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Nitrogen Source Preference in Maize at Seedling Stage Is Mainly Dependent on Growth Medium pH

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Abstract: To improve crop nitrogen recovery efficiency (NRE), plants must be supplied with their preferred form of nitrogen (N). However, whether pH affects crop N-form preference remains unclear. Here, we aimed to explore how maize (*Zea mays* L.) preference for NH_4^+ and NO_3^- is affected by pH and to determine the critical pH controlling this preference. Maize plants were grown with NH_4^+ or NO_3^- in different soils (pH 4.32–8.14) and nutrient solutions (pH 4.00–8.00). After harvest, plant dry weights, N content, N uptake, NRE, soil pH, and exchangeable aluminum (Al) were measured. Compared with the effect of NO_3^-, NH_4^+ decreased maize dry weight, N uptake, and NRE by 28–94% at soil pHs of 4.32 and 4.36 and a solution pH of 4.00, whereas it increased these parameters by 10–88% at soil pHs of 6.52–8.02 and solution pHs of 7.00 and 8.00. NO_3^- increased soil pH and decreased soil exchangeable Al content at soil pHs of 4.32–6.68. Critical soil and solution pHs for changing plant growth and N uptake preference for NH_4^+ vs. NO_3^- ranged from 5.08 to 5.40 and from 5.50 to 6.59, respectively. In conclusion, the preference of maize seedling growth and N uptake for NH_4^+ vs. NO_3^- mainly depends on the pH of the growth medium, and maize seedlings generally prefer NO_3^- in strongly acid soils but NH_4^+ in neutral to alkaline soils.

Keywords: ammonium; nitrate; critical pH; nitrogen recovery efficiency; nitrogen uptake

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1. Introduction

Maize (*Zea mays* L.) is one of the world's major food and feed crops and is very important for food security. Nitrogen (N) is a macro-element nutrient essential for plant growth. Maize grain yield is highly dependent on supplemental N, and an estimated 80 kg/ha of N in the form of N fertilizer is applied annually during maize production worldwide [1]. Only 25% to 50% of applied N fertilizer is taken up by maize plants, with at least half of the applied amount lost to groundwater or emitted as gaseous compounds into the atmosphere [2]. The resulting low N recovery efficiency (NRE), which is the fraction of N fertilizer recovered by maize, leads to a series of environmental problems, including groundwater contamination, freshwater eutrophication, greenhouse gas emissions, and soil acidification [3]. Improving NRE is one of the most effective ways to increase crop productivity while reducing environmental degradation caused by N fertilizer input [3].

Studies have shown that plant biomass, N uptake, and NRE are increased when crop plants are supplied with their preferred form of N [4,5]. Ammonium (NH_4^+) and nitrate (NO_3^-) are the two main forms of inorganic N that can be directly taken up and used by plants. Previous studies have shown that different plant species have different responses to supplementation with NH_4^+ or NO_3^- [6,7]. Kováčik et al. [8] reported that the yield parameters of radish (*Raphanus sativus* var. *radicula* Pers.) were more dependent on the contents of NO_3^- than those of NH_4^+ occurring in the soil substrate. Compared with the effect of NO_3^-, for example, supplementation with NH_4^+ promotes the growth of highbush blueberry (*Vaccinium corymbosum* L.) [9], tea (*Camellia sinensis* L.) [10], and rice (*Oryza sativa* L. subsp. *japonica*) [11], which indicates that these plant species have a growth preference for NH_4^+ over NO_3^-. In contrast, the growth of wheat (*Triticum aestivum* L.) [12,13], barley (*Hordeum vulgare* L.) [14], pepper (*Capsicum annuum* L.) [15], and strawberry (Fragaria × ananassa Duch.) [9] is significantly inhibited when plants are fed with NH_4^+ compared with supplementation with NO_3^-, thus revealing the growth preference of these plant species for NO_3^- over NH_4^+.

Maize is typically grown in well-aerated dryland soils, in which NO_3^- is the major N source for plants. Maize is thus usually considered to be a traditional NO_3^--preferring plant. However, extensive studies on the response of maize growth to NH_4^+ and NO_3^- have yielded inconsistent results under different pH conditions. Maize plants grown in acidic soils (pHs of 4.60 and 4.54) have been found to have greater root and shoot biomass under NO_3^- than NH_4^+ [16,17]. Al toxicity is the primary factor limiting crop productivity in acidic soils [7,11]. The growth preference of maize plants for NO_3^- over NH_4^+ in acidic soils was attributed to NO_3^--alleviated Al toxicity since NO_3^- relative to NH_4^+ increased soil pH and decreased soil exchangeable Al concentrations [17]. In contrast, maize has been reported to exhibit a growth preference for NH_4^+ over NO_3^- in pH 6.50 soil and solution culture [18,19]. Similarly, the growth of maize is enhanced by NO_3^- compared with NH_4^+ in a pH 5.50 solution, whereas NH_4^+ has a more beneficial effect than NO_3^- in soil at pH 6.40 [20]. Maize plants have been found to have a relative preference for NO_3^- in pH 4.30 soil, with NH_4^+ preferred in pH 5.50 and 7.10 soils [21]. No significant difference in maize dry weight between NH_4^+ and NO_3^- treatments has been observed at a soil pH of 4.9 [22]. As is well known, plants generally excrete excess H^+ and thus exacerbate acidification of the rhizosphere during NH_4^+ uptake, whereas NO_3^- uptake by plants requires the release of excessive anions (such as OH^-), which alkalifies the rhizosphere. Given that the supply of NH_4^+ and NO_3^- can alter the overall cation–anion relationship that influences alkali or acid production [23], the pH of the growth medium can be an important factor affecting the response of plant growth and N uptake to NH_4^+ and NO_3^-.

Although several studies, as described above, have hinted that the preferred N form of maize plants varies under acidic and alkaline conditions, complete information is still lacking on how maize growth and N uptake respond to NH_4^+ and NO_3^- under the changing kinetics of different pH levels. Furthermore, the critical pH for this response is also unknown. In this study, we therefore examined the response of maize growth, N uptake, and NRE to NH_4^+ and NO_3^- over a range of pH values through soil culture and hydroponic experiments. The objectives of the present study were two-fold: (1) to explore the relationship between growth-medium pH and the preference of maize for NH_4^+ and NO_3^- and (2) to determine the critical pH value at which maize N-source preference is altered and assess the contribution of such N preference to NRE. Our results should serve as a useful reference for choosing the appropriate form of N fertilizer according to soil pH, thereby improving the N-fertilizer use efficiency and growth of maize plants.

2. Materials and Methods

2.1. Soil Collection

Soils with pH values of 4.32–8.14 were collected for a soil culture experiment from different sites in China as follows: (1) three samples of Aric-Ferric Luvisols from a rapeseed field (pH 4.32), a pine forest (pH 4.36), and a paddy field (pH 5.02) in Yingtan; (2) three samples of Dystric gleysols from three fallow paddy fields in Nanjing; (3) one sample of Eum-Orthic Anthrosol from a winter wheat field in Yangling; (4) one sample of Haplic Kastanozems from a maize field in Baotou; and (5) one sample of Calcic Solonetz from a wheat field in Fengqiu. The experimental soils were collected down to a depth of approximately 20 cm with a shovel. All samples were mixed individually to ensure soil homogeneity. A part of mixed soil samples was taken out for determination of basic chemical properties, and the rest was air dried, pulverized, and filtered through a 4 mm mesh for a soil culture experiment. Basic information on the experimental soil samples is provided in Table 1.

Table 1. Basic information on experimental soils.

City	Sampling Site	pH	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)
Yingtan	116°55′48″ E 28°07′44″ N	4.32	12.48	9.70	7.24	168.14
	116°55′48″ E 28°12′07″ N	4.36	4.70	3.13	0.08	54.57
	116°55′37″ E 31°43′14″ N	5.02	19.37	19.13	12.75	124.74
Nanjing	118°46′37″ E 31°15′38″ N	6.52	4.37	3.13	19.49	104.81
	118°46′37″ E 31°05′27″ N	6.68	6.37	3.00	13.77	143.31
	118°46′37″ E 31°05′27″ N	6.91	4.97	3.07	66.16	147.22
Yangling	108°04′02″ E 34°15′48″ N	7.90	3.36	13.99	9.17	138.43
Baotou	109°49′05″ E 40°39′53″ N	8.02	3.62	20.79	10.60	71.19
Fengqiu	114°33′07″ E 35°01′14″ N	8.14	4.39	32.28	19.03	86.63

2.2. Soil Culture Experiment

A soil culture experiment was conducted from May 12, 2019, to June 12, 2019, in a greenhouse at the Institute of Soil Science, Chinese Academy of Sciences (28°12′ N, 116°55′ E). The temperature and humidity of the greenhouse during the experiment ranged from 20 to 35 °C and from 60 to 75%, respectively. Each collected soil sample with a unique pH was subjected to one of three treatments, namely, treatment with one of two forms of N—either NH_4^+, applied as $(NH_4)_2SO_4$, or NO_3^-, applied as $NaNO_3$—or withholding of N fertilizer (control; CK). The N fertilizers were applied at the rate of 200 mg N/kg soil. In addition, phosphorus (P) and potassium (K) were applied in the form of superphosphate and KCl at the rate of 100 mg P/kg and 200 mg K/kg, respectively, for all treatments. Each treatment was replicated three times, resulting in a total of 81 pots (9 soils × 3 N forms × 3 replicates). Each pot (depth = 12.7 cm; internal diameter = 12 cm) was filled with 1 kg of air-dried soil, and all fertilizers were thoroughly mixed into the soil. The pots were watered before the seed was planted.

Seeds of maize hybrid 'Zhengdan 958′ were surface sterilized with 10% H_2O_2 for 20 min and then rinsed repeatedly with deionized water. The seeds were germinated on moist filter paper in an incubator at 32 °C for 36 h. Germinated seeds were sown in each pot. After 2 days, upon reaching a height of 2 cm, seedlings were thinned to three plants per pot and then watered daily. Pot positions were changed every 3 days to avoid the influence of other environmental factors. Weeds were removed manually when identified.

Maize seedlings were harvested after 30 days of growth. Plant roots and shoots lifted from the soil were immediately separated from the soil with scissors, washed several times with deionized water, kept at 105 °C for 30 minutes, and oven dried to a constant weight at 60 °C. The oven-dried plant materials were pulverized and then passed through a 0.149 mm sieve for N content determination. Soil samples in each pot were collected after maize harvest and air dried for determination of soil pH and exchangeable aluminum (Al) content as previously described by Wang et al. [17].

2.3. Hydroponic Experiment

A hydroponic experiment was conducted in a controlled-environment growth chamber with day and night temperatures of 30 °C and 23 °C, respectively, and a relative humidity of 65 ± 5%. Two factors were used in the experiment: (1) solution pH (4.00, 5.00, 6.00, 7.00, and 8.00) and (2) N source (NH_4^+, applied as 0.5 mM NH_4Cl, and NO_3^-, applied as 0.5 mM $NaNO_3$). Seeds of maize 'Zhengdan 958' were germinated as described above. The germinated seeds were placed on a net floating on a solution of 0.5 mM $CaCl_2$ for 2 to 3 days. Uniform seedlings were then selected and randomly divided into two groups: one treated with NH_4^+ nutrient solution at different pHs, and the other treated with NO_3^- nutrient solution at different pHs. The solution pH was adjusted using 1 M HCl and 1 M NaOH. The composition of NH_4^+ and NO_3^- nutrient solutions was as follows: 0.5 mM NH_4Cl or 0.5 mM $NaNO_3$ plus 0.2 mM NaH_2PO_4, 1.0 mM KCl, 1.0 mM $CaCl_2{\cdot}2H_2O$, 0.4 mM $MgSO_4{\cdot}7H_2O$, 3.0 μM H_3BO_3, 0.5 μM $MnCl_2{\cdot}4H_2O$, 1.0 μM $Na_2MoO_4{\cdot}2H_2O$, 0.4 μM $ZnSO_4$, 0.2 μM $CuSO_4{\cdot}4H_2O$, and 20.0 μM Na-Fe-EDTA. To control pH changes in the nutrient solution, 4-morpholineethanesulfonic acid hydrate (5 mM) was added as a pH buffer to all treatments. After 15 days of growth, the maize plants were harvested. The shoots and roots were separated, washed, oven dried, pulverized, and passed through a 0.149 mm sieve as described above.

2.4. Analytical Methods

The basic properties, including soil pH, NH_4^+-N, NO_3^--N, available P and K, were determined according to the methods described by Bao et al. [24]. The air-dried soil samples were sieved to pass through a 2 mm mesh to determine pH, available P, and K contents. The soil pH of a 1:2.5 soil:water mixture was measured with a pH meter (PB-21, Sartorius, Göttingen, Germany). Soil available P was extracted from acidic soils (pH 4.32–5.02) with 0.03 M ammonium fluoride-hydrochloric acid and from neutral and alkaline soils (pH 6.52–8.14) with 0.5 M $NaHCO_3$ (pH 8.5). The P content of the extraction solution was measured by the molybdenum blue method. Soil available K was extracted with 1 M CH_3COONH_4 (pH 7.0) and measured by flame photometry (FP640, Shanghai Precision & Scientific Instrument, Shanghai, China). Fresh soil samples were used to determine NH_4^+-N and NO_3^--N contents. Soil NH_4^+-N and NO_3^--N were extracted with 2 M KCl, and their contents were measured by indophenol blue colorimetry and dual wavelength spectrophotometry, respectively.

Following maize harvest, soil samples were collected and used to determine soil pH and exchangeable Al content. The soil pH was measured as described above. The soil exchangeable Al content was extracted with 1 M KCl (1:50 soil:solution ratio) and measured by an inductively coupled plasma-atomic emission spectrophotometry (ICP-AES, Optima 8000, PerkinElmer, Waltham, MA, USA) as described by Wang et al. [17]. The N content of plant materials was measured with a CNS elemental analyzer (Vario MAX, Elementar, Hanau, Germany) as described by Zhang et al. [25].

2.5. Calculation Methods

The amount of accumulated N in maize shoots (or roots) was calculated as the product of shoot (or root) N content and dry weight as described by Fageria and Baligar [26].

The NRE (%) of maize plants was calculated according to the following equation [17]:

$$NRE(\%) = \frac{accumulated\ N\ in\ maize\ under\ N\ fertilization\ (mg/pot) - accumulated\ N\ in\ CK\ maize\ (mg/pot)}{N\ fertilizer\ application\ rate\ (mg/pot)}$$

2.6. Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows v19.0 (IBM, Armonk, NY, USA). Significant differences in the dry weight and N accumulation of maize plants among CK, NH_4^+, and NO_3^- treatments were assessed by one-way analysis of variance (ANOVA) followed by Duncans' multiple range test at the 5% level. Significant differences in maize NRE in the soil culture experiment and in maize plant dry weight and N accumulation in the hydroponic experiment between NH_4^+ and NO_3^- were assessed by one-way ANOVA followed by an independent sample t-test at the 5% level. Regression analyses were performed and R^2 and *p*-values were calculated using SPSS. All figures were created using Origin 2019.

3. Results

3.1. Maize Growth in Soil Culture

The maize growth after harvest was shown in Figure 1. In the two strongly acidic soils, which had pHs of 4.32 and 4.36, NO_3^- fertilization had no discernable effect on maize root, shoot, and plant dry weights because of inhibitory effects of strong acidity on maize growth, whereas NH_4^+ fertilization inhibited maize growth compared with CK (Figure 2a–c). At pHs of 4.32 and 4.36, NH_4^+ treatments reduced maize root, shoot, and plant dry weights by 35–75% and 48–67% compared with CK and NO_3^- treatments, respectively. Nitrogen fertilization significantly enhanced maize growth in all soils except for the two soils with pHs of 4.32 and 4.36. No significant differences in root, shoot, and whole plant dry weights were observed between NH_4^+ and NO_3^- treatments in soil with a pH of 5.02, whereas NH_4^+ significantly improved these parameters compared with NO_3^- in soils with pHs of 6.52−8.02. In soil with a pH of 8.14, however, no significant differences in maize growth were observed between the two N forms. These results indicate that maize plants grew better when supplemented with NO_3^- compared with NH_4^+ in strongly acidic soils (pH 4.32–4.36), whereas maize plants grew better under NH_4^+ than under NO_3^- in neutral and alkaline soils (pH 6.52–8.02).

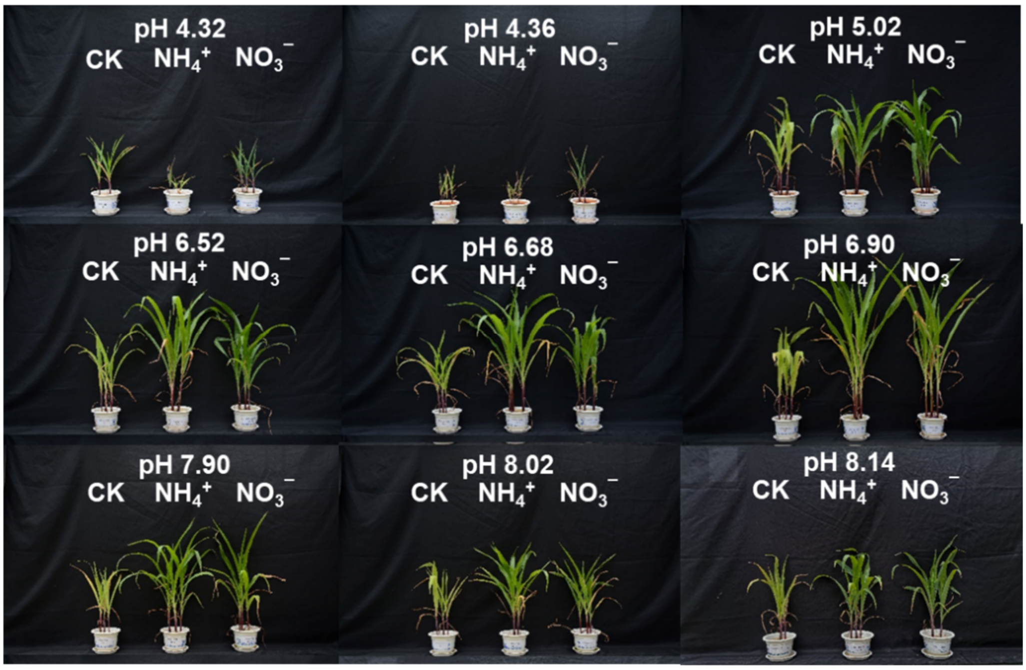

Figure 1. Maize seedlings after 30 days of growth with non-N (CK), NH_4^+, or NO_3^- at different soil pHs.

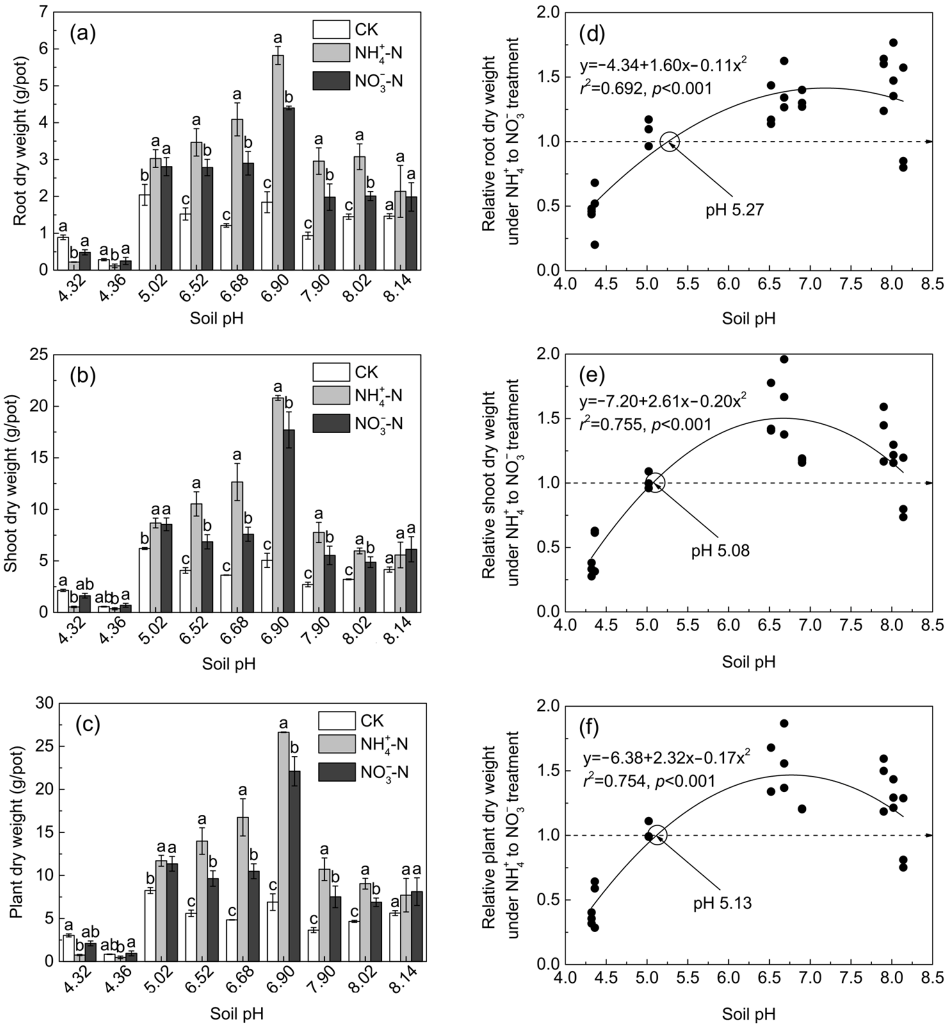

Figure 2. Effect of soil pH on the growth response of maize to ammonium (NH_4^+) and nitrate (NO_3^-) fertilizers. (**a**–**c**) Dry weights of the root (**a**), shoot (**b**), and total plant (**c**). (**d**–**f**) Relative dry weights of the root (**d**), shoot (**e**), and total plant (**f**). Maize seedlings were harvested after 30 days of growth. Relative dry weights were calculated as the ratio of dry weight under NH_4^+ treatment to that under NO_3^- treatment. Different letters above bars indicate significant differences among NH_4^+, NO_3^-, and control (CK) treatments in the same soil.

Root, shoot, and plant dry weights under NH_4^+ supplementation relative to these parameters under NO_3^- treatment were further used to compare the growth preference of maize for NH_4^+ vs. NO_3^-. We conducted a nonlinear regression analysis of soil pH and relative root, shoot, and plant dry weights of maize (Figure 2d–f). As soil pH increased, relative root, shoot, and plant dry weights increased and reached a plateau before eventually decreasing. When the value of relative root, shoot, or plant dry weight was 1.0 in the regression equation, the corresponding soil pH was 5.27, 5.08, or 5.13, which indicates that the growth of maize under NH_4^+ treatment was equivalent to that under NO_3^- treatment in soils with a pH of 5.08–5.27. This equation also predicted that maize plants would show a growth preference for NO_3^- when grown in soils at a pH below 5.08–5.27, with a growth preference for NH_4^+ indicated in soils with a pH above this range.

3.2. Nitrogen Accumulation and NRE of Maize in Soil Culture

In the two strongly acidic soils with pHs of 4.32 and 4.36, N accumulation in roots, shoots, and whole plants of maize treated with NO_3^- fertilizer was 2.5–5.6 times higher than that under the CK treatment (Figure 3a–c). In contrast, no significant differences in maize N accumulation were observed between NH_4^+ and CK treatments. In other soils, N accumulation in maize roots, shoots, and whole plants was significantly improved by both NH_4^+ and NO_3^- fertilizers compared with CK. The N accumulation in maize shoots and whole plants under NO_3^- treatment was 1.3–3.4 times higher than that under NH_4^+ treatment at pHs of 4.32–5.02 (Figure 3b,c), thus resulting in a significantly higher NRE for maize treated with NO_3^- compared with NH_4^+ in these three soils (Figure 4a). In soils with pHs of 6.52–8.02, in contrast, the application of NH_4^+ fertilizer improved N accumulation in maize shoots and whole plants by 8.9–67.2% compared with treatment with NO_3^- (Figure 3b,c). As a result, the NRE of maize was significantly higher under NH_4^+ than under NO_3^- in soils with pHs of 6.52–8.02 (Figure 4a). No significant differences were observed in root, shoot, and plant N accumulation or NRE between NH_4^+ and NO_3^- treatments at a pH of 8.14 (Figures 3a–c and 4a). These results indicate that the N uptake and NRE of maize plants were enhanced by NO_3^- compared with NH_4^+ in strongly acidic soils (pH 4.32–5.02), whereas these parameters were enhanced by NH_4^+ relative to NO_3^- in neutral and alkaline soils (pH 6.52–8.02).

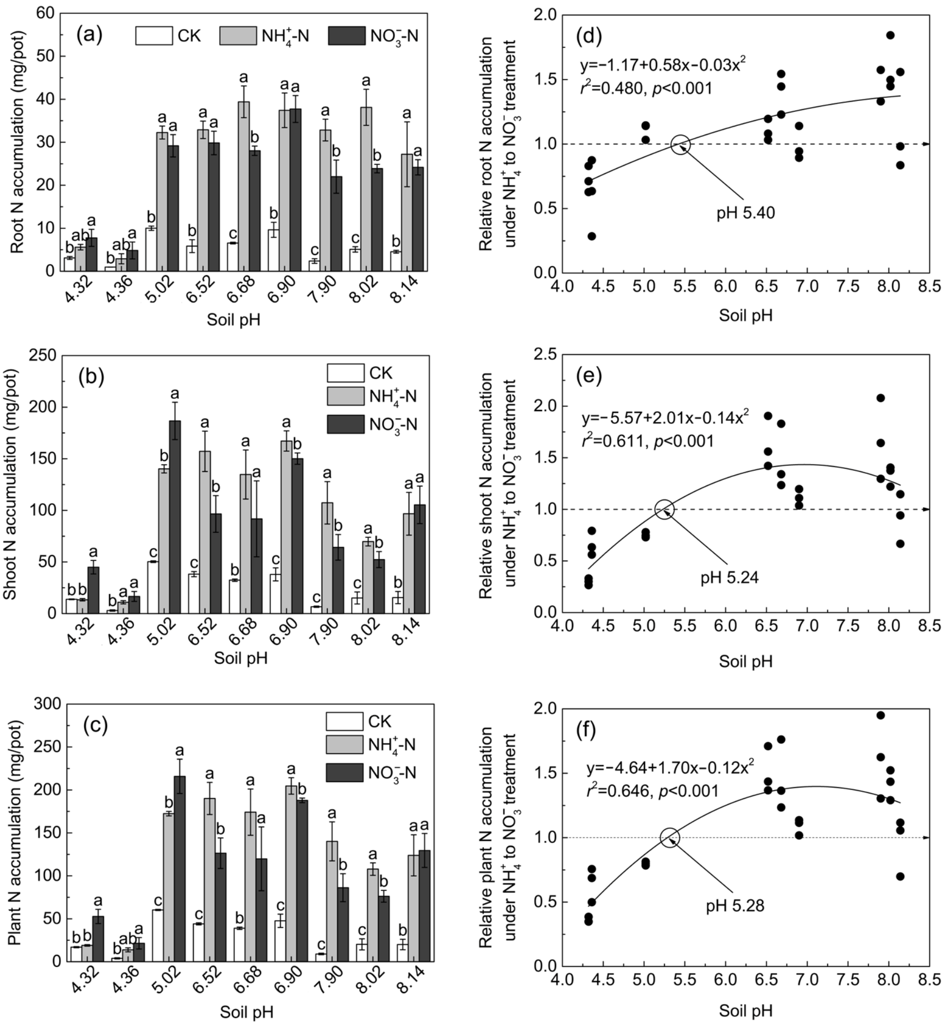

Figure 3. Effect of soil pH on the nitrogen (N) accumulation response of maize to ammonium (NH_4^+) and nitrate (NO_3^-) fertilizers. N accumulations in the root (**a**), shoot (**b**), and total plant (**c**). (**d**–**f**) Relative N accumulations in the root (**d**), shoot (**e**), and total plant (**f**). Maize seedlings were harvested after 30 days of growth. Relative N accumulation in maize was calculated as the ratio of N accumulation under NH_4^+ treatment to that under NO_3^- treatment. Different letters above bars indicate significant differences among NH_4^+, NO_3^-, and control (CK) treatments in the same soil.

According to the nonlinear regression analysis, N accumulation in maize roots, shoots, and whole plants (Figure 3d–f) and maize NRE (Figure 4b) under NH_4^+ treatment relative to NO_3^- followed the same trends as did maize dry weight (Figure 2d–f). When the value of the relative N accumulation of maize roots, shoots, or whole plants was 1.0 in the regression equation, the corresponding soil pH was 5.40, 5.24, or 5.28, thus indicating that maize N uptake under NH_4^+ treatment was equivalent to that under NO_3^- treatment in soils with a pH of 5.24–5.40 (Figure 3d–f). Moreover, a relative NRE value of 1.0 corresponded to a soil pH of 5.23, which indicates that maize NRE under NH_4^+ treatment was equivalent to that under NO_3^- treatment in soils with a pH of 5.23 (Figure 4b). Furthermore, NO_3^- fertilizer would enhance maize N uptake and NRE compared with response to NH_4^+ in soils with pHs below 5.23–5.40; in contrast, NH_4^+ fertilizer would have this effect in soils with pHs above 5.23–5.40.

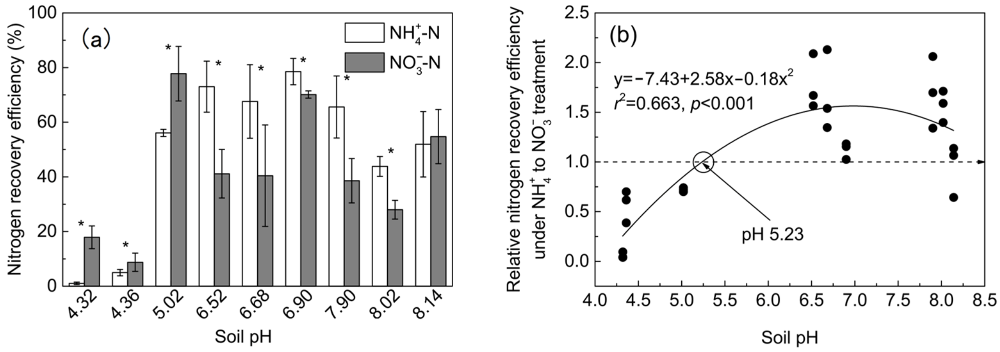

Figure 4. Effects of soil pH on the nitrogen recovery efficiency (NRE) response of maize to ammonium (NH_4^+) and nitrate (NO_3^-) fertilizers. NRE (**a**) and relative NRE (**b**). Maize seedlings were harvested after 30 days of growth. Relative NRE of maize was calculated as the ratio of NRE under NH_4^+ treatment to that under NO_3^- treatment. Asterisks indicate significant differences between NH_4^+ and NO_3^- treatments in the same soil.

3.3. Soil pH and Exchangeable Aluminum Content

The results of the present study confirmed that soil pH under treatment with NO_3^- was higher than that under treatment with NH_4^+ at soil pHs of 4.32–8.14 (Figure 5a). In contrast, soil exchangeable Al content under treatment with NO_3^- was lower than that under treatment with NH_4^+ at soil pHs of 4.32–6.68 (Figure 5b). The soil exchangeable Al content was the highest at pHs of 4.32 and 4.36, then decreased with pH increasing up to 6.9, and finally remained undetectable up to pH 8.14.

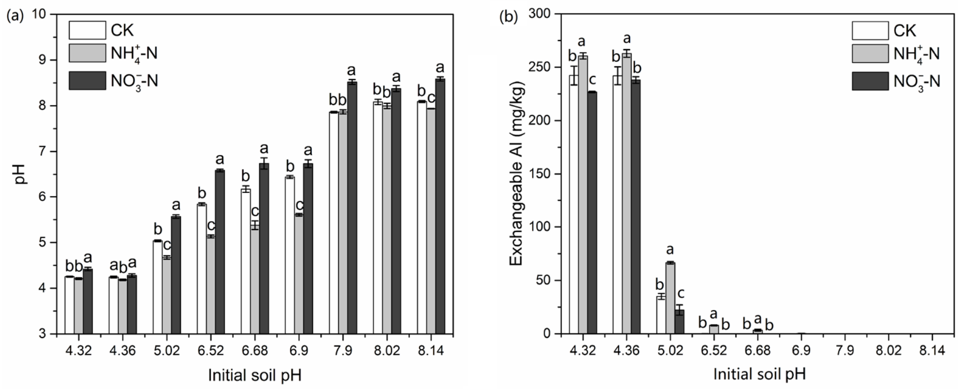

Figure 5. Soil pH and exchangeable aluminum content after maize harvest. (**a**) soil pH; (**b**) soil exchangeable Al. Maize seedlings were harvested after 30 days of growth. Different letters above bars indicate significant differences among NH_4^+, NO_3^-, and control (CK) treatments in the same soil.

3.4. Maize Growth in the Hydroponic Experiment

In the hydroponic experiment, root, shoot, and plant dry weights of maize treated with NH_4^+ initially increased with increasing solution pH and then leveled off, whereas a consistent decrease in these parameters was observed with increasing pH under treatment with NO_3^- (Figure 6a–c). Application of NO_3^- increased maize root, shoot, and plant dry weights by 3.6–4.1 times compared with NH_4^+ at pH 4.00, whereas NH_4^+ fertilizer increased these parameters by 33.7–87.9% compared with NO_3^- at pH levels of 7.00 and 8.00. No significant difference was observed in maize dry weight between NH_4^+ and NO_3^- treatments at pH 5.00 or 6.00.

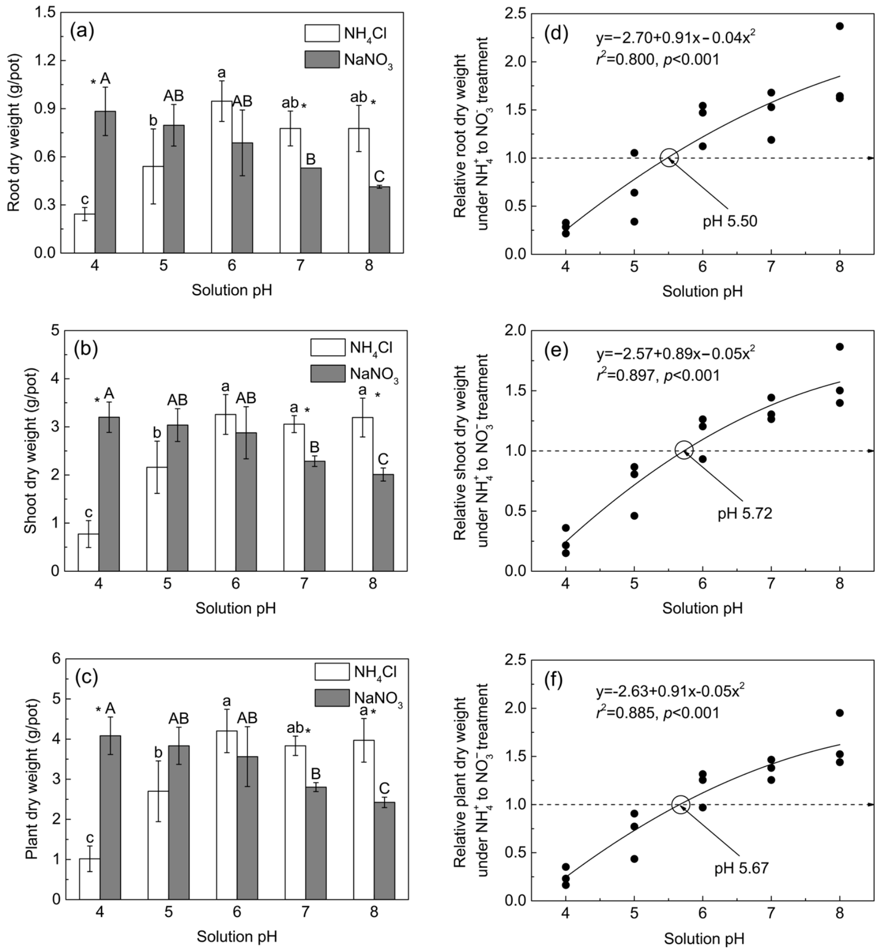

Figure 6. Effect of solution pH on the growth response of maize to ammonium (NH_4^+) and nitrate (NO_3^-) fertilizers under hydroponic conditions. (**a**–**c**) Dry weights of the root (**a**), shoot (**b**), and total plant (**c**). (**d**–**f**) Relative dry weights of the root (**d**), shoot (**e**), and total plant (**f**). Maize seedlings were harvested after 15 days of growth. Relative root, shoot, and plant dry weights of maize were calculated as the ratio of dry weight under NH_4^+ treatment to that under NO_3^- treatment. Different lowercase and capital letters above bars indicate significant differences among different solution pHs under NH_4^+ and NO_3^- treatments, respectively. Asterisks indicate significant differences between NH_4^+ and NO_3^- treatments at the same solution pH.

In the hydroponic experiment, the dry weight of maize under NH_4^+ treatment relative to that under NO_3^- gradually increased with increasing solution pH, which indicates that NH_4^+ had a continuously rising contribution to maize growth relative to NO_3^- as solution pH increased (Figure 6d–f). A relative root, shoot, or plant dry weight value of 1.0 corresponded to a solution pH of 5.50, 5.72, or 5.67, respectively, thus indicating that the dry weight of maize under NH_4^+ treatment was equivalent to that under NO_3^- treatment at solution pHs of 5.50–5.72. In addition, application of NO_3^- would result in a relatively higher maize dry weight when the solution pH was below 5.50–5.72, whereas the effect of NH_4^+ on maize weight would exceed that of NO_3^- when the solution pH was higher than 5.50–5.72 in the hydroponic experiment.

3.5. Nitrogen Accumulation by Maize in the Hydroponic Experiment

In hydroponically grown maize treated with NH_4^+, N accumulation in shoots and whole plants was significantly lower at pH 4.00 than at pH 5.00–8.00 (Figure 7b,c). When maize plants were treated with NO_3^-, root, shoot, and whole plant accumulation of N was unaffected by solution pH (Figure 7a–c). In the solution at pH 4.00, the N accumulation of maize roots, shoots, and whole plants was significantly improved under NO_3^- treatment, by 158%, 180%, and 176%, respectively, compared with NH_4^+. In contrast, N accumulation in shoots and whole plants of maize grown at pH 7.00 or 8.00 was 20–59% higher under NH_4^+ treatment compared with NO_3^- (Figure 7b,c). No significant differences were observed in root N accumulation between NH_4^+ and NO_3^- treatments at pH 5.00–8.00 (Figure 7a).

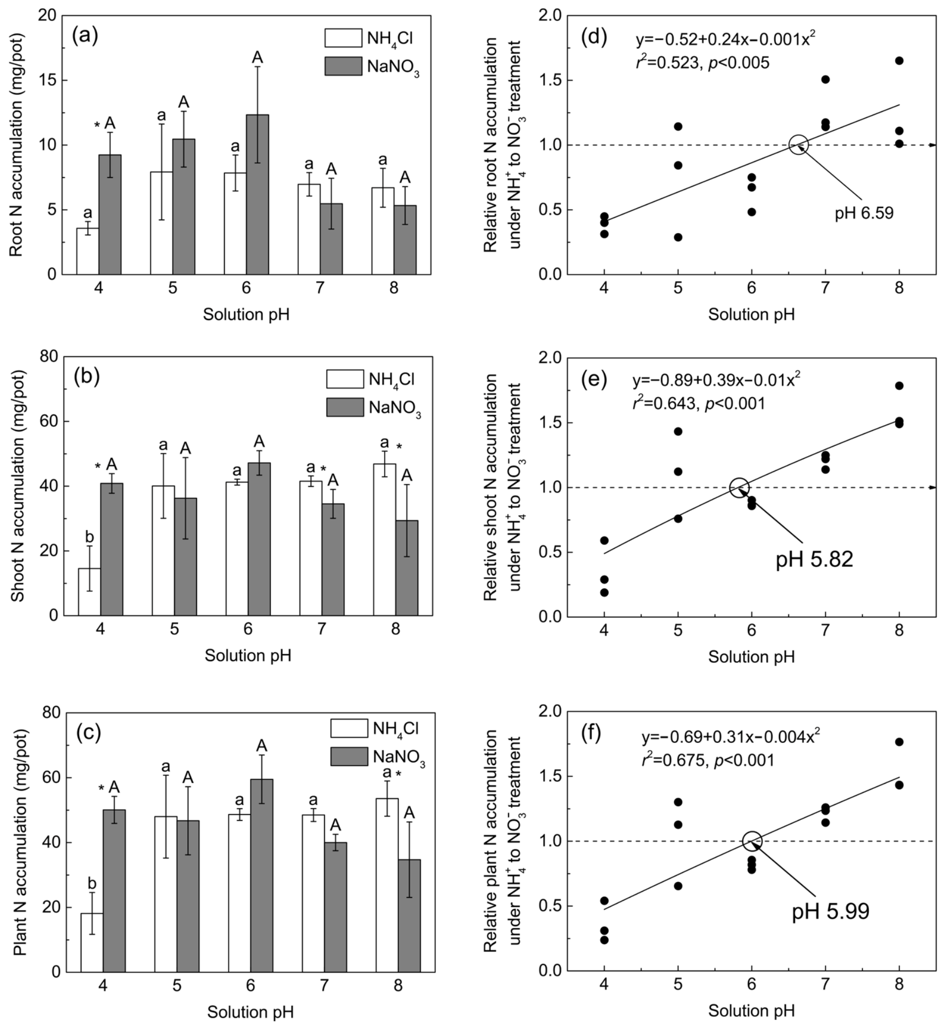

Figure 7. Effect of solution pH on the nitrogen (N) accumulation response of maize to ammonium (NH_4^+) and nitrate (NO_3^-) fertilizers under hydroponic conditions. (**a**–**c**) N accumulations in the root (**a**), shoot (**b**), and total plant (**c**). (**d**–**f**) Relative N accumulations in the root (**d**), shoot (**e**), and total plant (**f**). Maize seedlings were harvested after 15 days of growth. Relative N accumulations in roots, shoots, and whole plants were calculated as the ratio of N accumulation under NH_4^+ treatment to that under NO_3^- treatment. Different lowercase and capital letters above bars indicate significant differences among different solution pHs under NH_4^+ and NO_3^- treatments, respectively. Asterisks indicate significant differences between NH_4^+ and NO_3^- treatments at the same solution pH.

Nitrogen accumulation in maize roots, shoots, and whole plants under NH_4^+ treatment compared with that under NO_3^- treatment constantly increased with increasing solution pH, which indicates that the relative contribution of NH_4^+ fertilizer to N accumulation in hydroponically grown maize increased with increasing solution pH (Figure 7d–f). As indicated by the regression equations, a solution pH of 6.59, 5.82, or 5.99 respectively corresponded to a root, shoot, or whole-plant relative N accumulation of 1.0; N accumulation in maize under NH_4^+ treatment was thus equivalent to that under NO_3^- treatment at a solution pH of 5.82–6.59. Furthermore, NO_3^- enhanced maize N uptake relative to NH_4^+ when the solution pH was below 5.82–6.59, whereas NH_4^+ better promoted the N uptake of hydroponically grown maize at a solution pH above 5.82–6.59.

4. Discussion

4.1. Growth and N Accumulation of Maize under Ammonium and Nitrate Treatments at Different pHs

In the present study, maize plant growth and N accumulation were improved by NO_3^- application compared with that of NH_4^+ at soil pHs of 4.32–5.02 and a solution pH of 4.00. This maize NO_3^- preference under acidic conditions is consistent with previous reports [16,17,21]. NO_3^- uptake is driven by proton influx and depends on the H^+/NO_3^- transport system [27]. Conversely, equal amounts of H^+ are released from roots to balance charges within plants, absorbing NH_4^+. Although they facilitate NO_3^- uptake, external protons, when present in excess, inhibit the absorption of cations (including NH_4^+) by roots [28]. External pH may therefore have an impact on the uptake of NH_4^+ and NO_3^- by plants. In previous studies, maize plants accumulate more NO_3^- at a solution pH of 4.0, whereas more NH_4^+ is taken up at a solution pH of 7.0 [29]. Spring wheat absorbs more NO_3^- than NH_4^+ at a solution pH of 5.0; almost equal amounts of NO_3^- and NH_4^+ are accumulated at a solution pH of 6.5, while NH_4^+ uptake is greater than NO_3^- at a solution pH of 8.0 [30]. Pakchoi (*Brassica chinensis* L.) has a relatively higher NO_3^- uptake rate than NH_4^+ at a solution pH of 4–7 and a higher NH_4^+ uptake rate than NO_3^- at a solution pH of 8 [31]. The NO_3^- uptake rate of *Coix lacryma-jobi* L. is higher at solution pHs of 3.5 and 5.0 but significantly lower at pHs of 6.5 and 8.5 compared with NH_4^+ [32]. These studies consistently show that low pH may promote NO_3^- uptake and decrease NH_4^+ uptake, which may explain why the N accumulation of maize under NO_3^- is higher than that under NH_4^+ in acidic soil in the present study. In addition, the present study showed that NO_3^- increased the rhizosphere pH and then alleviated Al toxicity in acidic soils compared with NH_4^+, a phenomenon that may also contribute to the higher biomass and N accumulation of maize under NO_3^- compared with NH_4^+ observed in acidic soils [16,17].

In this study, the growth and N accumulation of maize under NH_4^+ treatment compared with that under NO_3^- was enhanced at soil pHs of 6.52–8.02 and nutrient solution pHs of 7.00–8.00, thereby revealing the preference of maize for NH_4^+ over NO_3^- under neutral to alkaline soil conditions. This observation is consistent with the findings of previous studies [18,19]. The preference of maize for NH_4^+ at a higher soil pH (6.52–8.02) is probably due to three factors: (1) reduced energy consumption for the assimilation of NH_4^+ compared with NO_3^- [33]; (2) higher uptake capacity of maize for NH_4^+ than for NO_3^- [34,35]; and (3) increased availability of some insoluble nutrient elements, such as P and iron, due to rhizosphere acidification during NH_4^+ uptake [36–38]. We note that the enhancement effects of NH_4^+ on maize growth relative to NO_3^- in our soil culture experiment decreased as soon as the soil pH was higher than 6.82. In the hydroponic experiment, however, the enhancement effects of NH_4^+ on maize growth relative to NO_3^- constantly increased as the solution pH was increased to 8.00. These inconsistent trends may be attributed to soil N transformation processes, such as ammonia volatilization and nitrification, during the soil culture experiment. These processes consume NH_4^+ and decrease the ratio of NH_4^+/NO_3^- in soil, as their transformation rates are often positively related to soil pH [39,40]. Consequently, an increase in soil pH decreases the match between maize preference for NH_4^+ and the availability of NH_4^+ in the soil. When maize is grown in soils with a pH above 6.82, the application of nitrification inhibitor together with NH_4^+ fertilizer may therefore be useful to reduce the nitrification rate and maintain a high NH_4^+/NO_3^- ratio in the soil to match maize NH_4^+ preference.

4.2. The Critical pH Changing the N Accumulation and Growth Preference of Maize for NH_4^+ and NO_3^-

In a recent study, the critical soil pH for maize growth in clay and sandy Ultisols was reported to be 4.8 and 5.0, respectively [41]. Above the critical soil pH, changes in soil pH did not affect maize growth; below the critical soil pH, however, a decreasing soil pH significantly inhibited maize growth, with soil exchangeable Al increasing sharply with the decrease in soil pH [41]. In the soil culture experiment in our study, we similarly found that the critical pH affecting the N accumulation and growth preference of maize for NH_4^+ vs. NO_3^- was around 5.1. Aluminum from soil minerals is soluble and causes phytotoxicity when the pH is less than 5.0 [42], and the availability of Al increases with decreasing soil pH [42,43]. A link potentially exists between the response of maize growth to NH_4^+ and NO_3^- and the solubility of Al in soil. Below the critical soil pH, H^+ release during the uptake of NH_4^+ by maize further acidifies its rhizosphere and exacerbates Al toxicity, which strongly inhibits root growth and plant N uptake [44]. In contrast, the uptake of NO_3^- by maize increases rhizosphere pH and alleviates Al toxicity to some extent, thereby resulting in higher maize dry weights and N uptake compared with the effects of NH_4^+. In soil with a pH higher than 5.0, where Al toxicity is no longer a limiting factor for plant growth, application of NH_4^+ improves maize growth compared with application of NO_3^-, as discussed above. The critical pH controlling the growth preference of hydroponically grown maize for NH_4^+ vs. NO_3^- is approximately 5.6, while that for N accumulation is around 5.9. These critical pHs are higher than those in the soil culture experiment, possibly because of differences in the growth medium components—such as the absence of Al toxicity and the availability of micro-elements.

4.3. Implications for Improvement of Maize NRE and Growth

NRE can be increased by matching crop preference for a specific N form with soil N transformation characteristics [4,5]. According to the present study, maize seedling preference for NH_4^+ vs. NO_3^- is affected by soil pH. The critical soil pH controlling the NRE preference of maize for NH_4^+ and NO_3^- is 5.23. Below this critical soil pH, maize plants have a higher NRE under NO_3^- than under NH_4^+, whereas the opposite is true above this pH. This finding may have great practical significance for N fertilizer management. Maize is cultivated on 25.8 million hectares of acid soils (pH < 5.5), occupying about 20% of maize production worldwide [45]. However, maize yield is reduced by 71% in acidic soils [46]. N fertilizer-induced soil acidification exacerbates maize yield reduction in acidic soils [47,48]. Thus, nitrate fertilizer may be applied to improve the NRE and growth of maize seedlings in soils with a pH lower than 5.23. This strategy has implications for decreasing N fertilizer application amounts and soil acidification while simultaneously increasing maize productivity in acidic soils. Conversely, NH_4^+ fertilizer may be applied in soils with a pH higher than 5.23 for the improvement of maize growth and the decrease of N fertilizer application amounts. We note that soils with a higher pH usually exhibit fast nitrification rates and applied NH_4^+ can be nitrified into NO_3^- in a short time. In high-pH soils, the addition of nitrification inhibitors and the control of ammonia volatilization is thus important to maintain a high NH_4^+/NO_3^- ratio. Since the present study investigated the N source preference only at the maize seedling stage due to the limitation of soil volume in the pot experiment, it is important to further examine maize N source preference at different soil pHs under field conditions until the maize maturity stage.

5. Conclusions

As consistently shown by the soil culture and hydroponic experiments in this study, the response of maize seedling growth and N uptake to NH_4^+ and NO_3^- mainly depends on the pH of the growth medium. Maize seedlings generally prefer NO_3^- in strongly acid soils but NH_4^+ in neutral to alkaline soils. A soil pH between 5.08 and 5.40 and a nutrient solution pH between 5.50 and 6.59 were the critical pH ranges in which a change in maize preference for NH_4^+ vs. NO_3^- occurred in soil culture and hydroponic experiments, respectively. Below the critical pH, maize plant growth, N uptake, and NRE were significantly improved by NO_3^- compared with NH_4^+, thus implying that application of NO_3^- may be an important strategy for improving maize NRE in acidic soils. Above the critical pH, these parameters were significantly improved by NH_4^+ compared with NO_3^-, therefore indicating that supplementation with NH_4^+ may be a useful approach for boosting maize NRE in neutral to alkaline soils.

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