Uptake of glycine by field grown wheat

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Summary

• Uptake of glycine, a simple organic nitrogen (N) source, directly from the soil is shown here in a conventionally cropped wheat (*Triticum aestivum*) field.

• Wheat plants were harvested after tracer injections into the soil of two forms of dual-labelled amino acid; [$^{13}C_2$], [^{15}N]-glycine and 2-[^{13}C], [^{15}N]-glycine. Uptake of intact amino acid was analysed by stable isotope–, and gas chromatography–, mass spectrometry.

• Significant increases in ^{13}C were found in root extracts for all glycine-treated plants. Regression analysis of excess ^{13}C vs excess ^{15}N for the two glycine forms showed that at least 20% of absorbed glycine-N was derived from uptake of intact glycine. Gas chromatography–mass spectrometry was used to verify the presence of intact dual-labelled glycine in wheat roots. Results also indicated that glycine decarboxylase had a minor role in metabolism of absorbed glycine in wheat roots. Microbial metabolism in the soil did, however, result in rapid decarboxylation of added glycine.

• Field-grown wheat takes up glycine directly from the soil; the dependence of agricultural plants on nitrate and ammonium as the only forms of available N is therefore questionable.

Key words: amino acid uptake, ammonium, ^{13}C, ^{15}N-glycine, GC-MS, nitrate, wheat (*Triticum aestivum*).

Introduction

Nitrogen is the mineral element needed in largest amounts by plants. In agriculture, large efforts are spent to augment N availability through supply of industrially fixed N. Indeed, the use of inorganic N fertilizers has been one of the cornerstones in the development of efficient, high-technology agriculture (Matson *et al.*, 1998). However, recent awareness of human impacts on global N cycling and negative effects of excessively high N additions (Matson *et al.*, 1997; Vitousek *et al.*, 1997) has raised questions about alternative crop-production systems (Drinkwater *et al.*, 1998; Tilman, 1998). Uptake of different N sources by agricultural plants is a critical issue in this context.

The conventional view in agronomy is that N-uptake by crops is restricted to inorganic N sources; that is ammonium and nitrate (Mengel & Kirkby, 1987; Haynes, 1986; Loomis & Connor, 1992). But a number of studies have shown that some nonagricultural plants can absorb several organic N forms, in particular different amino acids (Chalot & Brun, 1998). Such use of organic N sources was believed to be important for plants with ericoid mycorrhiza or with ectomycorrhiza that grow in soils of forest and heathland ecosystems, where rates of mineralization of organic N are low. Recent studies have shown, however, that the range of plants that can access organic N also includes nonmycorrhizal (Chapin *et al.*, 1993; Kielland, 1994; Raab *et al.*, 1996) and arbuscular mycorrhizal plants (Näsholm *et al.*, 1998, 2000). Laboratory studies have also shown that agricultural plants can absorb organic N (Virtanen & Linkola, 1946; Shobert & Komor, 1987; Jones & Darrah, 1994). Furthermore, a range of amino acid transporters have been identified in plants, for example transporters enabling uptake of acidic, neutral and basic amino acids in roots of *Arabidopsis thaliana* (Fischer *et al.*, 1998). Clearly, both mycorrhizal and nonmycorrhizal plants have a capacity to

absorb amino acids. Additionally, significant levels of amino acids have been shown to occur in some agricultural soils (Mengel, 1996; Schulten & Schnitzer, 1998).

Although the studies cited above show that a number of plant species, including agricultural plants, possess a capacity to absorb a range of organic N compounds, the extent to which this capacity is realized in the field, where plants compete with microorganisms for these substrates, remains a matter of dispute (Jones, 1999; Hodge *et al.*, 2000a,b).

We tested the hypothesis that an agricultural crop can take up a simple organic N source directly from the soil. Dual (^{13}C, ^{15}N) labelled glycine was used in order to differentiate between uptake of mineralized glycine N and uptake of intact glycine.

Materials and Methods

The experimental field

The experiment was conducted on May 27 1998, in a conventionally cropped winter wheat (*Triticum aestivum* L. cv. Tarso) field at Alnarp (55°38′N, 13°4′E), Sweden. The weather was cloudy and the temperature was 15°C. The soil was a clay loam with a pH (H_2O) of 7.2 ± 0.02, a C content of 1.3 ± 0.04%, a C : N ratio of 9 ± 0.1 and a water content of 0.14 ± 0.01 g g^{-1} (mean ± SE, n = 11). The field had alfalfa in 1991–1994, and winter wheat, sugar beet and oat in 1995, 1996 and 1997, respectively. The winter wheat studied was sown on September 18, 1997. The field was fertilized on 2 April and 12 May 1998 with a total of 170 kg N ha^{-1}. At the time of the experiment, approx. 10 d before heading, the stand was 0.5–0.6 m high, had 670 stems m^{-2}, a leaf area index of 4.8 and an aboveground biomass of 570 g (dry matter) m^{-2}. The N concentrations of shoots and roots were 3.1 ± 0.1 and 1.0 ± 0.01 (mean ± SE), respectively. The grain yield, in September 1998, amounted to 7.7 tons (dry matter) ha^{-1}.

Tracer experiment

Tracers (96–99% ^{15}N and 98% ^{13}C), $^{15}NH_4^+$; $^{15}NO_3^-$; 2-[^{13}C], [^{15}N]-glycine, U-[$^{13}C_2$], [^{15}N]-glycine or water only were added as 1 mM solutions to 10 cm by 20 cm plots containing one row of wheat plants. In each plot, 120 ml of one tracer solution (corresponding to 0.84 kg N ha^{-1}) was distributed by six injections, three at each side of the row of plants, approx. 3 cm from the shoot bases and at a depth of approx. 2–3 cm. Plots were distributed on 11 30 m^2 blocks, each block containing the five treatments.

Shoots (uppermost 10 cm) of wheat plants were cut 4 h after additions of tracers. Roots and soil were brought to a laboratory and separated by careful sieving. Roots were cleaned under tap water and then immersed in 0.5 mM $CaCl_2$ three times to assure removal of tracer adsorbed on root surfaces. Cleaned roots were immediately (approx. 8 h after tracer additions) frozen on dry ice and kept frozen at −23°C until further processed. Soil samples for analysis of ^{13}C and ^{15}N were preserved in the same way. Additionally, soil samples from control plots were extracted with de-ionized water (soil : solution ratio, 10) by shaking in 20 ml screw cap-polyethylene tubes for 10 min. One ml of this extract was transferred to a second vial, frozen on dry ice and stored at −23°C until analysed.

Analyses

Analyses of ^{13}C and ^{15}N in solid material and extracts were conducted on a automated nitrogen and carbon analyser equipped with a stable isotope mass spectrometer (ANCA-MS) as earlier described (Näsholm *et al.*, 1998; Ohlson & Wallmark, 1999). Gas chromatorgraphy-masspectrometry (GC-MS) analyses of root extracts were used to confirm the presence of labelled glycine in plants. One hundred mg of frozen and milled roots was extracted in 10 ml 0.01 M HCl, and amino acids were separated from other compounds on a cation exchange resin. Tert.-butyldimethylsilyl derivatives of amino acids were prepared (Woo & Lee, 1995) and analysed in the chemical ionization mode on a Varian Saturn 2000 GC-ion-trap MS system (Varian Chromatography Systems, Walnut Creek, CA, USA). The samples were injected splitless onto a 30-m × 0.25 mm id fused silica capillary column with a chemically bond 0.25 μm CP-SIL 19 CB stationary phase. The injector temperature was 260°C, and the column temperature was held at 130°C for 2 min, then increased by 70°C min^{-1}–145 °C, and by 30 °C min^{-1}–280 °C. Helium was used as carrier gas, and the head pressure was 12 pounds per square inch (psi). The interface and the ion source temperatures were 260 °C. The samples were analysed by chemical ionization using methanol as reagent gas. The mass spectrometer run detected the molecular ion of tBDMS-glycine at m/z 304 and the $[M + 2]^+$ and $[M + 3]^+$ ions at m/z 306 and 307, corresponding to tBDMS – 2-[^{13}C], [^{15}N]-glycine and tBDMS-U-[$^{13}C_2$], [^{15}N]-glycine, respectively. Ratios m/z 306 : 304 and m/z 307 : 304 of root extracts were compared with standards to assess incorporation of labelled glycine.

Analyses of ammonium and amino acids (including glycine) in soil extracts were carried out by HPLC according to Näsholm *et al.* (1987). Nitrate was analysed by flow injection analysis on a Tecator 5012 device and according to application note 110–01/92 (Foss, Tecator Sollentuna, Sweden).

To check for re-fixation of soil-respired $^{13}CO_2$, small potted plants of *Impatiens wallerana* Hook.f. were placed on the ground of 4 plots of each of the 2-[^{13}C], [^{15}N]-glycine, U-[$^{13}C_2$], [^{15}N]-glycine and control treatments immediately after injections of solutions into the soil. Shoots from these plants were collected at the time of the harvest of the wheat plants and thereafter treated identically to these.

Ethanol preserved roots were cleared in 10% Potassium hydroxide (KOH) at 87 °C for 45 min, rinsed in running tap water for 30 s, treated with 2% HCl for 5 s and stored in 70% glycerol for 1 wk. Roots on microscope slides were stained with toluidine blue (0.05% in 70% glycerol) and examined for

colonization by mycorrhizal hyphae in a light microscope at 100–1000 × magnification.

Results and Discussion

In the current study, uptake of glycine was studied using two forms of labelled amino acid; U-[$^{13}C_2$], [^{15}N]-glycine, in which both C atoms are labelled, and 2-[^{13}C], [^{15}N]-glycine labelled only at the α-C position. These two tracers allowed assessment of the fraction of glycine-N derived from uptake of intact amino acid. Moreover, the different labelling patterns of the two glycine tracers provided additional information on the metabolism of glycine in plants and in soil. Analyses of dried and milled wheat roots showed very small increases of ^{13}C for glycine treated plants (Table 1) and assessment of the fraction of glycine N absorbed as intact amino acid was not possible from these analyses. Plots of excess ^{13}C vs excess ^{15}N of root extracts, however, showed significant regressions for both glycine forms ($P < 0.0001$), but not for the two inorganic N species (Fig. 1). The slopes of the regression lines differed significantly between the two glycine treatments (analysis of covariance; $P < 0.005$) and were 0.43 for U-[$^{13}C_2$], [^{15}N]-glycine and 0.20 for 2-[^{13}C], [^{15}N]-glycine. The theoretical slopes for 100% of glycine N taken up as intact amino acid corresponds to 2.0 for U-[$^{13}C_2$], [^{15}N]-glycine and to 1.0 for 2-[^{13}C], [^{15}N]-glycine. The fractions of glycine N absorbed as intact amino acid are thus estimated at 0.22 and 0.20 for the respective glycine forms. A recent study of four agricultural plants (*Ranunculus acris*, *Phleum pratense*, *Trifoilum repens* and *T. hybridum*) growing in pots and with relatively low levels of inorganic N supply provided very similar estimates (0.19–0.23) of the fraction of glycine N absorbed as intact amino acid (Näsholm *et al.*, 2000). In contrast, a study of boreal forest plants (Näsholm *et al.*, 1998) showed that ectomycorrhizal conifer trees, an arbuscular mycorrhizal grass and an ericoid mycorrhizal shrub took up a fraction of 0.42, 0.64 and 0.91, respectively, of the absorbed glycine-N as intact amino acid. Thus, compared to plants typical of the boreal forest and growing in soils with low rates of N mineralization, wheat absorbed a smaller, but still significant fraction of intact glycine.

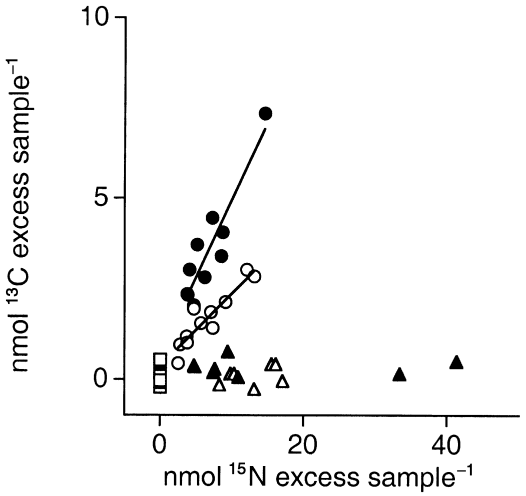

Fig. 1 The relationship between excess ^{15}N and excess ^{13}C of root extracts of field grown wheat plants supplied with either $^{15}NH_4^+$ (closed triangles); $^{15}NO_3^-$ (open triangles); U-[$^{13}C_2$], [^{15}N]-glycine (closed circles) or 2-[^{13}C], [^{15}N]-glycine (open circles) or water only (open squares) through the soil. (Each symbol represents one analysis of a root extract from 8 to 10 plants.) Lines indicate linear regression for U-[$^{13}C_2$], [^{15}N]-glycine ($y = 0.43x + 0.7$, $r^2 = 0.86$) and for 2-[^{13}C], [^{15}N]-glycine ($y = 0.20x + 0.3$, $r^2 = 0.84$), $P < 0.001$ for both. For data on roots and shoots before extraction see Table 1.

Theoretically, simultaneous labelling of both C and N of plant roots can result not only from uptake of intact amino acid but also from uptake of products of amino acid metabolism in soil, and for glycine, deamination would yield NH_4^+ and glyoxylate. In the current study, plant uptake of intact glycine was therefore studied using GC-MS. Small but significant increases in the abundance of the ions $[M + 2]^+$ ($P \leq 0.05$) and $[M + 3]^+$ ($P \leq 0.001$) were detected in root extracts of 2-[^{13}C], [^{15}N]-glycine and U-[$^{13}C_2$], [^{15}N]-glycine treated plants, respectively. Thus, the mean ratios of m/z 307 : m/z 304 were 0.04, and 0.07 for control and U-[$^{13}C_2$], [^{15}N]-glycine treated plants, respectively, and ratios m/z 306 : m/z 304 were 0.13 and 0.15 for control and of 2-[^{13}C], [^{15}N]-glycine treated plants, respectively. These ratios correspond to a fraction of 2% and 3% of 2-[^{13}C], [^{15}N]-glycine and of

Table 1 ∂^{15}N and ∂^{13}C of roots[1] and shoots of wheat plants supplied with either of the tested nitrogen sources ($^{15}NH_4^-$; $^{15}NO_3^-$; 2-[^{13}C], [^{15}N]- or U-[$^{13}C_2$], [^{15}N]-glycine) or water (control)

Treatment (per mil)	Root ∂^{15}N (per mil)	Root ∂^{13}C (per mil)	Shoot ∂^{15}N (per mil)	Shoot ∂^{13}C
Control	−0.5 (0.2)^a	−28.1 (0.08)^a	2.2 (0.2)^a	−27.7 (0.1)^a
$^{15}NH_4^-$	220 (60)^b	−28.2 (0.05)^a	6.5 (3.1)^{ab}	−27.1 (0.3)^a
$^{15}NO_3^-$	188 (30)^b	−28.1 (0.1)^a	8.2 (1.0)^b	−27.5 (0.1)^a
U-[$^{13}C_2$], [^{15}N]-glycine	164 (21)^b	−27.5 (0.1)^b	2.8 (0.3)^a	−27.8 (0.1)^a
2-[^{13}C], [^{15}N]-glycine	126 (21)^b	−27.9 (0.08)^a	2.9 (0.4)^a	−27.5 (0.1)^a

[1]Root data in the Table refers to dry, milled roots, while Fig. 1 shows data pertaining to root extracts. Mean ± SE, $n = 8–11$. Numbers followed by different letters are significantly different (ANOVA followed by Tukeys test, $P < 0.05$).

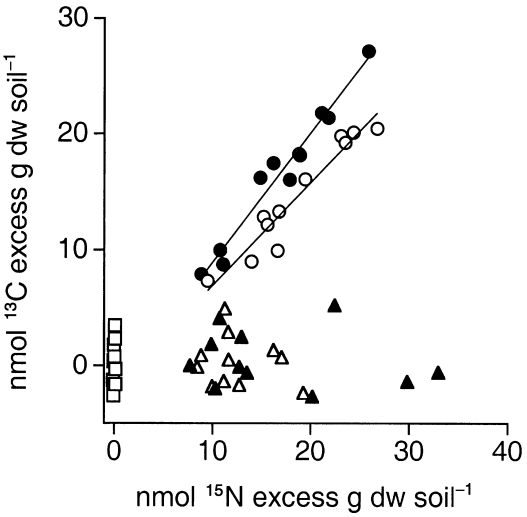

Fig. 2 The relationship between ^{15}N and ^{13}C labelling of sieved soil treated with either $^{15}NH_4^+$ (closed triangles); $^{15}NO_3^-$ (open triangles); U-[$^{13}C_2$], [^{15}N]-glycine (closed circles); 2-[^{13}C], [^{15}N]-glycine (open circles) or water only (open squares). Each symbol represents one soil sample. Lines indicate linear regression for U-[$^{13}C_2$], [^{15}N]-glycine ($y = 1.11x - 2.16$, $r^2 = 0.96$), and for 2-[^{13}C], [^{15}N]-glycine ($y = 0.88x - 1.86$, $r^2 = 0.93$), $P < 0.001$ for both.

U-[$^{13}C_2$], [^{15}N]-glycine in the respective glycine pool of roots and confirm the presence of the respective labelled glycine source in wheat plant roots.

For sieved soil from the treated plots (Fig. 2), significant regressions were again found for the two glycine forms, when plotting excess ^{13}C vs excess ^{15}N. As opposed to plants, however, the slopes corresponding to U-[$^{13}C_2$], [^{15}N]-glycine and 2-[^{13}C], [^{15}N]-glycine (1.11 and 0.88, respectively) were not significantly different (analysis of covariance; $P > 0.05$) in these regressions, despite the twofold higher addition of ^{13}C in the U-[$^{13}C_2$], [^{15}N]-glycine treatment. Thus, in relation to the amount of recovered ^{15}N, only 12% of the ^{13}C label was lost in the 2-[^{13}C], [^{15}N]-glycine treatment, while 45% of the ^{13}C label was lost from the U-[$^{13}C_2$], [^{15}N]-glycine plots. This likely resulted from rapid decarboxylation of the carboxy-C of glycine during microbial metabolism of glycine (Fokin *et al.*, 1993; Yan *et al.*, 1996).

The contrasting results between soil and plant roots gives further support to our claim that wheat plants took up intact glycine. If extracellular decarboxylation of glycine preceded plant uptake, identical slopes would have been found for both U-[$^{13}C_2$], [^{15}N]-glycine and 2-[^{13}C], [^{15}N]-glycine regressions on data from root extracts. Metabolism of absorbed glycine in roots could result in losses of ^{13}C through respiration of products of glycine metabolism (Schmidt & Stewart, 1999). Therefore, our values of the fraction of glycine N taken up as intact amino acid by the plants are conservative.

Elevated levels of ^{13}C could also result from re-fixation by shoots of root- or soil-respired $^{13}CO_2$. Introducing small potted plants of *Impatiens wallerana* on the treated plots during the labelling period tested this possibility. Analyses of shoots showed that $\partial^{13}C$ were -26.7 ± 1.4; -26.8 ± 1.4 and -26.6 ± 0.8 (mean $\pm$ SE, $n = 4$) per mil for potted plants placed on 2-[^{13}C], [^{15}N]-glycine, U-[$^{13}C_2$], [^{15}N]-glycine and control treatments, respectively, indicating that re-fixation of soil-respired $^{13}CO_2$ from the tracers was insignificant. Microscopic investigation of wheat roots failed to detect any mycorrhizal infection, a situation not uncommon for wheat before flowering (Hetrick & Bloom, 1983). Thus, glycine detected in roots must have been taken up intact by the plants themselves and not by arbuscular mycorrhizal fungi. Measurements of stable isotopes in wheat shoots revealed a small but significant increase in ^{15}N in nitrate treated plants. The small increases in ^{15}N in shoots of the glycine treated plants showed that detection of ^{13}C tracer in shoots of these plants would not be possible (Table 1).

There were no significant differences in ^{15}N labelling of roots supplied with the three different N-sources (Table 1, Fig. 1). Measurements of soil solution concentrations of the different N sources showed high levels of nitrate, intermediate levels of ammonium and low levels of glycine (i.e. 10.4 ± 0.4; 0.4 ± 0.1 and 0.008 ± 0.001 mM, respectively, average $\pm$ SE, $n = 10$). Thus, added, labelled nitrate would be diluted by nonlabelled endogenous nitrate and hence, ^{15}N values of nitrate treated plants underestimate uptake of this source. The dilution of the different labelled compounds cannot, however, be accurately calculated because the exact soil volume in which the labelling solution was injected and the rate of mixing are unknown. Assuming that the injected amount of tracer (120 ml) was mixed with 200 ml of endogenous soil water to a degree of 25–50% we would underestimate nitrate uptake by a factor 5–10. However, we cannot accurately determine the isotopic exchange with the respective endogenous unlabelled sources. Thus, it is not possible to assess the actual rates of uptake of the different N sources. The values of the fractions of glycine-N derived from uptake of intact amino acid are, however, not affected by dilution of tracers because these fractions are calculated from the relationship between ^{13}C and ^{15}N, and not the actual amounts of tracers.

Accelerated use of industrially fixed N has been a major driver behind the tremendous increase in agricultural production during the 20th century, but has also caused pollution of agricultural and nonagricultural ecosystems. Undesirable environmental impact can be limited by closely matching N supply with crop N demand but this requires that sources of N available for crops are identified and quantified. We demonstrate here that wheat, one of the most important crops globally, also takes up organic N, in our case represented by the amino acid glycine, directly from the soil, and thus that soil N mineralization can be by-passed. The extent to which organic N can supply N needed for growth by agricultural plants will depend, except on plant capacities for absorption of organic N, also on factors such as soil turnover rates and rates of mass flow and diffusion of different organic N compounds. A study of soil turnover

rates of a range of amino acids showed half-lives of a few hours for most compounds, implying that (nonmycorrhizal) plants might be poor competitors with respect to soil amino acids (Jones, 1999). Our study shows that nonmycorrhizal wheat can absorb glycine when competing with microbes. The relatively low fraction (approx. 20%) of glycine-N absorbed as intact amino acid suggests that the majority of glycine –N absorbed by plants had been mineralized in the soil before uptake. Hence, plants acquired only a small fraction of the added amino acid N directly. It should be stressed, however, that the estimate of the fraction of glycine N derived from uptake of intact amino acid (20%) is conservative. A study of plant and microbial utilization of a range of amino acids (Lipson *et al.*, 1999), indicated that glycine was a poor substrate for microbes. Thus, it has been argued (Lipson *et al.*, 1999; Hodge *et al.*, 2000b) that plants may compete better for glycine than for other amino acids. The rapid soil metabolism of glycine shown in the present study to some extent contradicts that glycine should be a poor substrate for soil microbes. Clearly, studies using other amino acids are needed before generalizations about plant uptake of these compounds can be made. The importance of organic N forms as N sources for plants in conventional cropping systems, as in this study, as well as in systems based on other forms of N input deserves further studies.

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