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Biogeosciences Discussions

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

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We thank Referee #1 for the positive comments and constructive suggestions which have helped us to improve our paper.

In the revision we have been able to address all the questions and to incorporate all the suggestions of Referee #1 as explained below:

Referee comment #1. The manuscript is a rather short and straight forward and may benefit with the addition of some references concerning amino acid uptake by wheat (such as Näshom et al, New Phytologist, 2001) or the interaction between organic and inorganic N uptake by other pants (such as Persson and Näsholm, 2002). The iconography is sufficiently clear.

Response: The references have been added in the Introduction and Discussion

Referee comment #2. Abstract Complete and concise. It is mentioned in this section that amino acid uptake and inorganic N uptake were measured with very different techniques (15N, 13C labelling for amino acids, rate of removal from the solution for nitrate and ammonium). This is of major importance for interpretation of the data, and I am surprised that this point is very quickly mentioned in the material and method section and discussed nowhere.

Response: The following discussion of the strengths and weaknesses of the two different techniques has now been added in a separate section: "Two different methods, viz. stable isotope labelling with 15N-13C amino acids and NO3-/NH4+ depletion in the nutrient solution, were used to measure the separate contribution of organic and inorganic N sources to total plant N acquisition. Technically, the two methods differ in that plants are harvested destructively and subsequently analysed to obtain the uptake of the isotopically labelled compound, while the nutrient solution in the root medium is sampled and analysed at different time intervals to determine the rate of solute removal by the roots. However, the two methods basically reflect the same underlying process viz. root uptake, and the two methods will give similar results provided other experimental and analytical factors are not interfering. Uptake rates of isotopically labelled amino acids might be underestimated if N losses during their assimilation in the plant, while on the other hand inorganic N uptake rate could be overestimated if there were N losses from the solution via e.g. volatilization. As discussed in section 4.2 we do not consider these sources of error to have had a major impact on our results. Rasmussen and Kuzyakov (2009) and Rasmussen et al. (2010) recently used 14C/13C/15N triple-labelling to show that the simultaneous uptake of inorganic C interfered with root uptake of dual-labelled organic N. Unfortunately, a similar methodological option is not available for N because the only isotope that can be implemented is 15N. The radio-isotope 13N with a half-life just below 10 min has been used for measurement of short-term uni-directional fluxes in plant roots (Ter Steege et al., 1998) but

can not be used in studies that also aim at revealing interactions associated with N assimilation and growth responses which are manifested over a period of time which is longer than a few minutes or hours. There is a methodological challenge in proving the uptake of intact amino acids in experiments where their uptake is studied over an extended period of time. Persson and Nasholm (2002) used different metabolic inhibitors to prevent assimilation of the absorbed amino acids in order to analyse their accumulation inside the plant. This approach may lead to secondary feed-back processes affecting the absorption of both inorganic and organic N forms."

Referee comment #3. Material and methods and results taken. These sections are not always clear, and the scientific methods are not always clearly outlined. It would be of interest to explain the specific aim of each experiment. Also I am confused about the incubation time used in each experiment. In experiment I, I do not see where the duration of the labelling experiment is explained. 4 time points are mentioned p 11316 but we do not know which one was used in the figures where no more information is added. In experiment II it is said that gly uptake was measured over 4 days, but a 3-day period is mentioned in figure 4 legend. I presume the authors did use single 15N labelled Gln, but it could be interesting in which position was the N was labelled, as gln can be taken up as glutamate.

Response: In the introductory part of the methods section there is an explanation of the specific aim of each experiment. The duration of the labelling period in experiment I has been added to the text in the methods section. The confusion about the length of the glycine exposure in experiment II has been corrected in the method section. The requested information about the 15N labelled glutamine has been added (both N atoms were labelled) although we suppose that glutamine was taken up as the intact molecule, not as glutamate as discussed on p. 11323. The calculation of uptake rates based on the 4 time points mentioned p 11316 has been clarified in section 2.3.

Referee comment #4. Discussion I agree with the major conclusions here but the authors should be more careful in some places. For example p 11320, the authors cannot

write that uptake rates were twice higher for NO3 and NH4 compared to amino acids, as NH4 uptake rates and gly uptake rates shown on figure 2 are not significantly different. Also, it is clearly established that amino acid uptake is concentration dependant, and the comparison between figure 2 and 4 cannot be used to discuss this point as plants were different (not the same age as far as I can see) and no statistics are shown.

Response: Statements on differences in uptake rates have been made more specific at p 11319 lines 16-18 and at p 11320 lines 20-22. Plants used in Experiment I and II had the same age (they were both grown for 30 days before treatments were started). Although we agree that the literature shows examples of correlations between concentration and amino acid uptake we did actually not find substantial differences between uptake rates in Experiments I (2 mM glycine) and Experiment II (1mM glycine). Generally, uptake rates of inorganic N were about twice as high as those of organic N compounds.

Referee comment #5. P 1322 lines 24-28. I do not find this point very convincing or very clear. The low amount of labelled C remained in the solution does not mean that no de-amination of amino acids occurred before uptake, as it is likely that this C has been respired by microorganism and released in the atmosphere, as suggested by the authors and by numerous studies.

Response: These aspects were already discussed at p 11322-11323: We have now clarified the wording, and substantiated the documentation of our interpretation. The following paragraphs have been added to the Discussion section: Lower excess 13C:15N ratios in plant tissues relative to applied amino acids might also result from de-amination of amino acids before uptake, followed by uptake of inorganic 15N. It does not seem likely that this process contributed significantly in our experiments, because only a few percentages of the 13C were recovered in solution at the end of the experiment (Fig. 6). If the amino acids had been decomposed by microorganisms and the N released in inorganic form to the solution and subsequently taken up by the roots, a significant portion of the labelled C would have remained in the bacteria without being

lost by respiration during the 3 day experimental period. The utilization efficiency of easily decomposable substrates typically varies between 0.4-0.7 within the first days to week of decomposition (Parton et al., 1987; Steinweg et al., 2008; Thiet et al., 2006). This means that the amount of C lost by microbial respiration would be roughly similar to the amount retained in the microbial biomass. Consequently, taking into account the fact that only a few percentage of 13C was recovered in the solution at the end of the 60-h experimental period (Fig. 6), losses of C following microbial decomposition are only likely to have been responsible for a few percentages of the unrecovered 13C.

Referee comment #6. Conclusions Line 5. The conclusion that ammonium causes a down regulation and vice versa is very surprising, as this is shown nowhere convincingly in the paper. This is a neat paper and I have full confidence the authors can respond constructively to these comments.

Response: We agree that out data cannot support literature showing down-regulation of ammonium by amino acids (cited p 11321), and we have modified the conclusion section accordingly.

Thanks for the very nice concluding comment!

Interactive comment on Biogeosciences Discuss., 8, 11311, 2011.