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Letter to the Editor

Carbon isotopes as proof for plant uptake of organic nitrogen: Relevance of inorganic carbon uptake

Dear Editor

Ten years ago the paper of Näsholm and co-workers (Näsholm et al., 1998) renewed the discussion of the 60ties (e.g. Birt and Hird, 1958; McKee, 1962; Borstlap, 1974; Fokin et al., 1993) about direct uptake of organic nitrogen (N) by plants. Thereafter, an increasing number of studies were pointed on the possibility of direct uptake of organic N sources (mainly amino acids) by plants in various ecosystems. The most of these studies used a similar methodology to that of Näsholm et al. (1998), cf. uptake of ^{14}C or ^{13}C either alone or in dual-labelling with ^{15}N, as evidence of organic N uptake. The prerequisite assumed, but not proven in all these studies is that the total labelled C from the added amino acid recovered in the plant corresponds to the amount of the initial amino acid taken by the plant, i.e. not mineralized to CO_2 and NH_4^+ or not transformed to other organic metabolites.

The possibility of root uptake of inorganic ^{14}C or ^{13}C is not discussed in these studies (e.g. Streeter et al., 2000; Rains and Bledsoe, 2007; Xu et al., 2008; Biernath et al., 2008). However, root uptake of HCO_3^- and its incorporation into plant tissue C via phosphoenolpyruvate carboxylase (PEP carboxylase) is well documented (Cramer and Lips, 1995; Wanek and Popp, 2000; Ford et al., 2007). In addition, a study by Govindarajulu et al. (2005) showed that mycorrhizal uptake of dual-labelled organic N can be the reason for the presence of labelled C in roots, as intact organic N was found to be transported from the extraradical mycelium to the intraradical mycelium, but the transfer to the host roots took place in form of NH_4^+. Thus in a recent study by Harrison et al. (2008) addressing the uptake of dual-labelled glycine, serine, and phenylalanine in five grass species, the ^{15}N-enrichment in the shoots was found to be greater than the ^{13}C-enrichment of the shoots for glycine and serine, whereas the opposite was the case for phenylalanine. The authors discussed the uptake of inorganic ^{15}N from mineralization of the amino acids, which implies that ^{13}C in inorganic form must have been present in soil air and soil solution. However, the potential incorporation of neither $^{13}CO_2$ via photosynthesis (Streeter et al., 2000; Rasmussen et al., 2008) nor root uptake of $H^{13}CO_3^-$ from soil solution were discussed, although these possible pathways serve as likely explanations for the higher ^{13}C- than ^{15}N-enrichment found for phenylalanine in the study of Harrison et al. (2008). Bicarbonate might have the same entry mechanisms into the plant root as anions such as nitrate and chloride via mass flow and co-transport with H^+ (Marschner, 1995).

Biernath et al. (2008) studied the uptake of organic N in maize using ^{14}C and ^{15}N tracers. They found that $<1\%$ of the ^{14}C added as alanine to the rhizosphere was found in the leaf material after 9 days. Based on a pH value of 6.9 in the soil used, it can be calculated that ^{14}C activity found in the leaf material corresponds to less than 0.1% of the HCO_3^-, which dissolves in the soil water at that pH (see Appendix). Furthermore the finding of more than 60% of the ^{14}C added leaving the soil–root system as $^{14}CO_2$ strongly suggests that dissolved $H^{14}CO_3^-$ has been present in the soil solution. In the study of Biernath et al. (2008) the tubes with the roots were ventilated, which might have reduced the calculated amount of HCO_3^- dissolved in the soil solution.

Ford et al. (2007) found that approximately 1% of total plant C originated from root uptake of inorganic C. Although this contribution may seem insignificant for the C budget of the plant, it may account for a significant proportion of labelled C when root uptake of dual-labelled organic N is studied and may strongly bias the real uptake of organic N. Furthermore, Ford et al. (2007) found a higher incorporation of inorganic C from HCO_3^- in root than shoot tissue, the fact frequently reported also from dual-labelled organic ^{15}N and ^{13}C or ^{14}C studies (e.g. Näsholm et al., 2000; Rains and Bledsoe, 2007; Biernath et al., 2008).

Jones et al. (2005) excellently reviewed the methodological difficulties when using dual-labelled amino acids to study the uptake of organic N, but did not discuss the aspect of uptake of labelled inorganic C. In future studies of organic N uptake it is necessary to include a treatment to evaluate the inorganic C uptake in order to justify whether uptake of labelled C is really taking place in organic form and to correct the values of organic N uptake. The inclusion of control treatments where $H^{14}CO_3^-$ or $H^{13}CO_3^-$ and $^{15}NH_4^+$ or $^{15}NO_3^-$ are added in the same ratios as the dual-labelled organic N compounds could be a method for testing this. A clear test of direct root uptake of amino acids would be compound specific ^{13}C or ^{15}N analysis of the added amino acid as recovered from roots and/or shoots if ^{13}C or ^{15}N ratios of added and recovered amino acids are equal (Glaser, personal communication).

Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2009.03.006.

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