

Interactive comment on “Interactions between uptake of amino acids and inorganic nitrogen in wheat plants” by E. Gioseffi et al.

Anonymous Referee #2

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The manuscript describes three experiments investigating the uptake of glycine, glutamine, NH_4^+ and NO_3^- by wheat plants. The subject matter is interesting and of importance to understanding the acquisition of N by plants. Unfortunately, the value of the manuscript and its conclusions would have been much greater if the experiments had been better designed for their stated purpose. There is also a need for much clarification of the description of the experimental methodology and there are some aspects of the data, which give me cause for concern. In my opinion, there is a great deal to do to this manuscript if it is to be suitable for publication in Biogeosciences. Some more specific points are:

In the Abstract, it is stated that the underlying hypothesis was that amino acids may lead to the down-regulation of inorganic N uptake. If this truly was the hypothesis, the

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experiments are poorly designed to test it. In my opinion, the only effect of one form of N on the uptake of another that is really tested is the effect of NO_3^- on glycine uptake. Only at this point is a single concentration of a solute applied with and without the presence of the potentially competing form of N. As the authors themselves point out, rates of solute uptake by roots are generally concentration-dependent and the effect of concentration varies between solutes. Thus, if the comparison of uptake rate with and without the competing N form is not carried out at the same concentration, it is hard to know how to interpret the results. In Fig. 4, uptake of NO_3^- alone is shown and alluded to in the Abstract, but I am unclear what this refers to as I can find no description of the methodology for this. I am also confused by the fact that uptake of NO_3^- is shown in this Fig. for a treatment where in the methods it states that glycine alone was used. There is no straightforward test of the effect of the presence of amino acids on uptake of NH_4^+ or the effect of the presence of NH_4^+ on amino acid uptake.

It is also stated in the Abstract that NH_4^+ was taken up at about twice the rate of organic N. According to the statistical information shown in Fig. 3, glycine N was taken up at the same rate as NH_4^+ . This kind of fundamental error gives me serious cause for concern about the quality of this investigation.

P11313 L2. adsorbed?

P11314 L8 and various places in the Introduction. I do not understand why Experimental is capitalised.

Materials and Methods Experiment 1.

It is not clear to me exactly what the experimental set up was here. It sounds as though four replicate containers were used for each N treatment, each with four pseudoreplicate plants. Is this correct? How large were the plants? In the description of the basic nutrient solution, NO_3^- is a counter ion for Mg, Ca, K and Fe. How was this altered in the N treatments? There are also questions regarding the concentrations of other ions. A complete breakdown of the nutrients in the different treatments would be helpful

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information for the reader.

Experiment 2. As I mention above, it is not at all clear what was done in this experiment. Was NO_3^- added alone? If so, at what concentration was it supplied? Why does Fig. 4 show NO_3^- uptake in a treatment where only glycine was supplied? Please clarify the replication as above.

Experiment 3. The description of this is not clear. What was the composition of the nutrient solutions without NO_3^- ? What is the source of the data shown for NO_3^- in Fig.7? Please clarify the replication.

P11317 L6-8 Please give more detail of the use of $^{15}\text{NH}_4^+$ and $^{15}\text{NO}_3^-$. I can find no other mention of this.

Table 1. It would help the reader if data for the whole plant were supplied as this is the real focus of the manuscript. Partitioning between root and shoot is interesting, but of secondary importance in my opinion.

P11319 L4-6 It seems contradictory that plants receiving NO_3^- and glutamine should have the highest N content when much is made of the fact that glutamine was taken up at a lower rate than NO_3^- . This requires some comment at least.

P11319 L8. The C to N ratios for glutamine and glycine are the wrong way round.

Fig.2 To which form of N do the error bars refer in the mixed treatments?

P11319 L16-18 As I mentioned above, Fig. 4 shows the uptake of glutamine N to be the same as NH_4^+ and all mixed N treatments.

P11319 L25-26 What form of statistical analysis is being referred to? How was this test conducted?

P11320 L2 Pre-starvation is not mentioned in the methods.

Fig.6. I find it rather worrying that recovery of the ^{15}N is so low. There seems to be

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no obvious reason for this. There also appears to be a tendency for the recovery to be lower in the glutamine treatments. I think that some explanation is necessary. I also wonder what effect there was of more-or-less completely depleting the solutions of organic N on the results i.e. the concentration of the solutes changes dramatically over the experimental period. Further, I wonder how there can be almost complete depletion of organic N from solution, yet have greater uptake of inorganic N (Fig.2). This does not seem possible and gives me severe reservations about the quality of the presented data. Could differences found between the rates of uptake of different forms of N be influenced by the different ways of measuring them utilised?

Fig.7. Why is data for shoots presented alone?

Discussion. In my opinion, the interpretation of the data needs completely re-evaluating with a much more critical eye. Because of the design of the experiments, I think that what can be said about the effect of organic and inorganic forms of N on each other is limited. Perhaps if the inconsistencies in the data are attended to and the description of the experiments is clarified, a clear message with scientific merit can be discussed.

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