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Short communication

Plant uptake of dual-labeled organic N biased by inorganic C uptake: Results of a triple labeling study

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ABSTRACT

Direct plant uptake of organic nitrogen (N) is often studied using the dual-labeling approach ($^{15}N + {}^{13}C$ or $^{15}N + {}^{14}C$). However, the method might be hampered by uptake of labeled inorganic carbon (C) produced by mineralization of labeled organic compounds. Here we report the results from a triple labeling experiment ($^{15}N + {}^{13}C + {}^{14}C$) investigating whether root uptake of labeled inorganic C can bias the results obtained in studies of organic N uptake using dual-labeled amino acids (^{15}N, ^{13}C). In a rhizosphere tube experiment we investigated ^{13}C and ^{14}C uptake by maize either supplied with labeled glycine or CO_3^{2-}, but found no differences in uptake rates between these C-sources. The uptake of inorganic C to the shoot tissue was higher for maize grown in full light compared to shading, which indicates a passive uptake of inorganic C with water. We conclude that uptake of inorganic C produced by mineralization of amino acids can significantly bias the interpretations of organic N uptake studies using dual-labeling.

For many years plant N acquisition have been assumed to mainly occur in the inorganic N forms, NO_3^- and NH_4^+. The finding of Näsholm et al. (1998) that plants take up N in organic form renewed the discussion of sources for plant N acquisition. Various methods have been used to document plant uptake of organic N such as amino acid depletion from liquid cultures (El-Naggar et al., 2009), bulk measurement of dual-labeled amino acid uptake (Fokin et al., 1993; Näsholm et al., 1998), and compound specific isotope analysis of intact dual-labeled amino acids in shoots and roots (Näsholm et al., 2001; Sauheitl et al., 2009a). Bulk measurement of uptake of dual-labeling with ^{13}C (or ^{14}C) and ^{15}N has been used for the last decade in a great number of studies (e.g. Sauheitl et al., 2009b; Streeter et al., 2000; Weigelt et al., 2005). The prerequisite is that the total amount of labeled C recovered in the plant corresponds to the amount of labeled amino acid taken up by the plant, and not in form of C mineralized to CO_2. However, root uptake of inorganic C has been studied since the 1950 (Graf and Aronoff, 1955) and the incorporation of inorganic C into plant tissue via phosphoenolpyruvate (PEP) carboxylase is also well described (Britto and Kronzucker, 2005; Vuorinen et al., 1992). We recently addressed the possibility that the uptake of labeled inorganic C might bias the results from organic N uptake studies when using bulk measurement of dual-labeling (Rasmussen and Kuzyakov, 2009). In the present study we aimed to test the significance of this inorganic C uptake for studies on organic N uptake using bulk measurement of C and N dual-labeling. That is, if uptake of added inorganic tracer-C is in a similar order as added organic tracer-C, it would be impossible to distinguish which process caused the tracer-C to enter the plant. Thus, the prerequisite assumption that the total amount of labeled C recovered in the plant tissue originates from uptake of the added organic compound is not valid.

We performed a triple labeling study with maize seedlings grown in rhizosphere tubes (Biernath et al., 2008; Jones et al., 2005) to study the uptake of ^{14}C, ^{13}C, and ^{15}N applied to the soil in inorganic and organic form. Our experiment resembled that of Biernath et al. (2008), using the same soil and experimental setup, except that in the present study we used glycine and excluded ventilation of the rhizosphere tubes in order to maintain the partial pressure of CO_2 in the soil. The soil was a loamy haplic Luvisol taken from the A_p horizon at the University of Hohenheim Agricultural Research Station at Fildern, Stuttgart, Germany. The soil had a pH (H_2O) of 6.9,

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Table 1
Characteristics of labeling solutions. A: U–[^{13}C]–[^{15}N]–glycine + U–[^{14}C]–glycine, B: U–[^{13}C]–[^{15}N]–glycine + $Na_2^{14}CO_3$, C: $K^{15}NO_3$ + $Na_2^{13}CO_3$ + $Na_2^{14}CO_3$.

	A	B	C
Added N concentration in soil (μg N g soil^{-1})	0.13	0.13	0.15
Added C concentration in soil (μg C g soil^{-1})	0.22	0.23	0.11
^{15}N abundance (%)	98.0	98.0	10.4
^{13}C abundance (%)	98.0	98.0	97.9
Total ^{14}C-activity added per rhizosphere tube (kBq)	126	180	181

The specific activity was 1.85 GBq mmol^{-1} for $Na_2^{14}CO_3$ and 4.14 GBq mmol^{-1} for U–[^{14}C]–glycine.

pH ($CaCl_2$) of 6.5, C_{tot} and N_{tot} contents of 1.5% and 0.14%, respectively (additional information are given in Biernath et al., 2008). The roots of maize seedlings grown in rhizosphere tubes were sealed off at the soil surface with silicone rubber paste before amended with a labeling solution of either:

(A) U–[^{13}C]–[^{15}N]–glycine + U–[^{14}C]–glycine, or
(B) U–[^{13}C]–[^{15}N]–glycine + $Na_2^{14}CO_3$, or
(C) $K^{15}NO_3$ + $Na_2^{13}CO_3$ + $Na_2^{14}CO_3$

with N concentration of 0.13–0.15 μg N g^{-1} soil and C concentrations for solution (A) and (B) of 0.22–0.23 μg C g soil^{-1} and 0.11 μg C g soil^{-1} for solution (C) (Table 1) in order to resemble a 50% release of tracer-C from mineralization of the organic compound. Nitrate ($K^{15}NO_3$) was used as the inorganic tracer-N source in solution (C) as ammonium has been shown to enhance the dark incorporation of inorganic C in maize roots compared to nitrate (Cramer et al., 1993). Thus nitrate was the conservative choice of tracer-N in relation to inorganic C uptake. After addition of the labeling solution plants were grown in a growth chamber under either full light (295 μmol m^{-2} s^{-1}) or shading (55 μmol m^{-2} s^{-1}) in order to study the impact of reduced water uptake on the uptake of tracer-C. Maize plants were destructively sampled 24 h after labeling and divided into shoots and roots and weighed; no significant differences in dry matter (DM) yield were found between treatments (Table 2). We chose to sample the plants after 24 h in order to reduce a possible volatilization of CO_2 added as Na_2CO_3 or produced from respiration and mineralization of glycine. Untreated control plants were grown between labeled plants in order to correct for incorporation of ^{14}C and ^{13}C-labeled CO_2. Root and shoot samples were grinded to fine powder before analysis for ^{14}C-activity by combustion in a Packard model 307 sample oxidizer (Packard Instrument Company, Meriden, CT, USA) and ^{13}C- and ^{15}N-enrichment by an elemental analyzer (Carlo Elba, NC 2500) coupled with IRMS (Delta^{plus}, Thermo, Bremen, Germany). All data were analyzed using the GLM procedure of SAS (SAS Institute, 1999) in a two-way analysis of variance with tracer solution and lighting conditions as fixed effects.

Tracer-C (^{13}C and ^{14}C) from all three labeling solutions was found in maize shoot and root tissues (Fig. 1, Table 2). We found no significant differences in the total uptake of tracer-C in shoot or root tissue between glycine (solution A) and carbonate (solution B) application (Fig. 1, Table 2), whatever the light treatment. Total uptake of tracer-N in root tissue differed significantly ($P = 0.0003$) between the three labeling solutions in the order B > A > C, irrespective of light treatment. Simultaneous uptake of tracer-C and tracer-N from glycine (Fig. 1, Table 2) is believed to show the uptake of intact organic compounds (Näsholm et al., 1998) and the conclusions of many studies are based on this assumption. Näsholm et al. (1998) investigated the uptake of glycine in a forest soil with a pH of 3.1. At such a low pH, the concentration of dissolved bicarbonate and carbonate is very small and should therefore not influence the calculated intact amino acid uptake. However, in a number of later studies, soil pH has been above 5 (e.g. Kielland et al., 2006; Näsholm et al., 2000; Weigelt et al., 2005) and in the present study it was 6.9 (in H_2O). At this pH inorganic C can be dissolved in significant amounts (see supporting online material in Rasmussen and Kuzyakov, 2009) in soil water and – as shown here – taken up by the plants.

The half-lives of amino acids in soil are within hours (Jones et al., 2005). This implies that for example glycine will be rapidly mineralized to its inorganic components, as shown by the presence of labeled CO_2 in controlled experiments (Biernath et al., 2008; Jones et al., 2005). In Biernath et al. (2008) study, less than 1% of the ^{14}C added in the form of alanine was taken up by the plants, whereas more than 50% was recovered as $^{14}CO_2$ in air leaving the root system after 24 h. In the present study, 5–10% of the ^{14}C added was recovered from the shoot and root tissue after 24 h of labeling. We explain the difference in the recovery of ^{14}C in the plant tissue between the two studies as being due to a reduction in the partial pressure of CO_2 in soil due to ventilation of the rhizosphere tubes in the Biernath et al. (2008) study, although differences in plant preference for the used amino acids may also influence the interpretation (Jones, 1999; Sauheitl et al., 2009b). The closed soil system used in the present study most likely invoked soil air to have a higher partial pressure of CO_2 from added CO_3^{2-} or mineralized glycine, than would be found in an open soil system, causing a likely overestimation of the inorganic tracer-C uptake. However, the high uptake rate of tracer-C clearly shows that in this study it is impossible to distinguish if tracer-C entered the plant in organic or inorganic form; this to greater or lesser extent also applies to more open systems depending on the soil air ventilation. Thus, the

Table 2
Dry matter yields, ^{14}C-activity, ^{13}C- and ^{15}N-enrichment under full light or shading for shoot and root tissues of maize plants labeled with solution (A), (B), and (C).

			Yield g DM tube^{-1}	^{14}C-activity kBq g DM^{-1}	^{13}C-excess nmol g DM^{-1}	^{15}N-excess nmol g DM^{-1}
Shoot tissue	full light	A	0.11 ± 0.01a	68 ± 31a	50 ± 88a	108 ± 16ab
		B	0.12 ± 0.01a	51 ± 3ab	12 ± 35a	98 ± 14b
		C	0.12 ± 0.02a	47 ± 6ab	76 ± 82a	37 ± 16c
	shading	A	0.10 ± 0.01a	27 ± 4b	109 ± 71a	100 ± 17b
		B	0.09 ± 0.02a	30 ± 2ab	62 ± 92a	154 ± 24a
		C	0.09 ± 0.01a	25 ± 3b	54 ± 60a	67 ± 4bc
Root tissue	full light	A	0.06 ± 0.01a	79 ± 2a	338 ± 108a	164 ± 9ab
		B	0.07 ± 0.01a	76 ± 5a	240 ± 65a	194 ± 31a
		C	0.08 ± 0.02a	58 ± 3b	135 ± 59a	21 ± 5c
	shading	A	0.06 ± 0.01a	69 ± 4ab	290 ± 79a	130 ± 13b
		B	0.08 ± 0.01a	65 ± 3ab	180 ± 157a	153 ± 9ab
		C	0.07 ± 0.01a	52 ± 8b	111 ± 69a	27 ± 3c

Mean and S.E. based on four replicate samples. Values with the same letter (a, b, or c) within each measured variable (^{14}C/^{13}C/^{15}N) and tissue type (shoot/root) are not significantly different ($P < 0.05$).

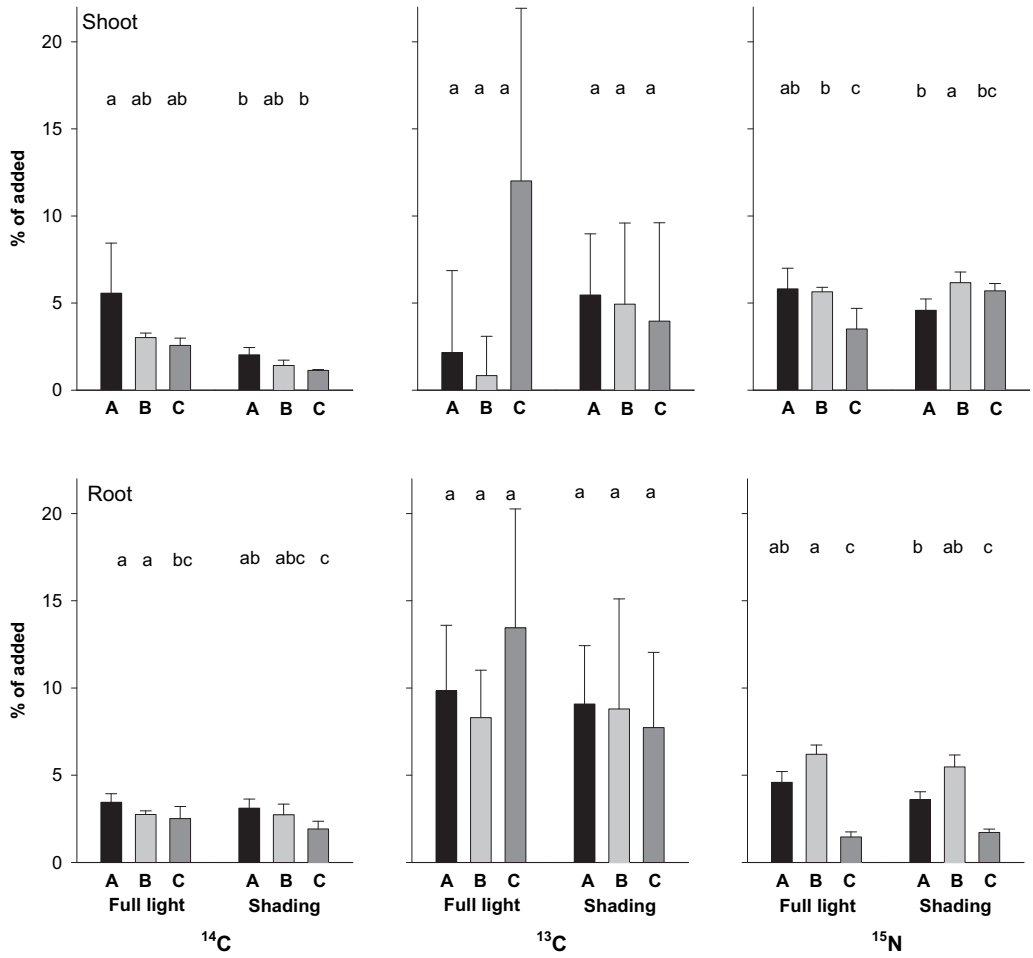

Fig. 1. Uptake of ^{14}C, ^{13}C, and ^{15}N in percent of added tracer in maize shoot and root tissue after 24 h of labeling in either full light or shading. Treatment A: all tracers in the form of glycine; B: ^{13}C and ^{15}N in glycine form and ^{14}C in carbonate form; C: all tracers in inorganic form. Error bars show the standard error based on four replicates. Bars with the same letter (a, b, or c) within each measured variable ($^{14}C/^{13}C/^{15}N$) and tissue type (shoot/root) are not significantly different ($P < 0.05$).

prerequisite assumption of dual-labeling bulk measurement studies of organic N uptake is not valid.

In the present study we found a significantly ($P = 0.039$) higher total uptake of ^{14}C in shoot tissue under full light than under shading independent of the labeling solution. The total uptake of ^{13}C or ^{15}N in shoot and root tissues was not significantly different between full light and shading, although the variability for ^{13}C was so high that a possible biological effect could have been masked. The uptake of ^{14}C from the carbonate tracer is in accordance with the study of Vuorinen et al. (1989) who found ^{14}C incorporation in willow from $NaHCO_3$ to be twice as high after 24 h in light than in darkness. Based on the ^{14}C uptake we suggest that the uptake of inorganic C in the shoot supports the conclusion of Vuorinen et al. (1989) that a part of the uptake is driven by transpiration and the increased water flow from root to shoot under full light.

The results of the present triple labeling study demonstrated that uptake of tracer-C in inorganic form can bias the results in studies using bulk measurement of dual-labeling to show organic N uptake. Our findings imply that the conclusions from many such studies of organic N uptake have to be reconsidered, as control treatments using inorganic C tracers have rarely been included. We anticipate that in the future, studies of organic N contribution to plant N nutrition will need to make use of compound specific isotope methods able to track the flow of intact organic N compounds and their metabolites from soil into the plant tissue.

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References

Biernath, C., Fischer, H., Kuzyakov, Y., 2008. Root uptake of N-containing and N-free low molecular weight organic substances by maize: a $^{14}C/^{15}N$ tracer study. Soil Biology & Biochemistry 40, 2237–2245.

Britto, D.T., Kronzucker, H.J., 2005. Nitrogen acquisition, PEP carboxylase, and cellular pH homeostasis: new views on old paradigms. Plant Cell and Environment 28, 1396–1409.

Cramer, M.D., Lewis, O.A.M., Lips, S.H., 1993. Inorganic carbon fixation and metabolism in maize roots as affected by nitrate and ammonium nutrition. Physiologia Plantarum 89, 632–639.

El-Naggar, A., de Neergaard, A., El-Araby, A., Høgh-Jensen, H., 2009. Simultaneous uptake of multiple amino acids by wheat. Journal of Plant Nutrition 32, 725–740.

Fokin, A.D., Knyazev, D.A., Kuzyakov, Y.V., 1993. Destruction of ^{14}C- and ^{15}N-labeled amino acids and nucleic bases in soil and the supply of their transformation products to plants. Eurasian Soil Science 25, 109–122.

Graf, G.E., Aronoff, S., 1955. Carbon dioxide fixation by roots. Science 121, 211–212.

Jones, D.L., 1999. Amino acid biodegradation and its potential effects on organic nitrogen capture by plants. Soil Biology & Biochemistry 31, 613–622.

Jones, D.L., Shannon, D., Junvee-Fortune, T., Farrar, J.F., 2005. Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biology & Biochemistry 37, 179–181.

Kielland, K., McFarland, J., Olson, K., 2006. Amino acid uptake in deciduous and coniferous taiga ecosystems. Plant and Soil 288, 297–307.
Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M., Högberg, P., 1998. Boreal forest plants take up organic nitrogen. Nature 392, 914–916.
Näsholm, T., Huss-Danell, K., Högberg, P., 2000. Uptake of organic nitrogen in the field by four agriculturally important plant species. Ecology 81, 1155–1161.
Näsholm, T., Huss-Danell, K., Högberg, P., 2001. Uptake of glycine by field grown wheat. New Phytologist 150, 59–63.
Rasmussen, J., Kuzyakov, Y., 2009. Carbon isotopes as proof for plant uptake of organic nitrogen: relevance of inorganic carbon uptake. Soil Biology & Biochemistry 41, 1586–1587.
SAS Institute, 1999. SAS/STAT Software. Release 8.02. SAS Institute Inc, Cary, NC, USA.
Sauheitl, L., Glaser, B., Weigelt, A., 2009b. Uptake of intact amino acids by plants depends on soil amino acid concentrations. Environmental and Experimental Botany 66, 145–152.
Sauheitl, L., Glaser, B., Weigelt, A., 2009a. Advantages of compound-specific stable isotope measurements over bulk measurements in studies on plant uptake of intact amino acids. Rapid Communications in Mass Spectrometry 23, 3333–3342.
Streeter, T.C., Bol, R., Bardgett, R.D., 2000. Amino acids as a nitrogen source in temperate upland grasslands: the use of dual labelled (C-13, N-15) glycine to test for direct uptake by dominant grasses. Rapid Communications in Mass Spectrometry 14, 1351–1355.
Vuorinen, A.H., Vapaavuori, E.M., Lapinjoki, S., 1989. Time-course of uptake of dissolved inorganic carbon through willow roots in light and in darkness. Physiologia Plantarum 77, 33–38.
Vuorinen, A.H., Vapaavuori, E.M., Raatikainen, O., Lapinjoki, S.P., 1992. Metabolism of inorganic carbon taken up by roots in salix plants. Journal of Experimental Botany 43, 789–795.
Weigelt, A., Bol, R., Bardgett, R.D., 2005. Preferential uptake of soil nitrogen forms by grassland plant species. Oecologia 142, 627–635.