Reviewer Comments:

Reviewer

Thank you very much for your careful review of our manuscripts and constructive suggestions. We use black text to mark your questions. The content of our answers to your questions is marked with blue text. The place where the article content is modified is marked with red text.

I believe the introduction is very well articulated and written, addressing the state of the art appropriately and outlining the objectives that this work aims to address correctly. I believe the article has substantial experimental content, and the results are presented and discussed correctly. However, it is true that studies of this kind are numerous in the literature with other types of plants, making the study itself less original. I don't see the authors referencing similar studies conducted with tomatoes and discussing their results in comparison with those available in the literature. In my opinion, the authors should improve on this aspect.

Response：

Dear Reviewer,

Thank you for your kind comments. In tomato, previous studies have found that nitrogen can significantly promote the growth and development of tomatoes. They only studied the ratio of ammonium to nitrate, and the effect of 25 % AN: 75 % NN treatment was the best. However, in our study, through the mixed application of nitrate nitrogen, ammonium nitrogen and urea, it was found that 25 % NN: 75 % UN had the best effect and was better than 25 % AN: 75 % NN treatment. We made a detailed reply in Response 5 and made corresponding modifications in the manuscript.

1. What nitrogen source was used for the control (CK)? Please specify the concentration and the type exactly.

Response 1：

Dear Reviewer,

Thank you very much for your comments. The control (CK) in this study did not add any nitrogen source, and the application amount of other elements was the same as that of normal treatment except for the absence of nitrogen source.

2. The materials and methods seem to be described in a very detailed, specific, and accurate manner. I just have one question; on many occasions, the authors mention: "three tomato plants with uniform growth were selected from each treatment" I think choosing only 3 plants for each of the treatments is too low a number. Why didn't the authors select a larger number to increase reproducibility?

Response 2：

Dear Reviewer,

Thank you for your careful review and constructive suggestions for our manuscripts. I have made the following response to your question:

In this study, seedlings with consistent growth were selected after germination, and transplanted to 50-hole seedling trays, respectively, and placed in an artificial intelligence climate incubator for seedling raising. Strict management processes were maintained during the seedling raising process. When the tomato seedlings were cultured in an artificial intelligence climate incubator to 4 leaves, we selected the tomato seedlings with consistent growth conditions for standardized transplanting, and waited for the tomato seedlings to grow for one week after transplanting. Then, on the first day of fertilization treatment, 10 tomato seedlings with consistent growth were selected for marking. All aspects of the whole growth and development period of tomato were carried out under our artificially controllable environmental conditions. Until the sampling, we selected the marked 5 tomato plants, and then mixed the 5 tomato plant samples of the same treatment. Because we strictly screened the samples with consistent growth for the determination of subsequent test indicators, the remaining tomato plants with more extreme growth conditions were not used for the determination of test indicators, which improved the fitting degree between the three replicates. This was consistent with the effect and purpose of determining five repetitive tasks.

3. I don't see it appropriate to include Figure 2, as differences are very difficult to discern at a glance, and moreover, they are unique examples for each condition. Additionally, the results are quantified in Table 3; I advise the authors to remove it,

as it contributes little scientifically. I also advise that the results in Table 3 be presented as column graphs to facilitate comprehension.

Response 3：

Dear Reviewer,

Thank you for your careful review and constructive suggestions on our manuscript. We have removed Figure 2 from the manuscript and revised the results in Table 3 to a bar chart. We have also made corresponding modifications in the manuscript. Our changes to the content of the article are marked in red text

Line:321

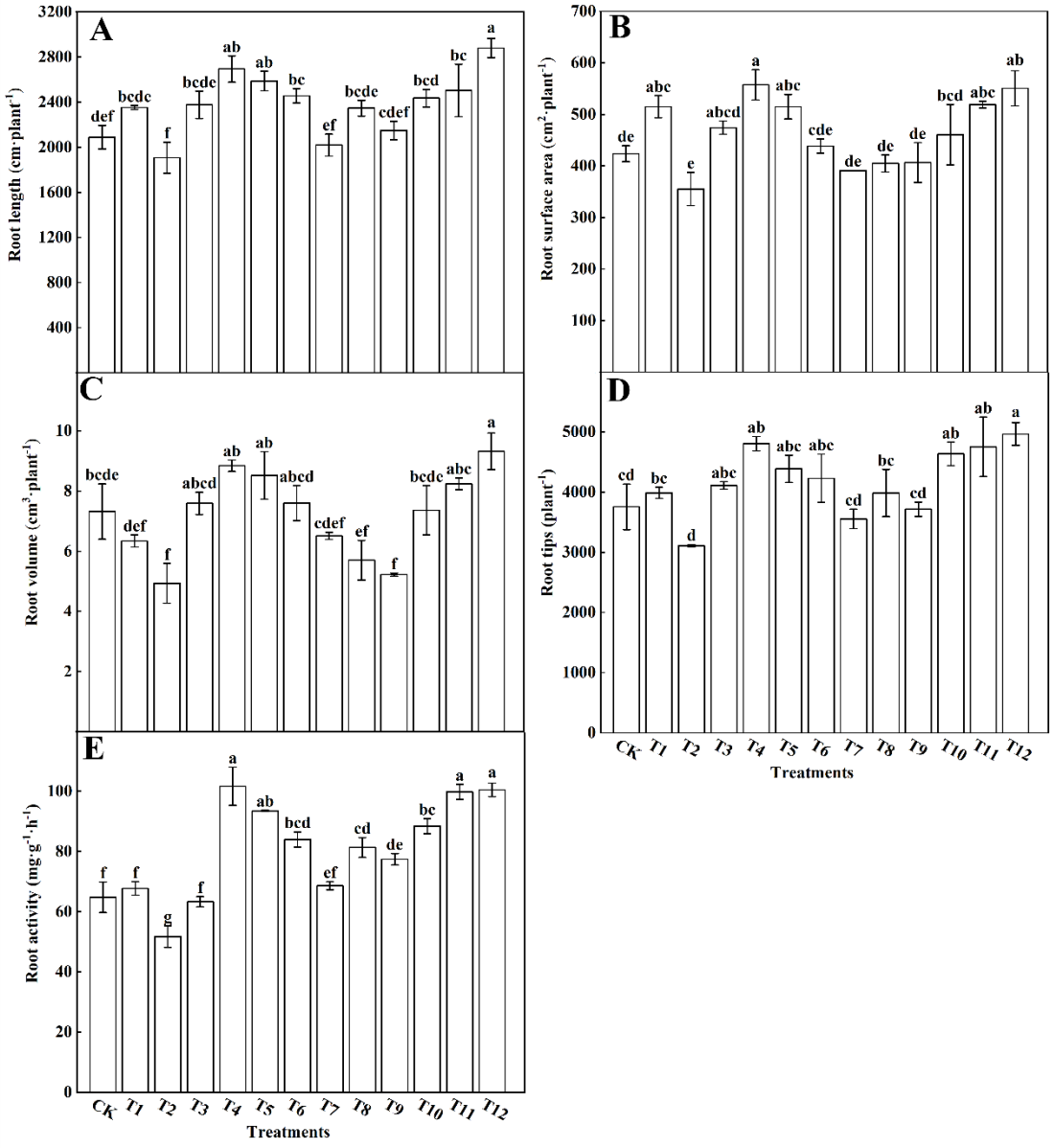

Figure 2. Effects of different forms of nitrogen on tomato root parameters. The short vertical line of the bar chart indicates the average value ±standard error (n = 3), and different letters in the same column indicate significant differences at the P< 0.05.

4. Change the order of Figures 3 and 4, as in the text, Figure 4 is mentioned before Figure 3.

Response 4：

Dear Reviewer,

Thank you very much for your suggestion. We have changed the order of Figure 4 and Figure 3 in the manuscript. And in the manuscript also made a corresponding modification.

Line:349-388

5. I believe the discussion is well-conducted, effectively comparing the obtained results with those of other authors in a very appropriate manner regarding other plants. However, concerning other studies that have also investigated the effect of different nitrogen fertilizers on tomatoes, this point needs to be substantially corrected.

Response 5：

Dear Reviewer,

Thank you very much for your careful review of our manuscript and constructive suggestions. "Regarding the fact that other studies have also investigated the effects of different nitrogen fertilizers on tomato, this needs to be substantially corrected", this is an issue that we have revised in the Discussion section of the manuscript and added references to studies conducted in tomato. Our changes to the content of the article are marked in red text.

Line:569-573

Kirkby et al. [40] discovered that when AN and UN were mixed, tomato plants exhibited increased lateral root length and lateral root numbers. In our study, 25%AN:75%NN treatment also significantly promoted root development in tomato plants. However, the 25%NN:75%UN treatment was more effective than the 25%AN:75%NN treatment.

Line:576-579

It indicated that the high proportion of UN and NN combined application could promote the absorption and utilization of nitrogen in tomato plants, and then improve the growth of tomato plants. The promotion effect of a high proportion of NN combined with UN on tomato growth was very weak.

Line:594-600

Zou et al. [48] studied tomato roots under different ammonium supply levels and found that when the NH_4^+ supply exceeded 0.5 mmol L^{-1}, the main root length, lateral root number and plant height decreased with the increase of NH_4^+, and 10 mmol L^{-1} NH_4^+ almost completely inhibited lateral root formation, which was consistent with our results. Therefore, the appropriate amount of NH_4^+ combined with NO_3^- can increase the absorption and utilization of nitrogen in tomato plants, reduce the toxicity of NH_4^+ to tomato, and promote the growth of tomato roots and plants.

Line:607-615

This may be due to the combined application of 25% AN and 75% NN, 25% NN and 75% UN significantly increased the flow of photosynthetic carbon to amino acids, which in turn led to an increase in photosynthetic enzyme content, while a high proportion of ammonium nitrogen inhibited the synthesis of photosynthetic products, resulting in a decrease in photosynthetic carbon content, which is consistent with the results of Golvano et al. [49]. Glaussen et al. [50]and Puritch et al. [51] also found the accumulation of ammonium nitrogen in plant leaves may lead to the uncoupling of electron transport in chloroplasts to form phosphorylation, which eventually leads to a decrease in photosynthetic rate.

Line:653-658

Singh et al. [65] found that nitrate nitrogen sources can protect photosynthetic pigments from ammonium nitrogen stress in tomato leaves, which competitively reduces the absorption of ammonium nitrogen by tomato plants. Moreover, the mixed application of nitrate nitrogen with urea or ammonium nitrogen had the best effect, and the high concentration of nitrogen source (15 mmol L^{-1}) was more conducive to the growth and development of tomato seedlings.

Line:673-685

Nasraoui-Hajaji et al. [70] observed that tomato plants receiving ammonium nitrogen or amide nitrogen exhibited lower rates of CO_2 assimilation, stomatal conductance, and transpiration compared to those supplied with exclusively NO_3^--N as the nitrogen source. Furthermore, the inclusion of nitrate in the nitrogen supply mitigated the reduction in these parameters. These findings suggest that a combination of low ammonium and high NO_3^--N supply is conducive to promoting the growth and development of tomato plants [71]. In our study, it was found that the application of 75% UN: 25% NN (T12) ratio significantly increased the gas exchange parameters, chlorophyll fluorescence parameters and net photosynthetic rate of tomato plants, and the effect was better than that of 75% NN: 25% AN (T4) ratio. Therefore, the effects of different nitrogen forms on photosynthesis of tomato were different.

Line:706-713

This phenomenon may be attributed to ammonium toxicity limiting the activity of Rubisco, which, in turn, results in decreased photosynthetic capacity and subsequently impacts the light utilization and overall performance of tomato plants [78,79]. In this study, single NN, AN and UN treatments reduced the expression of *SlRbcL* and *SlRbcS* genes. The addition of UN in-creased the expression of *SlRbcL* and *SlRbcS* genes. Therefore, we believe that UN can regulate the effect of a single nitrogen form on the activity of photosynthesis-related enzymes, thereby enhancing the photosynthetic capacity of tomato plants [80].

Line:718-724

However, we found that urea could not only reduce the effect of ammonium toxicity on tomato plants, but also significantly increase the enzyme activity and relative gene expression of Calvin cycle involved in photosynthesis of tomato plants through the study of NN combined with UN. In particular, the expression of Rubisco size subunit was elevated, which promoted the photosynthetic carbon cycle and organic matter synthesis. This provided a material basis for the plant to develop a larger leaf area and biomass, which ultimately promoted the growth of tomato.

Line:742-745

However, our findings were different from previous studies. We found that 25% NN: 75% UN treatment had better effect on the synthesis of tomato photosynthetic products than 25% AN: 75% NN treatment. This may be related to the sensitivity of different plants to nitrogen forms.

Line:759-764

The size of leaf area can directly reflect the nitrogen supply and can be used to evaluate any species [75]. The leaf area of tomato plants treated with 25% NN: 75% UN was larger than that of other nitrogen forms. The larger the leaf area, the more light-energy absorbed by the leaves. This effect was consistent with the increase in the accumulation of photosynthetic product content after mixed application of 25% NN: 75% UN treatment.

Line:841-852

Horchani F et al. [93] found that with the increase of external NH_4^+ concentration, the GDH activity showed an upward trend and *SlGDH* also up-regulated expressed. In our study, the enzyme activity and gene expression of GDH were the highest after single AN treatment, and the enzyme activity of GDH decreased with the decrease of AN application ratio. However, under the condition of 75% UN: 25% NN treatment, the GS activity of tomato leaves increased significantly, and the gene expression levels also increased. This indicated that the metabolism of NH_4^+ in tomato plants was mainly dependent on the GS-GOGAT pathway at normal levels. GDH may play an important role in metabolizing excessive ammonium and reducing ammonium toxicity in plants [94,95].

6. L733-L752: I advise the authors to replace this paragraph used as a summary with another that plays a role in presenting the main conclusions of the study.

Response 6：

Dear Reviewer,

Thank you very much for your kind comments and constructive suggestions, we have revised this passage in the manuscript.

Line:858-882

Nitrogen fertilizer plays a pivotal role in influencing the growth and development of tomato plants. As the global tomato cultivation area continues to expand, farmers often resort to excessive nitrogen fertilizer applications in a bid to boost yields. However, the indiscriminate choice of nitrogen fertilizers not only hinders the effective absorption and utilization of nitrogen by tomato plants but also exposes them to potential toxicity, with adverse environmental consequences. In summary, a nitrogen blend of 75% UN: 25% NN and 75% NN: 25% AN has been demonstrated to significantly enhance various growth parameters in tomato plants. These effects encompass increased plant height, stem diameter, leaf area, and root morphological attributes. Furthermore, these nitrogen ratios have also been found to enhance gas exchange parameters, chlorophyll fluorescence parameters, and the accumulation of photosynthetic products in tomato leaves. This results in an overall improvement in leaf photosynthetic capacity. Conversely, the combination of AN with UN has been shown to inhibit the growth of tomato plants. Additionally, the treatment involving 75% UN: 25% NN significantly elevates the activities of enzymes associated with nitrogen metabolism, the Calvin cycle, and sugar metabolism, along with their corresponding gene expression levels (Figure 14). This suggests that 75% UN: 25% NN can upregulate the expression of genes related to nitrogen metabolism, the Calvin cycle, and sugar metabolism, consequently increasing enzyme activities. This, in turn, promotes a higher net photosynthetic rate and greater accumulation of photosynthetic products in tomato plants. In contrast, a high proportion of AN has been observed to restrain root growth, subsequently impeding the overall growth and development of tomato plants. From a grower's perspective, it is advisable to consider the utilization of NN and UN in the fertilizer regimen to reduce the reliance on AN. This study provides a valuable practical foundation for tomato cultivation and sustainable agricultural fertilization management in the future.

7. L551: "Some researchers believe that the accumulation of ammonium nitrogen in plant leaves may lead to the uncoupling of electron transport in chloroplasts to form phos phorylation" I'm sorry, I don't understand this sentence. Could you please rephrase it?

Response 7：

Dear Reviewer,

Thank you very much for your careful review of our manuscript. I have made the following response to your question:

Photosynthesis in chloroplasts requires ATP to provide energy, and the process of generating ATP from the combination of ADP and Pi (phosphorylation) that occurs in the chloroplast-like vesicle membrane needs to be catalyzed by ATPase (coupling factor), which also catalyzes the hydrolysis of ATP and couples the transport of H^+ to the intravascular membrane of the vesicle. It was shown that the accumulation of NH_4^+ can lead to an increase in the permeability of the vesicle-like membrane to protons or an increase in the ability of coupling factors to permeate protons, with the result that the transmembrane H^+ electrochemical potential is eliminated. However, electron transfer could still take place, even at a rapid rate. Because the inhibition of electron transfers by high internal H^+ concentrations was removed. But phosphorylation the process in which biochemical reactions added phosphate groups and absorbed energy to rebuild high-energy bonds (ATP) no longer took place. Therefore, accumulation of ammonium nitrogen may lead to uncoupling of electron transfer in chloroplasts and formation of phosphate groups, reducing the photosynthetic rate of leaves. The results of the present study yielded similar results. In our study, 25% AN:75% NN (T4) significantly increased the photosynthetic rate of tomato leaves at the same N application rate. However, the net photosynthetic rate was significantly reduced when a single AN (T2) was applied or when more than 50% of AN was applied (Fig. 4), which could be attributed to the accumulation of ammonium nitrogen in the leaves leading to the uncoupling of electron transport in the chloroplasts, resulting in the formation of phosphorylation and the inhibition of ATP synthesis. This led to the decrease in photosynthetic rate of tomato plants (Claussen W and Lenz F, Plant and soil,1999; Puritch G S and Barker A V, Plant Physiology,1967).

Line：

8. L674: "ammonium is the primary storage and transportation form of ammonium" I don't see the point of this sentence. Could you provide a better explanation of what you mean?

Response 8：

Dear Reviewer,

Thank you for your kind comment and I am very sorry that there is some error in this sentence. I have made the following modifications to this sentence: Glutamine is the primary storage and transportation form of ammonium. And made corresponding modifications in the manuscript.

Line:785-788