Combinatorial Effects of Glycine and Inorganic Nitrogen on Root Growth and Nitrogen Nutrition in Maize (Zea mays L.)

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Abstract: Organic and inorganic nitrogen play important roles in plant nitrogen nutrition. However, how the coapplication of organic and inorganic nitrogen affects root growth, plant nitrogen metabolism, and soil nitrogen content is still unclear. Plant shoot and root growth, nitrogen uptake and metabolism, and soil nitrogen content were studied in maize (Zea mays L.) through pot experiments with different nitrogen treatments, including NH$_4^+$-N (Amm), NO$_3^-$-N (Nit), NH$_4^+$-N + NO$_3^-$-N (Amm + Nit), NH$_4^+$-N + NO$_3^-$-N + glutamate-N (Amm + Nit + Glu), and NH$_4^+$-N + NO$_3^-$-N + glycine-N (Amm + Nit + Gly). The results show that the shoot nitrogen uptake of maize treated with Amm + Nit + Gly was the highest among all the nitrogen treatments. In addition, the coapplication of glycine and inorganic nitrogen increased glutamine synthetase (GS) activity in the maize leaves, promoted nitrogen metabolism levels, and was conducive to the accumulation of amino acids and soluble protein in leaves. Compared with inorganic nitrogen, glycine combined with inorganic nitrogen increased the total root length and root surface area. A correlation analysis showed that total root length and root surface area had a significant positive effect on nitrogen uptake. When ammonium, nitrate, and glycine were applied together, the content of inorganic nitrogen and total nitrogen in soil was higher than that for other inorganic nitrogen treatments. Therefore, we conclude that glycine combined with inorganic nitrogen can increase soil nitrogen content, promote maize root growth, and thus facilitate nitrogen uptake and metabolism.

Keywords: nitrogen form; glycine; maize; nitrogen nutrition; root morphology

1. Introduction

Nitrogen is an essential elemental nutrient in plant growth and development, an important component of enzymes, chlorophyll, hormones, amino acids, proteins and other substances [1], and involved in plant physiological metabolism. Both inorganic and organic nitrogen can be absorbed and utilized by plants [2,3]. The nitrogen absorbed by plants is mainly inorganic nitrogen, including NH$_4^+$-N and NO$_3^-$-N. Nitrogen forms affect the development of root morphology [4], and a certain amount of NO$_3^-$-N significantly promotes lateral root growth [5,6], while NH$_4^+$-N inhibits root elongation and stimulates lateral root branching [7]. After NO$_3^-$-N enters the plant, it is reduced to NH$_4^+$-N by nitrate reductase (NR) and nitrite reductase (NiR), and under the action of glutamine synthetase—glutamate synthetase (GS-GOGAT), the organic nitrogen available to plants is formed [8,9]. The processes of nitrogen uptake, assimilation, transport, and utilization are involved in processes from soil to root, from root to shoot, and within the various organs of plants [10]. Generally speaking, terrestrial plants prefer NO$_3^-$-N, while high concentrations of NH$_4^+$-N inhibit plant growth and nitrogen metabolism [11]. Compared with a single supply of NH$_4^+$ or NO$_3^-$, an appropriate ratio of ammonium and nitrate in a mixed nitrogen source can increase plant growth rate, increase nitrogen uptake, and improve plant nitrogen metabolism and mineral element absorption [12,13].

In agricultural systems, research has found that crops such as wheat, maize, rice, and tomatoes can directly absorb organic nitrogen from the soil [14–17], which is not...
a process that requires traditional theoretical understanding of microbial decomposition into inorganic nitrogen [18]. In ecosystems with weak nitrogen mineralization, organic nitrogen thus becomes an important nitrogen source for plant growth [19,20]. The carbon bonus of organic nitrogen enhances the nitrogen use efficiency of plants [21]. Zhong et al. [22] found that the biomass, photosynthetic rate, and photosynthetic nitrogen use efficiency of Andrographis paniculata were increased by using organic nitrogen sources. In soil-soluble organic nitrogen, amino acids, as small molecular monomers, can be completely absorbed by plants and utilized in plants through transamination and deamination [23,24]. Amino acids are natural plant growth stimulants [25] that can regulate carbon and nitrogen metabolism and promote plant growth [22,26,27]. Amino acids, as the main constituents of organic nitrogen, affect root structure due to their different types and concentrations and are of great significance for root development [28]. The application of amino acids can promote plant root development and nitrogen fixation, thereby enhancing the uptake of nutrients on the root surface [29,30]. Chapin et al. [19] found that the amino acid nitrogen absorbed by field-collected roots of Eriophorum vaginatum accounted for at least 60% of the total nitrogen uptake, and when amino acids were used as the nitrogen source, the nitrogen uptake and biomass of Eriophorum were higher than when inorganic nitrogen was used instead.

Glycine and glutamate are common amino acids and are found in high levels in farmland soils [31]. Glycine is a model amino acid in plant absorption research and is considered an important nitrogen source for plants due to its low molecular weight, low carbon-to-nitrogen ratio, fast diffusion rate in soil, and resistance to microbial decomposition [32–34]. Glutamate is a multifunctional amino acid that plays an important role in nitrogen metabolism and serves as a nitrogen donor for the biosynthesis of amino acids and other nitrogen-containing compounds [35,36]. L-glutamate acts as an exogenous signal to modulate root growth and branching in Arabidopsis thaliana [37], while exogenous glycine inhibits root elongation and reduces nitrate-N uptake in pak choi [38]. The study of organic and inorganic nitrogen sources in plants can enrich the theoretical study of plant nutritional effects and promote the sustainable development of agricultural production. Previous studies have shown that a combination of inorganic nitrogen and organic nitrogen can enhance nutritional effects and improve crop growth compared to a single application of inorganic or organic nitrogen [39,40]. In hydroponic culture, the combined application of Gln-N and NO$_3^-$-N increased the pak choi quality by reducing NO$_3^-$ concentration and increasing mineral nutrient concentrations in shoots [40], and the combined application of Gly-N and NO$_3^-$-N provided nitrogen and carbon nutrients for spinach [41]. At present, it is not clear how the coapplication of amino acid and inorganic nitrogen affects soil nitrogen content, crop nitrogen nutrition and whether it changes root growth and shoot physiology, and its mechanism needs to be further studied.

Maize (Zea mays L.) is the main grain crop in China. It is a common nitrogen-loving crop, and its demand for nitrogen during growth and development is much higher than its demand for phosphorus and potassium [42]. Nitrogen not only affects the external morphological structure and growth of maize, but also plays an important regulatory role in its internal physiological characteristics [43,44]. Maize can directly absorb NH$_4^+$-N, NO$_3^-$-N, and amino acids from soil [15]. Therefore, this study aims to explore the effects of applying inorganic nitrogen alone and the combination of inorganic nitrogen and amino acids on maize plant growth, nitrogen uptake and metabolism, and soil nitrogen content.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

This experiment was carried out at the agricultural experiment base of Hainan University from August 2022 to October 2022. During the experiment, the daily and nightly temperatures were about 28 °C/22 °C, respectively, and the light was natural light. The tested maize (Zea mays L.) cultivar was “Zhengdan 958”, which has high yield, stable yield, and multiple resistances and is the main maize variety planted in China [45]. The
soil was latosol from Chengmai County, Hainan Province (19°74′ N, 110°01′ E). The soil was air-dried indoors and thoroughly mixed with river sand in a weight ratio of 1:1 after passing through a 2 mm sieve. The basic properties of the soil mixed with river sand were as follows: pH 7.05, organic matter 15.62 g/kg, alkali-hydrolysable nitrogen 35.00 mg/kg, available phosphorus 5.73 mg/kg, and available potassium 52.51 mg/kg.

2.2. Nitrogen Treatment

There were five treatments in the experiment: (1) NH$_4^+$-N 120 mg kg$^{-1}$ soil (Amm); (2) NO$_3^-$-N 120 mg kg$^{-1}$ soil (Nit); (3) NH$_4^+$-N 60 mg kg$^{-1}$ soil and NO$_3^-$-N 60 mg kg$^{-1}$ soil (Amm + Nit); (4) NH$_4^+$-N 40 mg kg$^{-1}$ soil, NO$_3^-$-N 40 mg kg$^{-1}$ soil, and glutamate-N 40 mg kg$^{-1}$ soil (Amm + Nit + Glu); (5) NH$_4^+$-N 40 mg kg$^{-1}$ soil, NO$_3^-$-N 40 mg kg$^{-1}$ soil, and glycine-N 40 mg kg$^{-1}$ soil (Amm + Nit + Gly). Each treatment had four repetitions, arranged in random blocks. The total amount of nitrogen in each treatment was the same. NH$_4^+$-N, NO$_3^-$-N, glutamate-N, and glycine-N were added in the form of (NH$_4$)$_2$SO$_4$, KNO$_3$, L-glutamate, and L-glycine. The planting containers were gallon pots (23 cm in diameter and 21 cm in height), each containing 6 kg of soil. Three seeds were planted in each pot, and only one remained after germination. Different forms of nitrogen fertilizer were supplemented once every 30 days and applied twice during the growth period of maize. In order to ensure that the maize was not stressed by the lack of other nutrients, the soil was mixed with basic nutrients at the following concentrations (expressed as pure nutrient mg kg$^{-1}$ soil): KH$_2$PO$_4$ 50, MgSO$_4$·7H$_2$O 50, CaCl$_2$ 100, MnSO$_4$·H$_2$O 3.25, ZnSO$_4$·7H$_2$O 0.79, CuSO$_4$·5H$_2$O 0.50, H$_3$BO$_3$ 0.17, and Fe-EDTA 3.25. The amount of water in each pot remained consistent throughout the experiment. The positions of all pots were changed every 7 days, and the maize was harvested after planting for 2 months.

2.3. Measurement of Shoot Growth Parameters

On the day of harvest, the SPAD value of the leaves was measured using a portable SPAD-502 measuring instrument (Konica Minolta, Tokyo, Japan), expressed as the average value of the chlorophyll meter reading on the fourth leaf at the top. The length from the soil surface to the top of the longest leaf in the plant was measured using a tape measure and was considered to be the plant height. The stem base was measured with an electronic digital vernier caliper (Links, China) and was considered to be the stem diameter. The length and width of each fully unfolded leaf were measured with a tape measure, and the leaf area = leaf vein length × maximum width × 0.75 [46]. Fresh leaves from the same part of each plant were stored at −80°C for further analysis. The shoot and root were separated, and the shoot was cut into pieces and put into the oven at 105°C for 30 min, then baked at 80°C until a constant weight, after which the shoot dry weight was determined.

2.4. Determination of Leaf Free Amino Acids, Soluble Protein, and GS Activity

The total free amino acids in the fresh functional maize leaves were determined by using the ninhydrin colorimetric method [47]. Free amino acids were extracted from 0.2 g leaves with 10% acetic acid solution, and then the extracts were diluted with distilled water and filtered. The amino acids in the filtrate react with ninhydrin under the condition of heating in water. The amino acid content was determined via the colorimetric method at 570 nm, with L-leucine as the standard.

The determination of soluble protein content was as follows: 0.2 g maize leaves were weighed, a small amount of quartz sand and distilled water was added to grind them into homogenate, and then the homogenate was centrifuged at 5000 r/min for 10 min; the supernatant was the protein extract. We used Coomassie brilliant blue G-250 staining to accurately absorb and determine the soluble protein content of 0.1 mL of protein extract [48].

The 0.1 g leaves were weighed, and a reaction buffer containing 80 mM Tris-HCl, 40 mM L-glutamate, 8 mM ATP, 24 mM MgSO$_4$, and 16 mM NH$_2$OH was added, after which the samples were homogenized in ice and centrifuged at 5000 r/min for 10 min at 4°C. The GS activity was determined using the supernatant solution [49].
2.5. Determination of Mineral Elements

The shoot samples were digested with H$_2$SO$_4$-H$_2$O$_2$ under heating conditions after drying and crushing, and the obtained digestion liquid was analyzed by using nesslerization for the nitrogen concentration and the molybdenum antimony anticolorimetric method for the phosphorus concentration [50]. The nitrogen and phosphorus uptake of the plants were calculated.

2.6. Measurement of Root Morphology

The roots were rinsed with deionized water and stored at −20 °C for root morphology analysis. The roots were scanned with a flat scanner (Epson Expression V800, Nagano, Japan) at a resolution of 600 dpi. The scanned images were analyzed using WinRHIZO Pro 2009b software (Regent Instruments Inc., Quebec, QC, Canada) to obtain parameters such as total root length, root surface area, and root diameter. Six roots were selected from each plant to measure its root hair length, and a small segment of about 1 cm long in the root hair area was cut from each root and stained with 0.05% toluidine blue solution. After cleaning with deionized water, they were photographed with a camera-mounted microscope (Motic, SMZ-168, Xiamen, China) and the root hair length was measured with Motic images plus 3.0 software.

2.7. Determination of Soil pH, Inorganic Nitrogen Content, and Total Nitrogen Content

After the plants were harvested, the soil in each pot was mixed and soil samples were collected. The contents of NH$_4^+$-N and NO$_3^−$-N were measured using fresh soil, and the sum of the two was the inorganic nitrogen content. Extraction from the soil was performed with 2 mol/L KCl solution, and the NH$_4^+$-N content was determined by using the indophenol blue colorimetric method [50], while the NO$_3^−$-N content was determined via ultraviolet absorbance correction at dual wavelengths (220 nm and 275 nm) [51]. The remaining soil samples were air-dried and sieved to determine soil pH and total nitrogen. The soil pH was measured with a pH meter (SevenCompact S220, Shanghai, China), and the ratio of soil to water was 1:2.5. The soil total nitrogen was digested with 5 mL H$_2$SO$_4$ and mixed catalyst (K$_2$SO$_4$:CuSO$_4$:Se = 100:10:1) to obtain a digestive solution, which was diluted and determined using a Kjeldahl nitrogen analyzer (KDN-816, Shanghai, China).

2.8. Statistical Analysis

The experimental data were processed and plotted using Excel 2019 and Origin 2020 software. IBM SPSS statistics 26 software was used to compare the data via one-way analysis of variance (ANOVA), and an LSD test was used for the significance between different treatments of each index in the figures and tables ($p < 0.05$). A redundancy analysis (RDA) was conducted with Canoco 5.0 software.

3. Results

3.1. Effect of Different Nitrogen Forms on Shoot Growth and Nitrogen Uptake of Maize

Maize plants treated with Ammonium (Amm), Ammonium + Nitrate (Amm + Nit), Ammonium + Nitrate + Glutamate (Amm + Nit + Glu), and Ammonium + Nitrate + Glycine (Amm + Nit + Gly) had significantly higher plant height and leaf area than plants treated with Nitrate (Nit) (Table 1). The Amm + Nit + Gly treatment had the highest stem diameter among all nitrogen treatments. Plants growing with Nitrate alone had a significantly lower leaf SPAD value than those with other nitrogen forms.

Nitrate (Nit) inhibited plant growth compared to other nitrogen sources (Figure 1a). Although there was no difference in shoot biomass between the Nit treatment and the Amm, Amm + Nit, and Amm + Nit + Glu treatments, it was significantly lower in the Nit treatment than in the Amm + Nit + Gly treatment (Figure 1b). Maize plants treated with Amm + Nit + Gly showed significantly higher nitrogen uptake than Nitrate alone and other inorganic nitrogen treatments (Figure 1c). Compared with Amm, Nit, Amm + Nit, and
Amm + Nit + Gly, the shoot nitrogen uptake values for plants treated with Amm + Nit + Gly increased by 21.6%, 59.1%, 35.3%, and 15.6%, respectively.

Table 1. Plant height, stem diameter, leaf area, and SPAD value of maize treated with different nitrogen forms.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant Height (cm)</th>
<th>Stem Diameter (mm)</th>
<th>Leaf Area (cm²)</th>
<th>SPAD Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amm</td>
<td>129.3 ± 6.8 a</td>
<td>14.91 ± 0.17 c</td>
<td>2432.3 ± 174.6 a</td>
<td>43.6 ± 0.7 a</td>
</tr>
<tr>
<td>Nit</td>
<td>109.3 ± 6.2 b</td>
<td>15.89 ± 0.42 c</td>
<td>1906.3 ± 66.2 b</td>
<td>38.0 ± 0.8 b</td>
</tr>
<tr>
<td>Amm + Nit</td>
<td>128.8 ± 6.0 a</td>
<td>16.06 ± 0.17 bc</td>
<td>2354.3 ± 77.0 a</td>
<td>41.7 ± 0.9 a</td>
</tr>
<tr>
<td>Amm + Nit + Glu</td>
<td>129.6 ± 6.5 a</td>
<td>17.15 ± 0.57 ab</td>
<td>2532.2 ± 59.2 a</td>
<td>43.1 ± 0.6 a</td>
</tr>
<tr>
<td>Amm + Nit + Gly</td>
<td>130.1 ± 6.0 a</td>
<td>17.27 ± 0.34 a</td>
<td>2524.4 ± 88.7 a</td>
<td>43.8 ± 0.4 a</td>
</tr>
</tbody>
</table>

Note: Amm, Nit, Glu, and Gly stand for NH₄⁺-N, NO₃⁻-N, glutamate-N, and glycine-N, respectively. Values in the table represent the mean ± standard error (n = 4). Different lowercase letters in the same column indicate significant differences between treatments (p < 0.05).

Figure 1. Phenotype (a), shoot biomass (b), and N uptake (c) of maize treated with different nitrogen forms. Amm, Nit, Glu, and Gly stand for NH₄⁺-N, NO₃⁻-N, glutamate-N, and glycine-N, respectively. Scale bar = 10 cm in (a). Error bars represent the standard errors of the means (n = 4). Different lowercase letters above the bars denote significant differences between treatments (p < 0.05).

3.2. Effect of Different Nitrogen Forms on Shoot Nitrogen Metabolism of Maize

Plants treated with Ammonium + Nitrate + Glycine (Amm + Nit + Glu) and Ammonium + Nitrate + Glutamate (Amm + Nit + Glu) had a significantly higher total amount of leaf free amino acids than plants treated with Ammonium (Amm), Nitrate (Nit), and Ammonium + Nitrate (Amm + Nit), but no difference in leaf free amino acids was found between plants treated with Amm + Nit + Glu and Amm + Nit + Gly (Figure 2a). Leaf soluble protein content was significantly increased in plants treated with Ammonium + Nitrate + Glycine (Amm + Nit + Glu) compared to those treated with Ammonium (Amm), Nitrate (Nit), Ammonium + Nitrate (Amm + Nit), and Ammonium + Nitrate + Glutamate (Amm + Nit + Glu). Specifically, plants treated with Amm + Nit + Gly showed an 18.0% increase in leaf soluble protein compared to Amm, a 50.2% increase compared to Nit, a
30.9% increase compared to Amm + Nit, and a 12.4% increase compared to Amm + Nit + Glu treatments (Figure 2b). Leaf GS activity was significantly increased in plants treated with Ammonium + Nitrate + Glycine (Amm + Nit + Gly) compared to those treated with Ammonium (Amm), Nitrate (Nit), and Ammonium + Nitrate (Amm + Nit) (Figure 2c). This indicates that the Amm + Nit + Gly treatment led to higher activity of GS, an enzyme involved in nitrogen metabolism, than other nitrogen treatments.

Figure 2. Leaf free amino acids (a), leaf soluble protein (b), and leaf GS activity (c) of maize treated with different nitrogen forms. Amm, Nit, Glu, and Gly stand for NH$_4^+$ -N, NO$_3^-$ -N, glutamate-N, and glycine-N, respectively. Error bars represent the standard errors of the means ($n = 4$). Different lowercase letters above the bars denote significant differences between treatments ($p < 0.05$).

3.3. Effect of Different Nitrogen Forms on Root Morphology of Maize

Nitrogen forms affected the root morphology of maize plants (Figure 3, Table 2). The coapplication of glycine and inorganic nitrogen (Amm + Nit + Gly) resulted in the longest total root length of maize among the nitrogen treatments. Compared with Ammonium (Amm), Nitrate (Nit), Ammonium + Nitrate (Amm + Nit), and Ammonium + Nitrate + Glutamate (Amm + Nit + Glu) treatments, Amm + Nit + Gly increased the total root lengths by 16.0%, 35.7%, 24.8%, and 0.2%, respectively. The combination of glutamate and inorganic nitrogen (Amm + Nit + Glu) led to the largest root surface area, followed by the combination of glycine and inorganic nitrogen (Amm + Nit + Gly). Amino acids combined with inorganic nitrogen significantly increased the total root length and root surface area of maize compared to the Nitrate (Nit) treatment. However, there was no significant difference in root surface area compared to the Ammonium (Amm) treatment. Different nitrogen forms did not have a significant effect on root diameter and root hair length.

Figure 3. Root morphology of maize treated with different nitrogen forms. Amm, Nit, Glu, and Gly stand for NH$_4^+$ -N, NO$_3^-$ -N, glutamate-N, and glycine-N, respectively. Scale bar: 10 cm.
3.4. Effect of Different Nitrogen Forms on Soil Nitrogen Nutrients

Nitrogen forms significantly affected soil pH, with notable differences observed (Figure 4a). The soil pH treated with Nitrate alone was significantly higher than that of soil treated with other nitrogen forms. This indicates that Nitrate application resulted in a more alkaline soil environment than other nitrogen forms. The soil inorganic nitrogen content of the Ammonium + Nitrate + Glycine (Amm + Nit + Gly) treatment was significantly higher than those of the Ammonium (Amm), Nitrate (Nit), and Ammonium + Nitrate (Amm + Nit) treatments (Figure 4b). Specifically, compared with the Amm, Nit, Amm + Nit, and Amm + Nit + Glu treatments, the Amm + Nit + Gly treatment had significantly greater soil inorganic nitrogen content by 25.2%, 53.5%, 22.8%, and 10.4%, respectively. The soil total nitrogen content in the Amm + Nit + Gly treatment was significantly higher than that in the Amm, Nit, and Amm + Nit treatments (Figure 4c). Compared with the Amm, Nit, and Amm + Nit treatments, the Amm + Nit + Gly treatment had a total nitrogen content greater by 15.8%, 29.4%, and 15.8%, respectively.

Figure 4. Effect of different nitrogen forms on soil pH (a), inorganic nitrogen (b), and total nitrogen (c). Amm, Nit, Glu, and Gly stand for NH$_4^+$-N, NO$_3^-$-N, glutamate-N, and glycine-N, respectively. Error bars represent the standard errors of the means ($n = 4$). Different lowercase letters above the bars denote significant differences between treatments ($p < 0.05$).

3.5. Correlations between Nitrogen Uptake and Different Indexes in Maize

Nitrogen uptake was significantly positively correlated with both total root length and root surface area (Figure 5a). This suggests that maize plants with longer roots and larger root surface areas tend to take up more nitrogen from the soil. For shoot indicators, nitrogen uptake showed a significantly positive correlation with the leaf SPAD value. This indicates that higher nitrogen uptake is associated with higher chlorophyll content in maize leaves, which can be an indicator of improved plant health and photosynthesis. There was a significantly positive correlation between nitrogen uptake and total free amino acid content in the plant. This suggests that increased nitrogen uptake is associated with higher levels of free amino acids in the plant. Both inorganic nitrogen content and total nitrogen content in the soil showed significantly positive correlations with nitrogen uptake by maize plants. This suggests that higher soil nitrogen levels are associated with increased nitrogen uptake by the plants. There was a significantly negative correlation between soil pH and nitrogen uptake.
uptake by plants. This indicates that as soil pH becomes more alkaline, nitrogen uptake by maize plants tends to decrease. The RDA results indicate that the root surface area was the most important factor influencing the changes in shoot physiological and biochemical indicators, explaining 37.9% of the variance (Figure 5b, Table 3). This suggests that the root surface area plays a crucial role in shaping the overall physiological responses of maize plants to different nitrogen forms. The influence of soil inorganic nitrogen content on changes in shoot physiological indicators was the second most important factor, explaining 15.0% of the variance. This underscores the significance of soil nitrogen availability in influencing plant responses.

Figure 5. Correlation analysis of shoot nitrogen uptake, root morphology, and soil indexes (a), and redundant analysis (RDA) of the effects of root morphology and soil properties on the shoot physiology of maize (b). TRL, total root length; RSA, root surface area; RD, root diameter; RHL, root hair length; SIN, soil inorganic nitrogen; STN, soil total nitrogen. ***: Significant at $p < 0.001$ level, **: Significant at $p < 0.01$ level, *: Significant at $p < 0.05$ level.
Table 3. Redundant analysis (RDA) results of the effects of root morphology and soil properties on the shoot physiology of maize.

<table>
<thead>
<tr>
<th>Name</th>
<th>Explains</th>
<th>Contribution</th>
<th>Pseudo-F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSA</td>
<td>37.9</td>
<td>54.0</td>
<td>11.0</td>
<td>0.004</td>
</tr>
<tr>
<td>SIN</td>
<td>15.0</td>
<td>21.4</td>
<td>5.4</td>
<td>0.006</td>
</tr>
<tr>
<td>RD</td>
<td>9.9</td>
<td>14.1</td>
<td>4.3</td>
<td>0.002</td>
</tr>
<tr>
<td>TRL</td>
<td>3.8</td>
<td>5.4</td>
<td>1.7</td>
<td>0.176</td>
</tr>
<tr>
<td>Soil pH</td>
<td>2.6</td>
<td>3.8</td>
<td>1.2</td>
<td>0.350</td>
</tr>
<tr>
<td>STN</td>
<td>0.6</td>
<td>0.8</td>
<td>0.3</td>
<td>0.892</td>
</tr>
<tr>
<td>RHL</td>
<td>0.3</td>
<td>0.5</td>
<td>0.1</td>
<td>0.986</td>
</tr>
</tbody>
</table>

Note: RSA, root surface area; SIN, soil inorganic nitrogen; RD, root diameter; TRL, total root length; STN, soil total nitrogen; RHL, root hair length.

4. Discussion

4.1. Coapplication of Glycine and Inorganic Nitrogen Promoted Shoot Nutrient Uptake and Metabolism in Maize

The exogenous application of different amino acids, mainly glycine, has a positive effect on crop growth and quality [26,52,53]. The application of reduced forms of nitrogen such as ammonium and amino acids has been evidenced to be more beneficial to plant growth than oxidized forms of nitrogen such as nitrate [54–56]. Similarly, in this study, the combined application of NH$_4^+$-N, NO$_3^-$-N, and glycine was more beneficial for shoot growth than the application of NO$_3^-$-N alone (Figure 1a, Table 1).

Glycine can improve the absorption and transportation of mineral nutrients [17,57,58]. The combined application of glycine and NO$_3^-$-N increased the nitrogen uptake of pak choi and onion [59,60], which may be related to the rational and efficient utilization of carbohydrates accumulated in various parts of the plant by the coapplication of glycine and NO$_3^-$-N, which can save energy and enable plants to store more nitrogen with less energy consumption [61]. The shoot nutrient uptake in this study showed that the combination of glycine and inorganic nitrogen was beneficial for maize in terms of absorbing nitrogen (Figure 1b). Furthermore, the leaf free amino acids and soluble protein contents in the Amm + Nit + Gly treatment was higher than that in the inorganic nitrogen treatment (Amm + Nit; Figure 2a,b).

Glutamate is also an important amino acid for plant nitrogen uptake. However, the addition of glutamate did not show greater nitrogen uptake than the inorganic treatment (Amm + Nit; Figure 1c), which was dissimilar with the results of glycine addition. This may be due to the fact that glycine is the smallest amino acid and much easier to be taken up by the root.

Ammonium and nitrate are both important nitrogen sources for plant growth. This study showed that NH$_4^+$-N better promotes the absorption and transport of total nitrogen in plants than NO$_3^-$-N, thus increasing the shoot biomass. It has also been reported that compared with NO$_3^-$, the application of NH$_4^+$ to maize is more conducive to nitrogen uptake and growth [62–64]. Zhang et al. [65] proposed possible reasons for maize’s preference for NH$_4^+$, including more energy-efficient absorption and assimilation of NH$_4^+$ by maize, better root development when providing NH$_4^+$, and the decrease in rhizosphere pH caused by NH$_4^+$ absorption being more conducive to nutrient uptake.

To a certain extent, the activity of key enzymes for nitrogen assimilation reflects the strength of nitrogen uptake and nitrogen assimilation capacity in plants, and nitrogen forms affect the activity of nitrogen-metabolizing enzymes [66–68]. GS is a key enzyme located at the center of nitrogen metabolism in the GS/GOGAT cycle, and its activity has a significant impact on plant growth and development, amino acid content, protein content, and yield [69–71]. Li et al. [72] found that the GS activity of wild peaches significantly increased, improving the nitrogen utilization efficiency of the peach trees, when glycine alone or glycine combined with urea were applied. In our study, the coapplication of NH$_4^+$-N, NO$_3^-$-N, and glycine nitrogen improved the GS activity of maize leaves, promoted the
accumulation of amino acids and soluble protein in leaves, and facilitated the accumulation of nitrogen (Figure 2). Thornton et al. [73] pointed that after glycine is absorbed by plants, on the one hand, it can directly participate in the synthesis of other amino acids through transamination; on the other hand, it can first synthesize aspartic acid through \( \text{NH}_4^+ \) generated by GS/GOGAT cycle metabolism, and then enter the plant free amino acid library to participate in the synthesis or transformation of other amino acids.

4.2. Coapplication of Glycine and Inorganic Nitrogen Promoted Root Growth in Maize

Root growth was highly promoted by the coapplication of glycine and inorganic nitrogen (Amm + Nit + Gly) in this study, although the shoot growth between Amm + Nit + Gly and Amm+ Nit was not significantly different (Figure 1, Table 2). Roots are important absorptive and metabolic organs in plants, affecting water and nutrient uptake and restricting shoot growth and development [74,75]. Glycine is involved in one of the production routes of betaine, and betaine can stabilize the structures of proteins and enzymes and protect the integrity of cell membrane, which are all conducive to root development [76]. Our results showed that compared with inorganic nitrogen, the combination of glycine and inorganic nitrogen increased the total root length by 24.8% in maize (Figure 3, Table 2). This is consistent with studies which found that amino acids enhanced rice and soybean root growth and nutrients uptake, thereby increasing crop yield [77,78]. The correlation analysis of root growth and plant nitrogen nutrition showed that the total root length and root surface area of maize have a highly significant positive impact on shoot nitrogen metabolism and nitrogen uptake (Figure 5), which indicates that the higher nitrogen uptake in the Amm + Nit + Gly treatment may be attributed to the longer roots. Glutamate is also an important amino acid, acting as a signal transducer through GLR receptors, which can regulate lateral root development [79]. Our results showed that the coapplication of glutamate and inorganic nitrogen also promoted root growth, which had a similar positive effect of root growth as glycine addition (Table 2).

The cell division rate of the maize root tip meristem is faster under \( \text{NH}_4^+ \) nutrition, which leads to higher root density and elongation of plants under the application of \( \text{NH}_4^+ \) compared to the application of \( \text{NO}_3^- \) [80]. In hydroponic systems provided with \( \text{NH}_4^+ \) or \( \text{NO}_3^- \), the shoot biomass of tomato plants was found to be similar, but all root parameters (biomass, length, branching, and area) were greater given \( \text{NH}_4^+ \) nutrition than \( \text{NO}_3^- \) nutrition [81]. Similarly, we found that the application of \( \text{NH}_4^+ -\text{N} \) was more beneficial to maize root growth than \( \text{NO}_3^- -\text{N} \) (Figure 3, Table 2).

4.3. Coapplication of Glycine and Inorganic Nitrogen Increased Soil Nitrogen Content

The coapplication of glycine and inorganic nitrogen significantly affected soil nitrogen content. Coapplication of glycine and inorganic nitrogen (Amm + Nit + Gly) showed the highest soil inorganic nitrogen and total nitrogen content, while nitrate applied alone (Nit) had the lowest soil inorganic nitrogen (Figure 4). Nitrogen fertilizer applied to the soil may be lost to the surrounding environment through denitrification, volatilization, surface runoff, and leaching processes [82]. Zhang et al. [65] found that the use of \( \text{NH}_4^+ \) can enhance plant nitrogen recovery in comparison to \( \text{NO}_3^- \), subsequently leading to a reduction in the loss of nitrogen fertilizer in the soil. In other words, nitrate fertilizer is more easily leached and lost in the soil than ammonium fertilizer. Therefore, the soil inorganic nitrogen and total nitrogen content were the lowest in the treatment of nitrate applied alone (Nit). In soil, amino acids can be mineralized into inorganic nitrogen [83–85]. Amino acids, as high-quality nitrogen sources, can stimulate the growth and activity of soil microorganisms when they are applied to soil [86,87]. During the proliferation process of microorganisms, a portion of inorganic nitrogen is converted into microbial nitrogen and fixed in the soil, and the microbial nitrogen can be released for the later growth of crops, thereby reducing nitrogen loss and facilitating the sustainable supply of soil nitrogen [88]. Therefore, when amino acids were combined with inorganic nitrogen, the soil inorganic nitrogen and total nitrogen contents were higher. Soil nitrification intensity is positively
correlated with nitrogen accumulation, and nitrogen use efficiency can be increased by improving nitrification [89]. Li et al. [72] found that high levels of glycine promoted the conversion of NH$_4^+$-N to NO$_3^-$-N and improved nitrogen utilization efficiency. It can be seen that the application of glycine is beneficial to improve the nitrogen nutrition of maize. Soil inorganic nitrogen content and soil total nitrogen content were found to be closely related to nitrogen uptake, and nitrogen uptake had a significant positive and direct effect on maize biomass (Figure 5). This indicates that both the transformation of nitrogen in the soil and the transportation of nitrogen absorbed by plants ultimately affect nitrogen nutrition and plant growth.

Soil pH value was affected by the nitrogen forms. Soil supplied with ammonium showed the lowest soil pH among the treatments. This was understandable since the pH of a 0.1 M (molar) solution of ammonium sulfate is around 5.5. Ammonium is positively charged and tends to adhere to negatively charged soil particles. For nitrate, it is easier to be leached on a neutral or alkaline soil than ammonium. In this study, the soil inorganic nitrogen content in the nitrate treatment is lower than that in the ammonium treatment. As a result, the nitrogen uptake of maize supplied with ammonium was higher than that supplied with nitrate. This preference of ammonium than nitrate was in line with the previous studies [65].

5. Conclusions

Our findings demonstrate that different nitrogen forms had a significant impact on plant nitrogen absorption and metabolism. Specifically, the soil inorganic nitrogen content was 22.8% higher in the treatment involving Amm + Nit + Gly than the Amm + Nit treatment, which had the effect of altering both soil nitrogen levels and root development. Furthermore, the treatment of Amm + Nit + Gly exhibited the longest total root length. Consequently, the maize plants in the Amm + Nit + Gly treatment exhibited a 35% improvement in nitrogen uptake compared to those in the inorganic treatment (Amm + Nit). We conclude that glycine combined with inorganic nitrogen is a preferable nitrogen source for maize growth that can increase soil inorganic nitrogen and total nitrogen content, promote root growth, and increase shoot nitrogen uptake, leaf GS activity, and organonitrogen compound content, thereby promoting plant nitrogen nutrition. Therefore, it is suggested that substituting certain inorganic fertilizers with organic alternatives like amino acids in agricultural production can not only enhance plant growth, but also may reduce the reliance on inorganic fertilizers and minimize the potential nutrient loss.

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