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# UPTAKE OF ORGANIC NITROGEN IN THE FIELD BY FOUR AGRICULTURALLY IMPORTANT PLANT SPECIES

TORGNY NÄSHOLM,[1,4] KERSTIN HUSS-DANELL,[2] AND PETER HÖGBERG[3]

[1]*Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden*
[2]*Department of Agricultural Research for Northern Sweden, Crop Science Section, Swedish University of Agricultural Sciences, S-904 03 Umeå, Sweden*
[3]*Section of Soil Science, Department of Forest Ecology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden*

*Abstract.* Uptake of glycine was studied in four plants commonly used in grasslands in northern Europe (*Phleum pratense, Trifolium hybridum, T. pratense,* and *Ranunculus acris*) and compared to uptake of ammonium and nitrate. The experiment was conducted in the field, but with plants transferred to pots with soil 8–10 d before the start of the experiment. Plant uptake of U-$^{13}C_2{}^{15}N$ glycine, $^{15}NH_4^+$, and $^{15}NO_3^-$ was studied by injecting dilute (1 mmol/L) solutions of respectively labeled N source into the pots and harvesting plants 21 h later. Measurements of $^{13}C$ and $^{15}N$ in roots showed that, in all plants, part of the glycine N was taken up in the form of intact amino acid. Hence, regressions of plots of excess $^{13}C$ against excess $^{15}N$ showed that a minimum of 19–23% of the glycine-derived N was taken up as intact amino acid; possible losses of labeled C atoms of glycine during its metabolism in the plants implies that these estimates are conservative. Uptake of the different N sources was similar in the two *Trifolium* species, while rates of nitrate uptake were comparably high in *P. pratense,* and rates of glycine uptake were comparably low in *R. acris.* $^{15}N$ labeling of shoots was detected in all species, whereas significant levels of $^{13}C$ tracer was only found in shoots of *P. pratense.* It is concluded that a capacity for uptake of organic N exists also in an agricultural setting, despite the rapid turnover of organic N usually found under such conditions. This adds to the growing knowledge of plant utilization of organic N sources in natural ecosystems and stresses the need for reexamining this step in the biogeochemical N cycle.

*Key words: agricultural plants; amino acid; ammonium; glycine; $^{15}N$ and $^{13}C$; nitrate; organic nitrogen uptake;* Phleum pratense; Ranunculus acris; *stable isotopes;* Trifolium hybridum; Trifolium pratense.

## INTRODUCTION

Soil nitrogen (N) availability is a major determinant of plant biomass production in many ecosystems, natural as well as agricultural. The early appreciation of this condition has stimulated extensive studies of soil N turnover and plant N uptake over decades. Today, industrial fixation of N and the use of fertilizers in agriculture have become a major issue of global concern (Vitousek et al. 1997). In spite of the long history of research, and the recent focus on N as an environmental problem, some basic principles of biogeochemical N cycling are still, however, not fully understood. Thus, the utilization of different N sources by plants is still a matter of dispute. Over the years, studies within this field have been focused on the relative importance of nitrate and ammonium, while utilization of organic N sources has attracted less research. This focus on inorganic N as being the only N forms used by plants has been questioned, however, and laboratory studies, as well as studies of natural systems, have given evidence for the importance of organic N forms (Melin and Nilsson 1953, Stribley and Read 1980, Abuzinadah and Read 1986, 1988, Finlay et al. 1992, Chapin et al.1993, Kielland 1994, 1995, Turnbull et al. 1995, 1996, Raab et al. 1996, Schimel and Chapin 1996, Schmidt and Stewart 1997, Lipson and Monson 1998). These studies have, in various ways, indicated that plants have the capacity to take up some organic N sources, notably the simple amino acids glycine, alanine, glutamic acid, and aspartic acid (e.g., Chapin et al. 1993, Kielland 1994, 1995), but also more complex organic N sources (e.g., Turnbull et al. 1995, 1996). In the field, however, plants compete with soil microorganisms for such substrates, and the potential for up-

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[1] E-mail: Torgny.Nasholm@genfys.slu.se

take of organic N sources by plants demonstrated in laboratory experiments might not be realized. In a recent study, Näsholm et al. (1998) showed that plants typical of the boreal forest take up a simple organic N source in the field (the amino acid glycine), and that ecto-, ericoid-, and arbuscular-mycorrhizal plants all had uptake rates similar to that of ammonium uptake. Thus, it is now established that organic N sources are important for plants growing in the boreal forest.

Agricultural plants were shown to absorb simple organic N sources in laboratory studies (Soldal and Nissen 1978, Shobert and Komor 1987, Jones and Darrah 1994). Moreover, carrier systems for amino acid uptake in roots have been documented (cf. Fischer et al. 1998), which supports that such plants can make use of organic N sources under laboratory conditions. In agricultural systems, rates of mineralization are typically much higher than in natural and forest ecosystems, and it can be questioned whether plants can compete for organic N under field conditions. A few studies have discussed the possible occurrence of organic N uptake in agricultural settings (e.g., Jones and Darrah 1994), and some data indicate that the actual pool sizes of some organic N forms might be large (Mengel 1996). Still, the prevailing idea is that inorganic N, in particular nitrate, is the only N source of importance for agricultural plants. The current study was undertaken to test if some plants commonly found in agricultural settings in northern Europe can take up a simple organic N source under field conditions. For this purpose, we used double-labeled (U-$^{13}C_2{}^{15}N$) glycine and compared rates of glycine uptake with uptake of $^{15}N$ labeled nitrate and ammonium.

## METHODS

### Plants

Timothy (*Phleum pratense* L. cv. Jonatan), buttercup (*Ranunculus acris* L.), alsike-clover (*Trifolium hybridum* L. cv. Stena), and red clover (*T. pratense* L. cv. Betty) were sown in early June 1996 on agricultural experimental farmland in Umeå, Sweden (63°49′ N, 20°15′ E). Plants were cut once in late August of the same year. During the period of 6–10 June 1997, plants were carefully dug up with a volume of soil surrounding the roots, and each plant was transferred into a plastic pot (upper diameter, 18 cm; soil depth, 15 cm; total soil volume, ~3 L). Each pot was put tightly back into the soil pits, such that the soil surface in the pot was at level with the soil surface of the field. Each pot was then given 0.4 L water.

### Experimental set-up

On 18 June, when all plants had grown in the pots for 8–12 d, 100 mL of 1 mmol/L N was added to each pot. This corresponded to 0.59 kg N/ha. Recalculated, each pot received 1.4 mg N, which corresponds to ~0.03% of the total N present in each pot (N concentration of soil was 0.13 ± 0.05% [mean ± 1 SE]; C. Mulder, P. Högberg, and K. Huss-Danell, *unpublished data*). The solution was injected at 3–4 cm soil depth and at five points in each pot. Great care was taken to prevent solution from contaminating shoots. Four plants of each species received $^{15}NH_4Cl$ (98 atom% $^{15}N$), four received $Na^{15}NO_3$ (98 atom% $^{15}N$), and four received U-$^{13}C_2$-$^{15}N$ glycine (98 atom% $^{13}C$; 96–99 atom% $^{15}N$; ''U'' denotes uniformly labeled). Thus, each treatment was repeated four times for each species ($n = 4$), and treatments were laid out so that there were four groups of plants, each containing one plant per treatment. The N additions were made in the afternoon at 1300.

By using an organic N compound labeled with both $^{13}C$ and $^{15}N$ as a tracer, any uptake of intact amino acid can be distinguished from uptake of N from mineralized amino acid. Thus, if both $^{13}C$ and $^{15}N$ are detected in plants, this shows that uptake of intact amino acid occurs. Moreover, the slope of the regression of excess $^{15}N$ to excess $^{13}C$ gives a conservative estimate of the fraction of N that was taken up in the form of intact amino acid (Näsholm et al. 1998).

### Harvest

The following day, ~21 h after addition of tracers, all pots were rapidly removed from the field, but kept outdoors in the shade. Shoots were collected, except for the basal 2–3 cm segments. These basal parts were discarded to avoid any contamination from labeled solutions. Shoots were kept on ice and then put in a freezer (−23°C). Soil was shaken off the roots, and roots were rinsed in tap water and then carefully washed twice in 0.5 mmol/L $CaCl_2$ for 5–7 min to remove any tracer adsorbed on root surfaces. Subsamples of roots for microscopic examination of mycorrhizae were taken arbitrarily from *P. pratense* and *Trifolium* spp. and kept in 50% ethanol at room temperature for up to 7 mo. The remaining roots were kept on ice until moved to a freezer.

### Sample treatment and analyses

Frozen shoots and roots were milled in liquid $N_2$ using a ball mill. Subsamples of this milled material were dried and masses were measured into tin capsules for mass-spectrometric analysis. Stable isotopes were determined in dual ($^{15}N$ and $^{13}C$) isotope mode by automated nitrogen carbon analyzer mass spectrometry (ANCA-MS) on a Model 20–20 Europa Scientific stable isotope analyzer (Crewe, Cheshire, UK). The standard deviation of repeated analyses of labeled samples was 8.3 per mil for $^{15}N$ and 0.1 per mil for $^{13}C$.

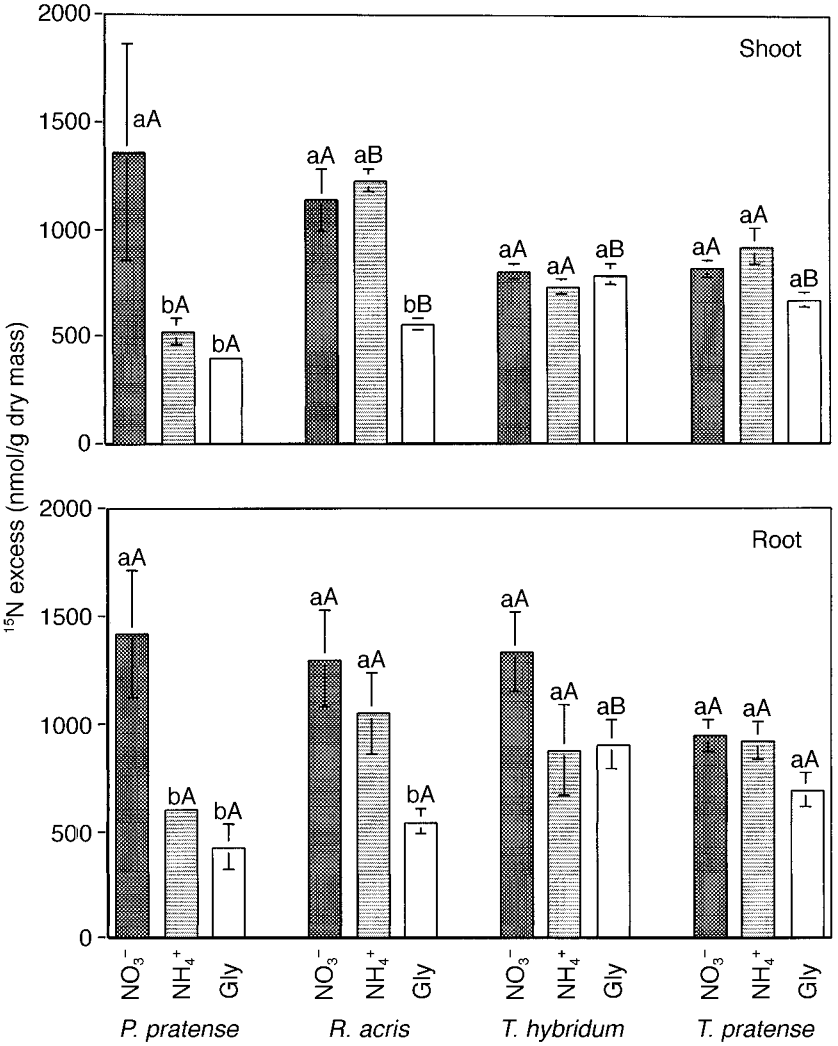

FIG. 1. $^{15}$N levels in shoots and roots of field-grown plants supplied with Na$^{15}$NO$_3$, $^{15}$NH$_4$Cl, or U-$^{13}$C$_2$$^{15}$N glycine. Values represent number of nanomoles of $^{15}$N in excess of the atomic standard (see *Methods* for explanation). Means ± 1 SE are shown, unless standard errors are too small to be visible; $n = 4$. Different capital letters indicate significant differences between species ($P < 0.05$); different lowercase letters indicate significant differences between treatments ($P < 0.05$).

Ethanol-preserved roots were cleared in 10% KOH at 87°C for 45 min, rinsed in running tap water for 30 s, treated with 2% HCl for 5 s, and were then kept in 70% glycerol for one week. Roots were placed on microscope slides, toluidine blue (0.05% in 70% glycerol) was added, surplus stain was rinsed with deionized water, and a cover slip was added prior to examination in a light microscope at 100–1000 × magnification. (A.-S. Hahlin, *unpublished method*).

### *Calculations*

Values of atom percent and concentrations of C and N were used to calculate moles excess of $^{13}$C and $^{15}$N. The atomic standard of $^{15}$N (N$_2$; 0.3663 atom%) was used as reference for calculations of excess $^{15}$N. Earlier studies had shown that the mean $\delta^{15}$N abundance of the plants were as follows: 0.9 ± 1.4 (mean ± 1 SD) for *T. hybridum*; 0.5 ± 1.1 for *T. pratense*; 5.1 ± 1.2 for *R. acris*, and 6.5 ± 1.9 for *P. pratense* (C. Mulder, P. Högberg, and K. Huss-Danell, *unpublished data*). Moreover, the maximum error introduced in the calculations by using the atomic standard instead of a measured reference (i.e., if the $^{15}$N value should be 10 per mil higher or lower than the atomic standard) would correspond to ~30 nmol $^{15}$N/gram dry mass, and would thus be negligible in relation to the values of excess $^{15}$N achieved in the treatments (cf. Fig. 1). Mean values of $^{13}$C abundances of ammonium- and nitrate-treated

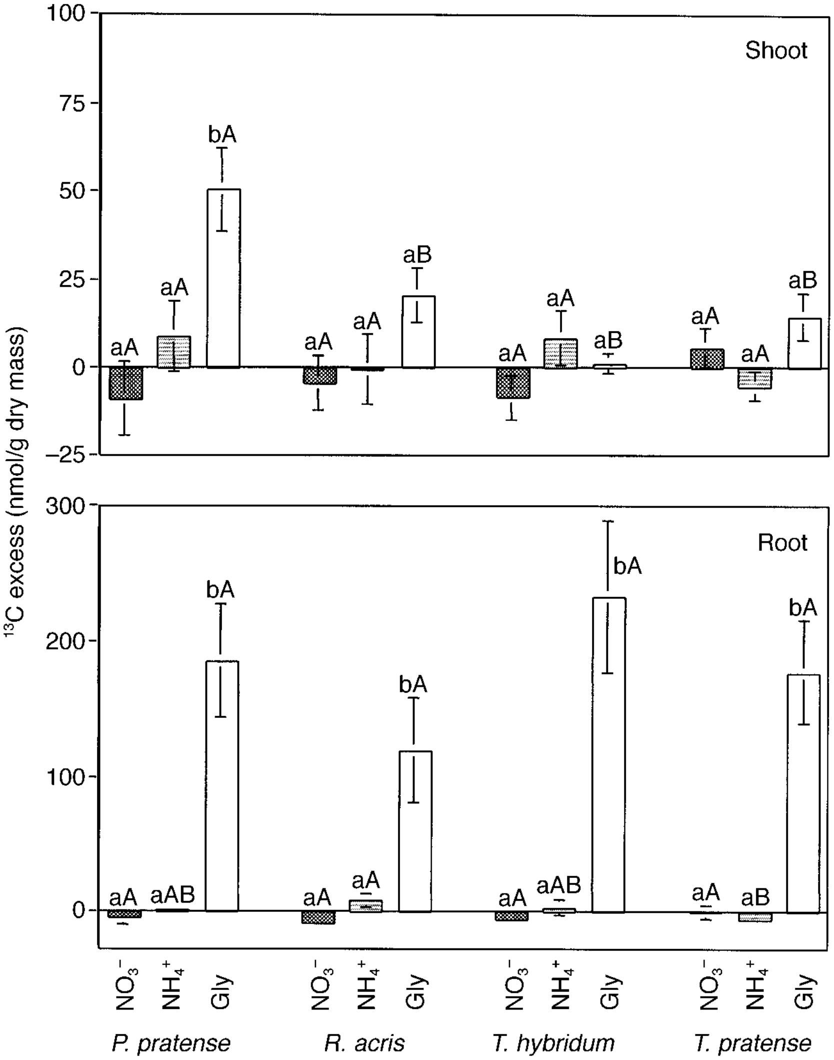

FIG. 2. $^{13}$C levels in shoots and roots of field-grown plants supplied with $Na^{15}NO_3$, $^{15}NH_4Cl$, or U-$^{13}C_2{}^{15}N$ glycine. Values represent the number of nanomoles of $^{13}$C in excess of the mean of nitrate- and ammonium-treated shoots and roots, respectively, of each species (see *Methods* for explanation). Means ± 1 SE are shown, unless standard errors are too small to be visible; $n = 4$. Note different scales for shoots and roots. Different capital letters indicate significant differences between species ($P < 0.05$); different lowercase letters indicate significant differences between treatments ($P < 0.05$).

plants were used as references for calculations of $^{13}$C excess. Thus, excess $^{13}$C refers to levels above the mean of ammonium- and nitrate-treated plants. By using treatments supplied with the same amount of N as references, shifts in $^{13}$C due to N uptake (Raven and Farquahar 1990) are taken into account when estimating $^{13}$C labeling of glycine-treated plants. Separate references were calculated for each species and for shoots and roots. Differences between treatments and species in moles excess of $^{13}$C and $^{15}$N was tested by ANOVA followed by Tukey's test.

## RESULTS

Elevated $^{13}$C abundance was found in roots of all glycine-treated plants showing that uptake of organic N occurred in all plants (Fig. 2). Moreover, an elevated level of $^{13}$C was found in shoots of glycine-treated plants of all species except *T. hybridum.* However, the difference was significant only for shoots of *P. pratense* (Fig. 2). $^{15}$N labeling was found in both roots and shoots in all species, and the pattern of labeling, as well as the amount of tracer recorded, were similar for roots and shoots (Fig. 1). Differences in $^{15}$N labeling between treatments were most obvious in *P. pratense,* for which $NO_3^-$-supplied plants had higher $^{15}N$ levels than those supplied with either $NH_4^+$ or glycine, indicating a high uptake of $NO_3^-$ in this species. For *R. acris* plants, a significantly lower $^{15}N$ level was found in both roots and shoots of glycine treated than of $NO_3^-$- or $NH_4^+$-treated plants (Fig. 1). In the two *Trifolium* species, similar patterns of labeling were found for all three N sources.

Regressions of excess $^{13}C$ against excess $^{15}N$ of roots were used both to illustrate and to calculate the fraction of uptake out of intact amino acid of total uptake of amino acid N. It was obvious that all plants studied absorbed approximately the same fraction of N as intact amino acid. The slopes of the regressions of excess $^{13}C$ against excess $^{15}N$ in roots (Fig. 3) ranged 0.38–0.46 for glycine-treated plants. Because each mole of the added glycine contains two moles of $^{13}C$ and one mole of $^{15}N$, the slope corresponding to 100% uptake of intact amino acid is 2.0. Thus, the slopes of 0.38–0.46 correspond to 19–23% uptake of glycine N as intact amino acid. Correlation coefficients were high for all plants, except for *R. acris,* due to one value with low $^{13}C$:$^{15}N$ ratio in this species. In shoots, no clear relationship between $^{13}C$ and $^{15}N$ labeling was found (data not shown). All plants screened for mycorrhizal infection were mycorrhizal, with arbuscules clearly present in all plant root samples.

## DISCUSSION

This study shows that organic N, in our case represented by the amino acid glycine, can be taken up intact by agricultural plants growing in the field. The fraction of glycine-derived N taken up as intact amino acid varied 19–23% for the different species (Fig. 3). However, these numbers should be interpreted as the minimum fractions of organic N uptake, because metabolism of glycine following uptake would probably lead to loss of $^{13}C$ label. One of the major metabolic pathways for glycine is that of serine synthesis, either through the combined actions of glycine decarboxylase and serine hydroxymethyltransferase (Oliver 1994), or through the action of an aminotransferase, serine glyoxalate aminotransferase (Ireland and Hiltz 1995). The former reaction would lead to loss of the carboxylic C, while the latter would produce glyoxylate that could be catabolized through the combined actions of the glyoxalate and citric acid cycles, and would thus lead to loss of both of the labeled C atoms. Elevated levels of $^{13}C$ in shoots of glycine-treated plants were found in all species except *T. hybridum* (Fig. 2), but the difference was significant only for *P. pratense.* The significant labeling of *P. pratense* shoots further strengthens our claim that part of the absorbed glycine N was taken up as intact amino acid in this species. Differ-

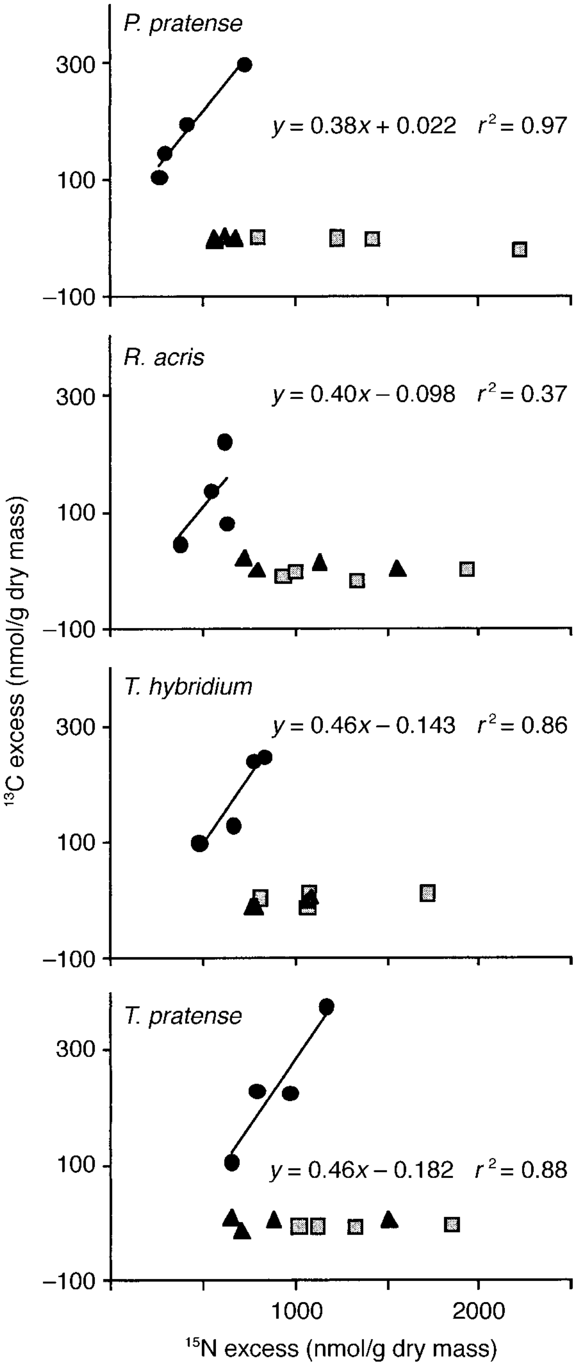

FIG. 3. The relationship between excess $^{13}C$ and excess $^{15}N$ in roots of field-grown plants supplied with $Na^{15}NO_3$ (squares), $^{15}NH_4Cl$ (triangles), or U-$^{13}C_2{}^{15}N$ glycine (filled circles). The regression of excess $^{13}C$ on excess $^{15}N$ of glycine-treated plant roots (solid line) is shown.

ences between species in shoot $^{13}$C labeling could be due to differences in metabolism of absorbed glycine, as well as differences between plants in transport of N from roots to shoots. Shoot $^{13}$C labeling could theoretically also result from refixation of soil- or root-respired $^{13}CO_2$. In the experimental setup, plant shoots from the different treatments were next to each other, and this would, if shoot refixation of root- or soil-respired $^{13}CO_2$ occurred, result in similar $^{13}$C labeling of shoots from all treatments. Therefore, it seems probable that the elevated $^{13}$C levels in glycine-treated *P. pratense* resulted from uptake of $^{13}$C label in roots. Thus, $^{13}$C levels of roots of all species and of *P. pratense* shoots suggest that a significant fraction of the recovered glycine N was due to root uptake of intact amino acid. The three species that were inspected for mycorrhizal colonization were all shown to be mycorrhizal. We cannot judge to what extent mycorrhizal and nonmycorrhizal parts of the roots contributed to uptake of organic N. From the perspective of plant acquisition of organic N, however, the important fact is that both C and N (Figs. 1 and 2, respectively) transfer to plants occurred either directly or via their respective fungal associates.

No significant differences in uptake of the different N sources were recorded for the two *Trifolium* species. *Phleum pratense* absorbed $NO_3^-$ at a higher rate than either glycine or $NH_4^+$ while in *R. acris*, uptake rates where significantly lower for glycine treated as opposed to both $NH_4^+$- and $NO_3^-$-treated plants. High uptake of $NO_3^-$ was also recorded in *Hordeum vulgare* (Chapin et al. 1993), and this is commonly assigned as a characteristic trait of plants growing in N-rich environments. Species inhabiting poor soils, on the other hand, have been shown to prefer ammonium (Kronzucker et al. 1997). In soil, diffusivity of ammonium is much smaller than that of nitrate (Raven et al. 1992). Moreover, the flux of nitrate towards roots is also governed by transpirationally induced mass flow of water in the soil. Thus, to increase acquisition of ammonium from surrounding soil, plants must develop high rates of uptake and high affinity for this N form (i.e., low $K_m$). These adaptations would also lead to high uptake rates of ammonium in environments, were diffusion is not limiting ammonium flux towards roots. Typically, studies of species preferences for different N sources have been conducted in solution culture. Consequently results from such studies indicating preference for ammonium might reflect different adaptations to maximize capture of ammonium, rather than reflecting actual uptake of N sources in the field. In the current study, we used plants in pots with agricultural soil. The recorded uptake rates of the different N sources are therefore dependent on both mobility of the different N forms in the soil and rates of uptake of these. Hence, we cannot determine if plants had preferences for the individual N forms. The advantage of our experimental setup is that it more accurately mimics the natural conditions under which plant N acquisition occurs than do solution cultures.

Obviously, our data imply that models describing N cycling in agricultural systems, or in other ecosystems where soil turnover rates of N are high, should include terms for uptake of organic N. The relative contribution of organic N to the N economy of plants is still not known, however. Two lines of evidence indicate that organic N can make a substantial contribution to plant N uptake. First, levels of soluble organic N are typically high and comparable to those of inorganic N, even in agricultural soils (e.g., Shobert and Komor 1987, Nemeth et al. 1988, Mengel 1996). Second, transporters of different amino acids have been shown to be present in plant cells (Bush 1993, Fischer et al. 1998). Thus, the physical precondition exists for a significant uptake of organic N. Earlier studies of plants from natural ecosystems have indicated that organic N can be an important N source (Stribley and Read 1980, Abuzinadah and Read 1986, Chapin et al. 1993, Turnbull et al. 1995, 1996, Raab et al. 1996, Schimel and Chapin 1996, Lipson and Monson 1998). These studies, as well as a few studies of agricultural species (Soldal and Nissen 1978, Shobert and Komor 1987, Jones and Darrah 1994) have indicated that organic N potentially is important for plant N acquisition. The actual realization of this potential depends, however, on the ability of plants and mycorrhizal fungi to compete with other organisms for organic N substrates (Chapin et al. 1986). It is generally held that microorganisms would outcompete plants for organic N substrates, and that plant N uptake should therefore be confined to inorganic N sources. Recent studies in natural ecosystems (Schimel and Chapin 1996, Lipson and Monson 1998, Näsholm et al. 1998) have shown that plants compete well for organic N. The current study extends these findings to agricultural settings, implying that organic N can be an important N source for plants also under conditions where soil processing of C and N sources may be much more rapid than in natural ecosystems in the temperate and arctic regions. The fractions of uptake of intact amino acid recorded in the current study are lower than those recorded for boreal forest plants, i.e., 19–23% vs. 45–61% (referring to data from the harvest one day after labeling; Näsholm et al. 1998). Although this indicates that organic N might be less important for agricultural plants than for forest plants, it is still evident that a substantial fraction of the total N uptake could, theoretically, be met by organic N sources in agricultural settings. Future studies should show to what extent plants utilize more complex organic N sources, as well as assessing the relative importance of organic N, as opposed to inorganic N, for plants under various conditions.

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