rate can facilitate leaf expansion. This study observed that the leaf area of the pineapple plants in the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 supply treatment significantly increased by 61.10%, whereas the supply of ammonium alone inhibited the growth of leaves (Figure 1D). Plant leaf area is a key factor determining the dry matter accumulation of pineapples, as it is directly related to photosynthesis and photosynthate fixation (An et al., 2022). Corresponding to this leaf area enlargement, the leaf dry matter

content was significantly enhanced by 24.40% in this treatment (Figure 6A), which aligned with the positive correlation between leaf area and dry matter accumulation.

Different NH_4^+ and NO_3^- ratios significantly altered the growth and nitrogen assimilation of pineapple in hydroponic systems, these variations further affected plant's nutrient uptake efficiency. It is well-documented that soil N transformation processes (e.g., nitrification, denitrification) and associated microbial activities regulate nitrogen availability in field environments, thereby mediating plant nitrogen acquisition (Zhang et al., 2018; Fu et al., 2025). However, our current study did not measure these soil N transformation dynamics or account for the role of microbial activities, as the hydroponic system lacks the soil matrix required for such processes. Therefore, future research should focus on validating our findings through pot experiments using field soils and *in situ* field trials. These studies should explicitly incorporate analyses of nitrogen cycling dynamics to clarify how soil-specific factors modulate the effects of $\text{NH}_4^+/\text{NO}_3^-$ ratios on plant performance, thereby enhancing the practical applicability of our results.

5 Conclusions

Supplying a low ammonium with a high nitrate concentration promoted nitrogen absorption and stimulated pineapple growth. Pineapple plants grown with $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 exhibited the highest biomass, as well as carbon and nitrogen accumulation, compared with other treatments (including the sole ammonium, sole nitrate, and other mixed nitrogen ratios). Specifically, the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 enhanced pineapple root growth and nitrate uptake ability, thereby promoted nitrogen assimilation. Furthermore, the concentrations of chlorophyll a and b in leaves of the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 treatment were significantly higher than those in the sole ammonium and sole nitrate treatments,



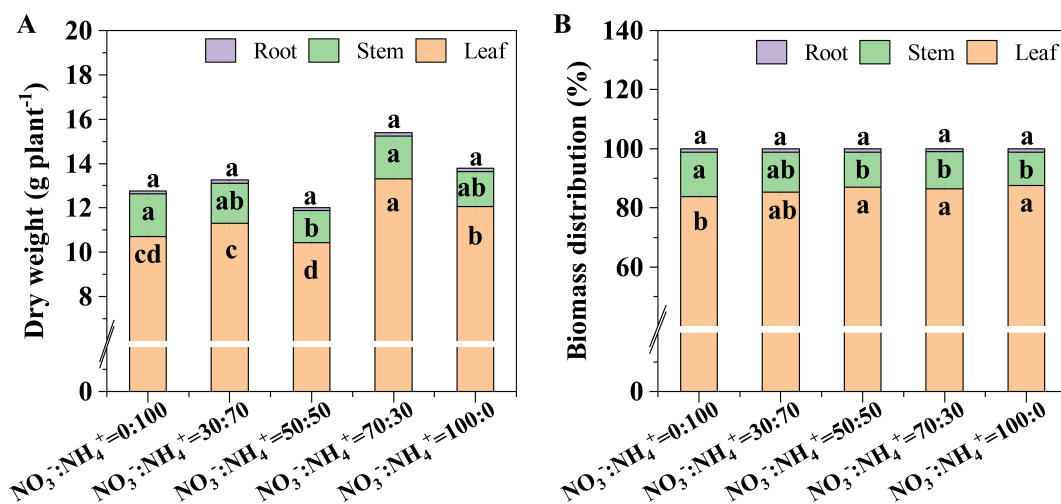


FIGURE 6

The biomass accumulation and distribution in pineapple that received three months of different NO₃⁻: NH₄⁺ ratio treatments. The dry weight (A) and biomass distribution (B). N = 4. The different letters above the columns represent significant differences between treatments (P<0.05).

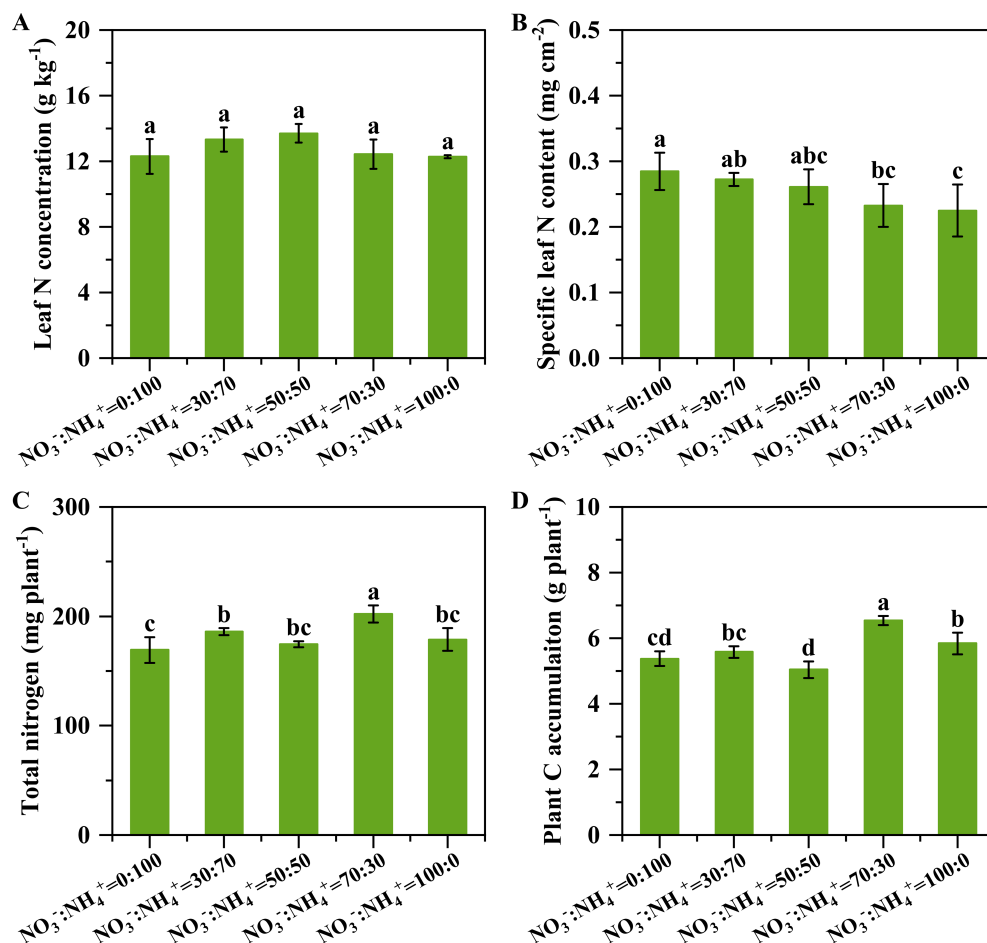
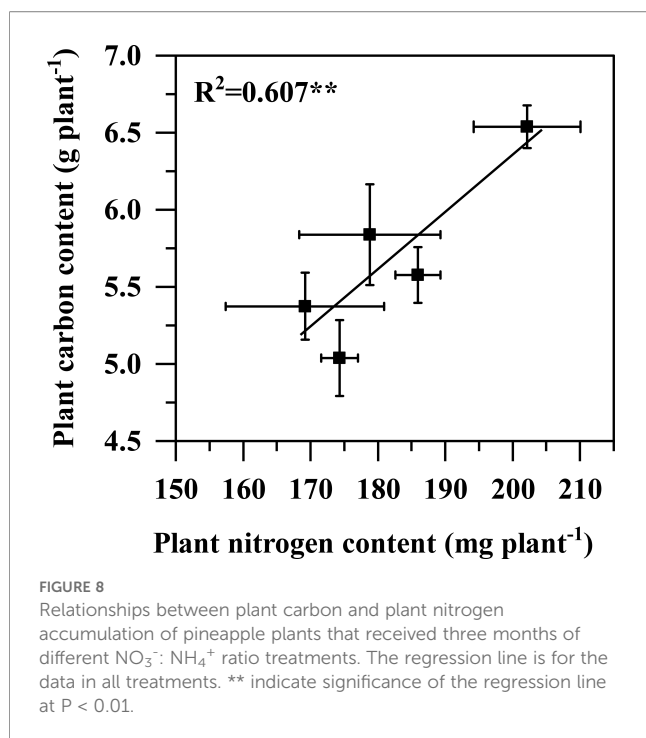


FIGURE 7

Leaf carbon and nitrogen concentration and accumulation in pineapple that received three months of different NO₃⁻: NH₄⁺ ratio treatments. The leaf N concentration (A), specific leaf N content (B), total nitrogen accumulation (C) and plant C accumulation (D). N = 4. The different letters above the columns represent significant differences between treatments (P<0.05).



indicating enhanced photosynthetic capacity of pineapple leaves and thereby facilitating the synthesis of more sugars. Consequently, leaf area expansion in pineapple plants under the $\text{NO}_3^-:\text{NH}_4^+$ ratio of 70:30 treatment was promoted. Corresponding to this leaf area enlargement, the leaf dry matter content in this treatment was significantly increased by 24.40%. The results suggest 70:30 as the optimum $\text{NO}_3^-:\text{NH}_4^+$ ratio, which can enhance the root and shoot growth, and C and N accumulation of pineapple.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

HM: Data curation, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. SL: Investigation, Software, Writing – review & editing. QZ: Data curation, Investigation, Writing – original draft. YL: Software,

Writing – review & editing. SS: Software, Writing – review & editing. LS: Data curation, Software, Writing – review & editing. HL: Data curation, Writing – review & editing. WS: Supervision, Validation, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

- An, D. S., Liu, Y. N., Yan, C. M., Zhao, B. S., Li, H. R., and Dou, M. A. (2022). Effect of supplementary fertigation on pineapple growth, yield and fertigation production. *Chin. J. Trop. Crops* 43, 1166–1173. doi: 10.3969/j.issn.1000-2561.2022.06.009
- Bassi, D., Menossi, M., and Mattiello, L. (2018). Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. *Sci. Rep.* 8, 1–13. doi: 10.1038/s41598-018-20653-1

- Bittsánszky, A., Pilinszky, K., Gyulai, G., and Komives, T. (2015). Overcoming ammonium toxicity. *Plant Sci.* 231, 184–190. doi: 10.1016/j.plantsci.2014.12.005
- Borland, A. M., and Griffiths, H. (1989). The regulation of citric acid accumulation and carbon recycling during CAM in *Ananas comosus*. *J. Exp. Bot.* 40, 53–60. doi: 10.1093/jxb/40.1.53
- Carr, N. F., Boaretto, R. M., and Mattos, D. (2020). Coffee seedlings growth under varied $\text{NH}_4^+:\text{NO}_3^-$ ratio: Consequences for nitrogen metabolism, amino acids profile, and regulation of plasma membrane H^+ -ATPase. *Plant Physiol. Biochem.* 154, 11–20. doi: 10.1016/j.plaphy.2020.04.042
- Chen, X., Xu, X., Fu, L., Pan, Y., Feng, Y., and Cai, Z. (2023). Nitrogen acquirement strategy of different nitrogen forms in two pineapple cultivars. *Chin. J. Eco-Agric.* 31, 895–903. doi: 10.12357/cjca.20220857
- Chen, J., Xu, M. G., Sun, G. M., Shi, W. Q., Xian, A. M., and Ma, H. Y. (2018). Effect of nitrate nitrogen on inhibiting the vegetative growth of pineapple. *Soil Fert. Sci. China* 4, 82–86. doi: 10.11838/sfsc.20180413
- Chen, M., Zhu, K., Xie, J., Liu, J., Qiao, Z., Tan, P., et al. (2023). Ammonium-nitrate mixtures dominated by NH_4^+ -N promote the growth of pecan (*Carya illinoensis*) through enhanced N uptake and assimilation. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1186818
- Coskun, D., Britto, D. T., Li, M., Becker, A., and Kronzucker, H. J. (2013). Rapid ammonia gas transport accounts for futile transmembrane cycling under $\text{NH}_3/\text{NH}_4^+$ to xicity in plant roots. *Plant Physiol.* 163, 1859–1867. doi: 10.1104/pp.113.225961
- Da Cunha, J. G., Da Silva, R. F., Miranda, R. D. S., Pereira, G. A., De Araújo Costa, E. W., Da Silva, J. F., et al. (2024). Ammonium and nitrate distinctly regulate photosynthetic and growth performance, eliciting antagonistic responses in yellow passion fruit plants. *Sci. Hortic.* 330, 113022. doi: 10.1016/j.scienta.2024.113022
- Darnaudery, M., Fournier, P., and Léchaudel, M. (2018). Low-input pineapple crops with high quality fruit: promising impacts of locally integrated and organic fertilization compared to chemical fertilisers. *Exp. Agric.* 54, 286–302. doi: 10.1017/S0014479716000284
- De Andrade-Santos, S. V., Young, J. L. M., Ramiro, L. K., Suzuki, R. M., Souza, S. R., and Tamaki, V. (2020). Effect of nitrate concentration on plant growth and anthocyanin accumulation in the ornamental bromeliad *Alcantarea imperialis*. *J. Plant Nutr.* 44, 181–195. doi: 10.1080/01904167.2020.1806300
- Endres, L., and Mercier, H. (2001). Ammonium and urea as nitrogen sources for bromeliads. *J. Plant Physiol.* 158, 205–212. doi: 10.1078/0176-1617-00170
- Food and Agriculture Organization of the United Nations (2023). *Crops and livestock products*. Available online at: <https://www.fao.org/faostat/en/data/QCL> (Accessed June 18, 2025).
- Fu, Y., Ren, X., and Zhu, B. (2025). Responses of soil nitrogen transformation and N_2O emission to soil pH and hydrothermal changes. *Agronomy* 15, 1005. doi: 10.3390/agronomy15051005
- Hachiya, T., and Sakakibara, H. (2017). Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *J. Exp. Bot.* 68, 2501–2512. doi: 10.1093/jxb/erw449
- Hu, L., Liao, W., Dawuda, M. M., Yu, J., and Lv, J. (2017). Appropriate $\text{NH}_4^+:\text{NO}_3^-$ ratio improves low light tolerance of mini Chinese cabbage seedlings. *BMC Plant Biol.* 17, 1–14. doi: 10.1186/s12870-017-0976-8
- Li, T., Li, W., Yi, J., Wang, Y., Yu, C., Rees, R. M., et al. (2025). A multi-level integration framework for sustainable nitrogen management in tropical agriculture. *One Earth* 8, 101308. doi: 10.1016/j.oneear.2025.101308
- Li, H. S., Sun, Q., Zhao, S. J., and Zhang, W. H. (2000). *Principles and techniques of plant physiological biochemical experiment (in Chinese)* (Beijing: Higher Education Press).
- Liu, G. Y., Du, Q. J., and Li, J. M. (2017). Interactive effects of nitrate-ammonium ratios and temperatures on growth, photosynthesis, and nitrogen metabolism of tomato seedlings. *Sci. Hortic.* 214, 41–50. doi: 10.1016/j.scienta.2016.09.006
- Ma, H. Y., Li, L., Liu, S., Shi, W., Wang, C., Zhao, Q., et al. (2022). Physiological response, phytohormone signaling, biomass production and water use efficiency of the CAM plant *Ananas comosus* under different water and nitrogen regimes. *Agric. Water Manag.* 266, 107563. doi: 10.1016/j.agwat.2022.107563
- Maia, V. M., Pegoraro, R. F., Aspiazú, I., Oliveira, F. S., and Nobre, D. A. C. (2020). “Chapter 50 - Diagnosis and management of nutrient constraints in pineapple,” in *Fruit Crops*. Eds. A. K. Srivastava and C. Hu (Amsterdam, Netherlands: Elsevier), 739–760. doi: 10.1016/B978-0-12-818732-6.00050-2
- Marschner, P. (2012). *Marschner's Mineral Nutrition of Higher Plants. 3rd Edition* (Cambridge: Academic Press), 135.
- Meister, R., Rajani, M., Ruzicka, D., and Schachtman, D. P. (2014). Challenges of modifying root traits in crops for agriculture. *Trends Plant Sci.* 19, 779–788. doi: 10.1016/j.tplants.2014.08.005
- Ortigosa, F., Valderrama-Martín, J. M., Urbano-Gómez, J. A., García-Martín, M. L., Ávila, C., Cañas, F. M., et al. (2020). Inorganic nitrogen form determines nutrient allocation and metabolic responses in maritime pine seedlings. *Plants* 9, 481. doi: 10.3390/plants9040481
- Pereira, P. N., and Cushman, J. C. (2019). Exploring the relationship between Crassulacean Acid Metabolism (CAM) and mineral nutrition with a special focus on nitrogen. *Int. J. Mol. Sci.* 20, 4363. doi: 10.3390/ijms20184363
- Pereira, P. N., Gaspar, M., Smith, J. A. C., and Mercier, H. (2018). Ammonium intensifies CAM photosynthesis and counteracts drought effects by increasing malate transport and antioxidant capacity in *Guzmania monostachia*. *J. Exp. Bot.* 69, 1993–2003. doi: 10.1093/jxb/ery054
- Pereira, P. N., Smith, J. A. C., and Mercier, H. (2017). Nitrate enhancement of CAM activity in two *Kalanchoë* species is associated with increased vacuolar proton transport capacity. *Physiol. Plant* 160, 361–372. doi: 10.1111/ppl.12578
- Rao, M. H., Subramanian, T. R., Murthy, H. K. S., Singh, H. P., Dass, H. C., and Ganapathy, K. M. (1977). Leaf nitrogen status as influenced by varying levels of nitrogen application and its relationship with yield in ‘Kew’ pineapple. *Sci. Hortic.* 7, 137–142. doi: 10.1016/0304-4238(77)90053-X
- Ravoo, A. A. (1973). Effects of root temperatures and nitrogen carriers on nutrient uptake, growth, and composition of pineapple plants, *Ananas comosus* (L.) Merr. University of Hawaii at Manoa:Honolulu.
- Rodrigues, M. A., Freschi, L., Pereira, P. N., and Mercier, H. (2014). “Interactions between nutrients and crassulacean acid metabolism,” in *Progress in Botany*, vol. 75. Eds. U. Lüttge, W. Beyschlag and J. Cushman (Springer, Berlin, Heidelberg), 167–186. doi: 10.1007/978-3-642-38797-5_6
- Roosta, H. R., and Schjoerring, J. K. (2008). Effects of nitrate and potassium on ammonium toxicity in cucumber plants. *J. Plant Nutr.* 31, 1270–1283. doi: 10.1080/01904160802135050
- Sideris, C. P., Krauss, B. H., and Young, H. Y. (1938). Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. *Plant Physiol.* 13, 489. doi: 10.1104/pp.13.3.489
- Stewart, G. R., Thomas, E. C., and Horner, J. (1925). The comparative growth of pineapple plants with ammonia and nitrate nitrogen. *Soil Sci.* 20, 227–242. doi: 10.1097/00010694-192509000-00002
- Tabatabaei, S. J., Fatemi, L. S., and Fallahi, E. (2006). Effect of ammonium: nitrate ratio on yield, calcium concentration, and photosynthesis rate in strawberry. *J. Plant Nutr.* 29, 1273–1285. doi: 10.1080/019041606006767575
- Wang, P., Wang, Z. K., Pan, Q. C., Sun, X. C., Chen, H., Chen, F. J., et al. (2019). Increased biomass accumulation in maize grown in mixed nitrogen supply is mediated by auxin synthesis. *J. Exp. Bot.* 70, 1859–1873. doi: 10.1093/jxb/erz047
- Wen, B. B., Li, C., Fu, X. L., Li, D. M., Li, L., Chen, X. D., et al. (2019). Effects of nitrate deficiency on nitrate assimilation and chlorophyll synthesis of detached apple leaves. *Plant Physiol. Biochem.* 142, 363–371. doi: 10.1016/j.plaphy.2019.07.007
- Xu, G., Fan, X., and Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. *Annu. Rev. Plant Biol.* 63, 153–182. doi: 10.1146/annurev-arplant-042811-105532
- Xu, X., Tang, Y., He, L., Sun, Y., Liu, D., Wang, Y., et al. (2025). Mixed nitrogen forms enhance growth and photosynthetic nitrogen use efficiency by optimizing nitrogen metabolism and leaf N allocation in Gisela 6 cherry rootstock. *Front. Plant Sci.* 16. doi: 10.3389/fpls.2025.1696713
- Yamori, W., Hikosaka, K., and Way, D. A. (2014). Temperature response of photosynthesis in C3, C4, and CAM plants: Temperature acclimation and temperature adaptation. *Photosynth. Res.* 119, 101–117. doi: 10.1007/s11120-013-9874-6
- Yang, Z. Q., Yan, H. F., Liu, H. W., Yang, L., Mi, G. H., and Wang, P. (2025). Enhancing crop nitrogen efficiency: The role of mixed nitrate and ammonium supply in plant growth and development. *Biology* 14, 546. doi: 10.3390/biology14050546
- Zhang, Y., Ding, H., Zheng, X., Cai, Z., Misselbrook, T., Carswell, A., et al. (2018). Soil N transformation mechanisms can effectively conserve N in soil under saturated conditions compared to unsaturated conditions in subtropical China. *Biol. Fertil. Soils* 54, 495–507. doi: 10.1007/s00374-018-1276-7
- Zhang, Y. Y., Tian, J. P., Cui, J., Hong, Y. H., and Luan, Y. S. (2021). Effects of different $\text{NH}_4^+/\text{NO}_3^-$ ratios on the photosynthetic and physiological responses of blueberry (*Vaccinium* spp.) seedlings growth. *J. Plant Nutr.* 44, 854–864. doi: 10.1080/01904167.2021.1871754
- Zhu, Z. B., Yu, M. M., Chen, Y. H., Guo, Q. S., Zhang, L. X., Shi, H. Z., et al. (2014). Effects of ammonium to nitrate ratio on growth, nitrogen metabolism, photosynthetic efficiency and bioactive phytochemical production of *Prunella vulgaris*. *Pharm. Biol.* 52, 1518–1525. doi: 10.3109/13880209.2014.902081