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Minireview

Plant acquisition of organic nitrogen in boreal forests

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Research on plant nitrogen (N) uptake and metabolism has more or less exclusively concerned inorganic N, particularly nitrate. Nevertheless, recent as well as older studies indicate that plants may have access to organic N sources. Laboratory studies have shown that ectomycorrhizal and ericoid mycorrhizal plants can degrade polymeric N and absorb the resulting products. Recent studies have also shown that some non-mycorrhizal plants are able to absorb amino acids. Moreover, amino acid transporters have been shown to be present in both plant roots and in mycorrhizal hyphae. Although both mycorrhizal and non-mycorrhizal plants appear to have a capacity for absorbing a range of organic N compounds, is this capacity realized in the field? Several lines of evidence show that plants are outcompeted by microorganisms for organic N sources. Such studies, however, have not addressed the issue of spatial and temporal separation between plants and microorganisms. Moreover, competition studies have not been able to separate uptake by symbiotic and non-symbiotic microorganisms. Qualitative assessment of organic N uptake by plants has been performed with dual-labelled glycine in several studies. These studies arrive at different conclusions: some indicate that plants do not absorb this organic N source when competing with other organisms in soil, while others conclude that significant fractions of amino acid N are absorbed as intact amino acid. These variable results may reflect species differences in the ability to absorb glycine as well as differences in experimental conditions and analytical techniques. Although theoretical calculations indicate that organic N might add significant amounts of N to plant N uptake, direct quantitative assessment of the fraction of plant N derived from uptake by organic N sources is a challenge for future research.

Introduction

Plant growth in boreal forest ecosystems is commonly limited by nitrogen (N) supply. In spite of this limitation, large stocks of N are usually found in boreal forest soils. This apparent contradiction is explained by the slow rates of production of plant-available N from complex soil N compounds, which are relatively stable against biological decomposition. Thus, N limitation is not caused by low amounts of N but by slow rates of production of N forms that plants can access.

Generally, it has been assumed that organic N must be converted into inorganic N, ammonium and nitrate, in order to be available for plant uptake. Thus, N mineralization has been considered the bottleneck process in plant N nutrition in boreal as well as in arctic, alpine and heathland ecosystems. This view, however, has been contradicted by a number of laboratory studies showing that plants may have access to organic N sources (e.g. Melin and Nilsson 1953, Stribley and Read 1980, Abuzinadah and Read 1988, Chapin et al. 1993, Kerley and Read 1994, Turnbull et al. 1995, Lipson and Monson 1998). A capacity to utilize organic N has been suggested for a variety of vascular plants as well as cryptogams growing in a range of different ecosystems, from arctic tundra to Australian subtropical *Eucalyptus* forests (Kielland 1994, 1997, Turnbull et al. 1996, Schmidt and Stewart 1999).

Although attempts have been made to estimate the relative importance of organic N in plant N nutrition (e.g. Kielland 1994, Schimel and Chapin 1996), direct assessments are still lacking. Consequently, this minireview cannot quantitatively estimate the role of organic N in plant N nutrition in the boreal forest. Instead, it is focused on some of the processes that affect the rate and extension of plant organic N acquisition. Moreover, part of it is devoted to methodological aspects, because the methods used in evaluating the role of organic N in plant N nutrition limit the probability of obtaining unambiguous results.

The term 'organic N' obviously encompasses a wide range of N substances. However, most of the studies cited here have been restricted to the common proteinaceous amino acids. Thus, in this context organic N is, unless otherwise specified, synonymous to proteinaceous amino acids. This minireview also complements reviews on competition for N between plants and microbes (Kaye and Hart 1997, Hodge et al. 2000a) and organic N acquisition by ectomycorrhizas (Chalot and Brun 1998).

Plant and mycorrhizal uptake of organic N

A prerequisite for plant utilization of organic N compounds is certainly a capacity to transport these compounds into the root, i.e. the presence of transporters in plasma membranes of plant roots and/or mycorrhizal hyphae. As regards plants, several reviews on amino acid transporters have been published recently (e.g. Bush 1993, Tanner and Caspari 1996, Fischer et al. 1998). Although most of the properties of amino acid transport systems have been elucidated from studies of yeast (*Saccharomyces cerevisiae*), recent studies have confirmed the presence of genes encoding similar transporters in plants (cf. Fischer et al. 1998). According to these studies, plants possess a number of amino acid proton symporters exhibiting different affinities and transport rates. These transporters also show varying specificities for the spectrum of amino acids they can transport (Table 1). Although a wide range of transporter genes has been identified in plants, only a few of these have been confirmed to be expressed in roots (Table 1).

The amino acid transport systems do not appear to be inhibited by the presence of inorganic N (Jones and Darrah 1994). Transport of amino acids, however, seems to be regulated in response to endogenous and exogenous substrate concentrations, similar to transport of inorganic N (Tanner and Caspari 1996). In yeast, the general amino acid transporter (GAP1) is reversibly inactivated by a rich supply of N, a phenomenon known as N catabolite inactivation (Tanner and Caspari 1996). The broad specificity of the transporters identified in plant roots suggests that regulation of uptake occurs synchronously for most amino acids. A possible exception to this is the uptake of basic amino acids, because this is not inhibited in the same manner as the transporters of neutral and acidic amino acids (cf. Frommer et al. 1995, Tanner and Caspari 1996).

Table 1. Organic nitrogen transporter genes shown to be expressed in roots of *Arabidopsis thaliana*.

Gene	Type of Transporter	Source
AtAAP3	Low affinity general amino acid transporter	Fischer et al. 1995
AtAAP5	Low affinity general amino acid transporter	Fischer et al. 1995
AtAAP6	High affinity neutral/acidic amino acid transporter	Rentsch et al. 1996
AtProT1	Proline transporter	Rentsch et al. 1996
AtProT2	Proline transporter	Rentsch et al. 1996
AtCAT1	High affinity transporter of basic amino acids	Frommer et al. 1995
AtLHT1	Lysine/Histidine transporter	Chen and Bush 1997
AtPTR2	Di- and tripeptide transporter	Steiner et al. 1994

Data on amino acid transporters in mycorrhizal fungi are scarce. Nevertheless, the available information suggests that transporter systems are similar to those found in yeast and plants (Chalot et al. 1996). A recent study of *Amanita muscaria* identified a general amino acid transporter with high affinity for basic amino acids and somewhat lower affinity for neutral and acidic amino acids (Nehls et al. 1999). Accumulation of this transporter was low in the presence of high N concentrations (of either ammonium or amino acids) but increased 10-fold when *A. muscaria* was grown with low N supply. Thus, a similar regulation of amino acid transport as that recorded for yeast seems to be operating in this common ectomycorrhizal fungus.

In summary, both plant roots and mycorrhizal hyphae possess a number of transporters that enable uptake of amino acids from an external medium. The broad specificity of some of these transporters suggests that most proteinaceous amino acids can be absorbed by plant roots or mycorrhizas. Therefore, it is not surprising that various studies have demonstrated amino acid uptake in sterile culture (Soldal and Nissen 1978, Schobert and Komor 1987, Jones and Darrah 1993, 1994). Several studies have also shown that some mycorrhizal plants have higher affinities and higher uptake rates for amino acids than for ammonium and nitrate (Jones and Darrah 1994, Wallenda and Read 1999). Because most transporters show such broad specificity, regulation of uptake from the soil may mainly concern the total uptake of amino acids and may not involve regulation of uptake of individual amino acids. The ubiquitous appearance of amino acid permeases also strengthens the claim that both non-mycorrhizal and mycorrhizal plants possess the capacity for amino acid absorption from soil. Therefore, the critical question is not if the potential for organic N uptake exists in different plants but rather to what extent this potential is realized in the field.

Factors affecting the importance of organic N in plant N nutrition

Acquisition of nutrients involves a series of processes, ending with root absorption. In natural ecosystems, levels of N in the soil solution are usually low, implying a subordinate role of quantitative (but not qualitative) root uptake capacity for plant N acquisition. Instead, calculations from theoretical models have pointed at rates of soil supply and rates of transport in soil as determinants for plant N capture (Leadley et al. 1997). Assessing the role of organic and inorganic N compounds for plants must, therefore, take these and other processes into account. We will discuss some of these processes here, namely the processes leading to production of amino acids and concentrations of these in the soil, rates of transport of these compounds towards roots or hyphae and competition between plants and microorganisms for these substances.

Soil N

As stated in the Introduction, boreal forest soils usually contain large amounts of N. Soil N is found in a number of different compounds (Schulten and Schnitzer 1998), of which a considerable part is relatively resistant to breakdown (Mengel 1996). The compounds are polymeric, such as proteins, peptides, DNA and RNA, chitin and lignin, and monomeric, such as amino acids, nucleic acids, aminosugars and quinones (Hart et al. 1993, Mengel 1996, Schulten and Schnitzer 1998). In a recent review of N fractions of humic substances in different soils, Schulten and Schnitzer (1998) summarized the following proportions: proteins – 40%; aminosugars – 5–6%; heterocyclic N compounds – 35%; and NH_3–N – 19%. Thus, proteins and heterocyclics (including purines and pyrimidines) are the major N containing compounds of the humic fraction of soils according to this review.

Ecto- and ericoid mycorrhiza have been proposed to be especially important in the utilization of organic N. This is partly because these types of mycorrhiza have been shown to secrete extracellular hydrolytic enzymes, in particular proteinases (e.g. Bajwa et al. 1985, Leake and Read 1989). These enzymes cleave proteins and polypeptides to produce amino acids that are subsequently absorbed by plants or microbes. Ericoid mycorrhizal plants of *Vaccinium macrocarpon* and *Calluna vulgaris* have also been found to utilize chitin-N hydrolysed by chitinolytic enzymes secreted by the fungi (Kerley and Read 1994). The capacity for secreting proteolytic or chitinolytic enzymes is probably of great importance in N cycling. However, hydrolysed proteins and polypeptides are not the only sources of amino acids in soil. Most perennial plants store N in the form of amino acids in below-ground structures, such as roots and rhizomes (Jaeger III and Monson 1992, Nordin and Näsholm 1997). Leakage from these structures or decay of old tissues could release appreciable amounts of amino acids to the soil solution. In fact, the amino acids that dominate as storage compounds in boreal forest plants, arginine, glutamine and asparagine (Nordin and Näsholm 1997) have correspondingly been found to be the prominent amino acids in soil (A Nordin, M Högberg, T Näsholm, unpublished data). Moreover, amino acids are also released during turnover of microbial populations. Specifically, some fungi store N as arginine polyphosphates (Ludwig II et al. 1977, Finlay et al. 1992) and leakage from such organisms might thus add arginine to the soil.

A number of studies have reported on concentrations of amino acids in forest soils (Grov 1963, Ivarson and Sowden 1969, Ktsoyev 1978, Näsholm et al. 1998), in heathlands (Abuzinadah and Read 1988) and in arctic tundra (Kielland 1994, 1995). These studies have shown that relatively large amounts of amino acids are present in soil solutions. In many cases, levels of amino acids are considerably higher than those of ammonium, the main inorganic N form (Abuarghub and Read 1988a, Kielland 1995, Näsholm et al. 1998).

The usefulness of soil N pool sizes is limited, however, because such data only describe bulk concentrations in soil and not the actual concentrations at the surfaces of plant roots and/or hyphae of mycorrhizal fungi. Calculations produced by theoretical models have also illustrated that the rate of production (i.e. supply rate) is a major determinant of plant N acquisition of various N sources (Leadley et al. 1997). Thus, a low soil solution concentration can simply reflect rapid absorption by plants and/or microorganisms. Furthermore, soil solution concentrations of organic N may show great temporal variation (Abuarghub and Read 1988b, Singh et al. 1989, Kielland 1995, Raab et al. 1999), again limiting our ability to make inferences of plant N acquisition from data on soil concentrations of amino acids.

Delivery to root or hyphal surfaces

Roots have been described as infinite sinks for nutrients such as N. Accordingly, in the soil-plant system, uptake of N largely depends on the rate of delivery to root or hyphal surfaces (Nye 1977, Robinson 1986, Leadley et al. 1997). The major processes that deliver nutrients to root surfaces are diffusion and mass flow, although diffusion is generally believed to be the most important process with respect to nutrients present in low concentrations in the soil solution (Nye 1977).

The rate of transport by mass flow is the product of soil solution concentration and mass flow of water. This mass flow is normally induced by transpiration from shoots. However, mass flow is also induced by gravitation, causing both lateral and vertical water movements in soil. Such movements could, under specific circumstances, also be of importance for the supply of nutrients to roots (Chapin et al. 1988).

The rate of transport by diffusion depends on several factors, including the gradient in concentration from the root surface outward into the soil and the effective diffusion coefficient of the particular compound. Generally, diffusion rates decrease with molecular mass. Because most organic N substances have higher molecular masses than either ammonium or nitrate, this fact would limit transport of organic N to roots compared with inorganic N. However, other factors affect diffusion rates much more than molecular mass differences. One such factor is the tendency of adsorption or fixation to solid soil material. Adsorption to surfaces occurs mainly as a result of electrical charge and diffusion rates are, in general, considerably lower for cations than for anions. Amino acids differ in their net charge depending on pH. In the pH range typical of boreal forest soils (3.5–6), basic amino acids, such as arginine, histidine and lysine, are cations, whereas acidic amino acids, such as aspartic and glutamic acid, are anions. In between, compounds, such as glycine, serine, glutamine and asparagine, will occur as zwitterions. We would therefore expect the diffusive flux to be highest for neutral amino acids, such as serine and glycine, somewhat lower for acidic amino acids, such as aspartic and glutamic acids (depending on anion exchange capacity of the soil), and considerably lower for basic amino acids, such as arginine and lysine.

The putative differences in diffusion coefficients between individual organic N compounds also lead to great differences in the extension of depletion zones around roots.

Again, basic amino acids such as arginine are expected to form narrow depletion zones around roots, while neutral amino acids such as glycine can be expected to form broad depletion zones. Implicitly, plant capture of basic amino acids is probably more dependent on rates of root or hyphal growth, somewhat similar to phosphate acquisition, than capture of neutral and acidic compounds.

Competition between plants and microbes

Generally, microorganisms are thought to be superior to plants in competing for N and the general theory assumes that only N in surplus of microorganism growth is available for plants. This implies that only small amounts of N are available for plants, since N generally is the limiting factor for growth in boreal forests. Competition for N between plants and microbes has also been shown to be severe in several studies that estimated microbial uptake to be up to 95% of added N tracers (e.g. Schimel and Chapin 1996, Kaye and Hart 1997, Hodge et al. 1999b).

When assessing competitive abilities of different species or groups of organisms, the temporal aspects are important (Kaye and Hart 1997, Hodge et al. 2000a). Long-lived plants, which dominate boreal forest ecosystems, compete for nutrients over extended periods of time, thus having several opportunities to acquire a specific nutrient molecule compared with organisms with shorter life spans, such as bacteria. Furthermore, several studies have shown a temporal separation between plant and microbial nutrient acquisition (Mancinelli 1984, Singh et al. 1989, Jaeger III et al. 1999, Lipson et al. 1999c) indicating that nutrients may be cycling between different organisms during the course of a year.

A major problem when studying competition between plants and soil microorganisms is the difficulty separating different kinds of microorganisms, i.e. those forming symbiotic relations with plants from those actually competing with plants. In boreal forests, mycorrhizal relations between plants and different fungi are very common. Mycorrhizal fungi have also been shown to constitute a large part of the microbial biomass in soil (Chalot and Brun 1998). Thus, techniques currently used in studies of competition between plants and microorganisms include mycorrhizal fungi in the group of organisms competing with plants. Obviously, a large part of the nutrients acquired by mycorrhizal fungi are transported later to the associated host plants. Thus, short-term studies of competition between plants and microorganisms tend to underestimate plant N acquisition, irrespective of which N source is studied. In addition, there are reports of mycorrhizal fungi having an antagonistic effect on neighbouring, competing microorganisms in favour of its own, and thus its associated plant's, nutrient acquisition (Gadgil and Gadgil 1975, Olsson et al. 1996).

That organic N should be of importance for plants has also been questioned in a number of studies. Jones (1999) found that turnover rates of amino acids in a range of soils were very rapid with half-lives in the range of 1–2 h and concluded that (non-mycorrhizal) plants may have little chance to absorb amino acids from the rhizosphere. Thus, proliferation of soil bacteria and fungi in the rhizosphere would create a filtering mechanism leading to very low access to amino acids (or other organic N compounds) for plant roots. However, and as also argued by this author, this conclusion might not apply to mycorrhizal plants. Because most boreal forest plants are mycorrhizal, a significant uptake of amino acids in these plants cannot be ruled out. Moreover, temporal specialization (McCane et al. 1990) and/or specialization for specific substrates (Lipson et al. 1999a) may enhance plant uptake of organic N. It can also be argued that the short life spans for some free-living microbes would lead to frequent releases of organic N compounds in the rhizosphere, thus enabling acquisition of organic N by plant roots (Hodge et al. 2000a).

As stated above, several studies suggest that competition between plants and microorganisms is intense for organic N as well as for inorganic N. A critical question in assessing the relative importance of organic N for plant N nutrition is whether the outcome of competition between soil microbes and plants differs for the organic versus the inorganic N forms. The available information suggests that plants are inferior to microbes in competing for N irrespective of N form. Thus, it should be asked if it is possible to make inferences of the importance of different N sources for plants from short-term tracer studies (Kaye and Hart 1997). Consequently, studies allowing longer time between tracer addition and harvest have shown that plants acquire a larger fraction of applied N with time (cf. Kaye and Hart 1997, Hodge et al. 2000a). Unfortunately, such studies do not provide information as to which N source the plants ultimately take up.

Methodological considerations

Because of the complexity of the problem of plant uptake of organic N, most studies within this research field have been conducted under controlled conditions in greenhouses or climate chambers. These studies have provided the framework for further investigations by identifying uptake mechanisms and elucidating the role of mycorrhiza. As stated above, the question is not whether plants possess the capacity for organic N acquisition but rather the extent to which this capacity is realized in the field. Assessing the role of organic N for plants in natural communities can, therefore, only be done if the physical, chemical and biological complexity of the plant environment is intact.

A number of different techniques have been used in studies of plant uptake of organic N. Among these, the use of dual-labelled (^{13}C, ^{15}N) amino acids to trace uptake offers special advantages and have been used in a number of recent studies (Schimel and Chapin 1996, Näsholm et al. 1998, Hodge et al. 1999b, Lipson et al. 1999b). By supplying plants with dual-labelled organic N compounds and measuring ^{13}C and ^{15}N levels, uptake of intact molecules of organic N can be determined. The number of studies that have used dual-labelled amino acids to assess plant uptake of organic is still limited, but different studies have come to clearly different conclusions. For example, application of dual-labelled glycine (1-^{13}C, ^{15}N-gly) to isolated soil cores contain-

ing plants of *Eriophorum vaginatum* did not result in significant ^{13}C labelling of plants, although the rapid rates (similar to that for NH_4^+ treated cores) of ^{15}N labelling indicated that glycine was absorbed intact by *E. vaginatum* (Schimel and Chapin 1996). On the other hand, *Kobresia myosurides* plants supplied with dual-labelled glycine (2-^{13}C, ^{15}N-gly) showed a clear ^{13}C enrichment, indicating uptake of intact glycine (Raab et al. 1996). Similarly, injection of dual-labelled glycine (U-^{13}C, ^{15}N-gly) into the mor layer of a boreal coniferous forest showed that two conifers (*Picea abies* and *Pinus sylvestris*), an ericaceous shrub (*Vaccinium myrtillus*) and a grass (*Deschampsia flexuosa*) had clearly elevated levels of ^{13}C in roots (Näsholm et al. 1998). Using the same approach in an agricultural setting, Näsholm et al. (2000) also showed that four common agricultural plants (*Phleum pratense*, *Ranunculus acris*, *Trifolium hybridum* and *Trifolium pratense*) absorbed intact glycine in the field, but the fraction of intact uptake was appreciably lower than for forest plants. In a series of experiments, Hodge et al. (1998, 1999a, 2000b) studied plant and microbial N acquisition in patches of different N sources. A range of dual-labelled organic N substrates (i.e. 1-^{13}C, α-^{15}N-lysine; ^{13}C, U-^{15}N urea; algal amino acid mixture; algal lyophilized cells and ^{13}C, ^{15}N *Lolium perenne* shoots) were used to determine if plants could access organic N. Because enrichment in ^{15}N but not in ^{13}C in plant tissues was recorded in these experiments, it was concluded that the studied plants depended on microbial mineralization to acquire N from the organic substrates.

The conflicting results obtained in the studies cited above may reflect differences between plant species in the specificity of amino acid uptake (e.g. glycine; Lipson et al. 1999a) or between experimental environments with varying rates of microbial mineralization (Näsholm et al. 1998, 2000). The conflicting results may also, to some extent, be related to differences in the experimental design and the analytical techniques used to measure tracer levels in plants. Because of these differences, a short review of the problems faced in using dual-labelled amino acids is presented below.

The most obvious problem when using dual-labelled amino acids to study plant uptake is that of detecting ^{13}C label in plants supplied with realistically low levels of substrate (Fig. 1). This is because of the very high dilution of ^{13}C label in plants, usually around two orders of magnitude greater than for ^{15}N. This dilution is caused both by the high concentration of carbon in plants (45–50% dry weight (DW) of leaves or fine roots) and by the relatively high level of natural ^{13}C in plants (ca 1.08 at.% in C3 plants). Thus, plant leaves or fine roots usually contain ca 0.45 mmol of ^{13}C per g DW, but only ca 0.003 mmol of ^{15}N per g DW. This means that the relative dilution of ^{13}C is in the range 60–150 times that of ^{15}N. When using dual (^{13}C, ^{15}N) labelled amino acids in order to evaluate intact uptake, measured values of ^{15}N labelling can be used to calculate the theoretical shift in ^{13}C corresponding to 100% N uptake as intact amino acid. By doing this, it can be determined whether the theoretical shift in ^{13}C is distinguishable from natural variation and analytical error.

Figs. 1–3 illustrate the results of an experiment where dual-labelled glycine was added to the soil of a boreal

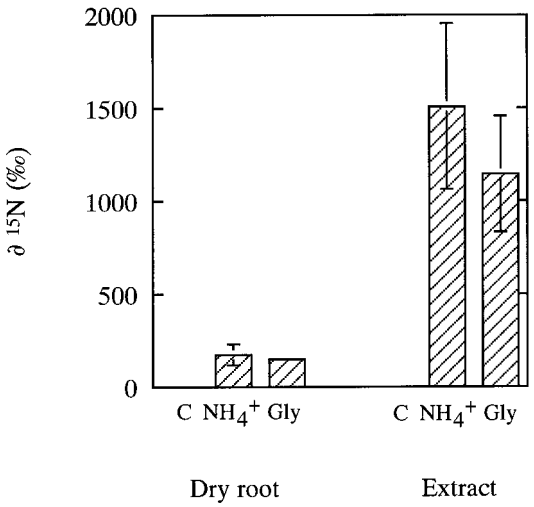

Fig. 1. $\partial^{13}C$ in conifer roots sampled from plots treated with water (C), $^{15}NH_4^+$ or U-^{13}C, ^{15}N-glycine. Left: values pertaining to dried and milled roots; right: values pertaining to root extracts. Data are presented as mean values ± SE, n = 8.

coniferous forest. Here, measurements of stable isotopes in dried and milled conifer roots indicated a shift in ^{15}N abundance of ca 100‰ in roots from glycine treated plots (Fig. 2). The corresponding shift in ^{13}C was very small, ca 0.5‰ (Fig. 1) and plotting excess ^{13}C versus excess ^{15}N did not produce significant regressions (Fig. 3). Thus, although a small shift in mean ^{13}C abundance of ^{13}C suggested that uptake of intact glycine could have occurred, the extremely low level of ^{13}C excess precluded assessment of the fraction of uptake as intact amino acid.

One solution to the problem of low levels of ^{13}C is to add larger amounts of tracers to the study object. Such an approach would, however, give unrealistically high levels of the substrate. Another approach, adopted in our laboratory,

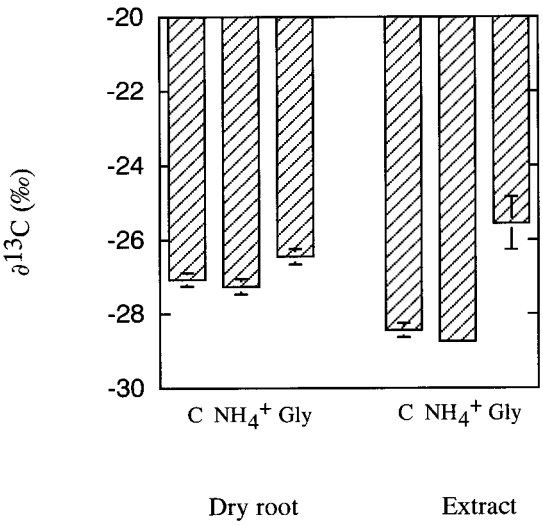

Fig. 2. $\partial^{15}N$ in conifer roots sampled from plots injected with water (C), $^{15}NH_4^+$ or U-^{13}C, ^{15}N glycine. Left: values pertaining to dried and milled roots; right: values pertaining to root extracts. Data are presented as mean values ± SE, n = 8.

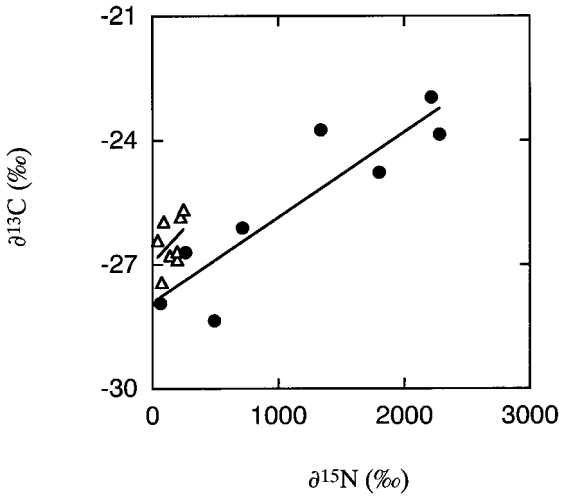

Fig. 3. Regression of $\partial^{13}C$ vs $\partial^{15}N$ for conifer roots sampled from plots treated with U-^{13}C, ^{15}N glycine. (△) Dried and milled roots ($R^2 = 0.17$); (●) root extracts ($R^2 = 0.87$).

is to concentrate the labelled fraction of the plant material. In short-term labelling experiments, tracers absorbed by roots are predominantly present in soluble compounds. By removing fractions that have a lower probability of incorporating tracers during short-term experiments, such as cell wall compounds, the tracer level can be increased. In our study, frozen and milled conifer roots of parallel samples to those shown in Figs. 1 and 2 were extracted with a buffer and the resulting extract was concentrated and analysed for ^{15}N and ^{13}C. The concentration of ^{13}C and ^{15}N increased ca 10-fold compared with concentrations of tracers in dried and milled roots by this procedure (Figs. 1 and 2). Moreover, plotting excess ^{13}C versus excess ^{15}N in extracts resulted in a highly significant regression (Fig. 4).

Another problem is that plant metabolism of labelled amino acid will, in many cases, lead to losses of ^{13}C in the form of $^{13}CO_2$. This risk is obviously greater when using

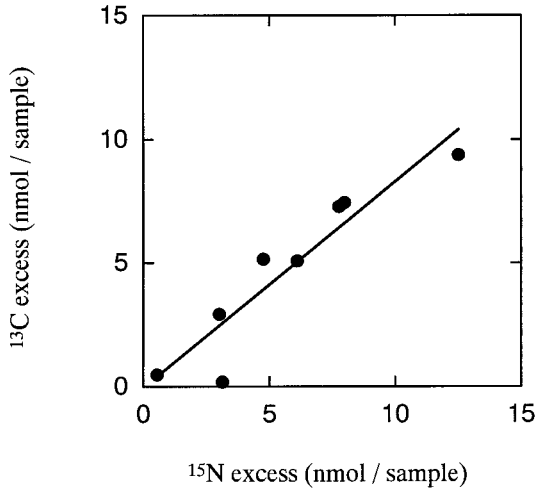

Fig. 4. Regression of nmol excess ^{13}C sample^{-1} vs nmol excess ^{15}N sample^{-1} for extracts of conifer roots sampled from plots treated with U-^{13}C, ^{15}N-glycine (slope = 0.84; $R^2 = 0.87$).

long incubation times. Additionally, this risk differs between amino acids and for different C positions of the individual amino acids. Loss of C from amino acids can occur through decarboxylation of the C-1 (i.e. the carboxy-C) atom and through deamination followed by breakdown of the C skeleton in the Krebs cycle. In the first case, a specific C atom is lost, while in the latter, losses of all C atoms in the former amino acid may occur. The choice of tracer should, therefore, be thoroughly considered. Because decarboxylation of amino acids is a rapid and common metabolic step in amino acid catabolism, compounds labelled only at the carboxy-C should be avoided.

Further problems occur when trying to detect a ^{13}C tracer in shoots. Transport of N from roots to shoots occurs in the form of specific amino acids, such as the amides asparagine and glutamine. If the tracer used is glycine, ^{15}N moving into the shoot would be connected to a new carbon skeleton and no relation between ^{15}N and ^{13}C label should be expected in the shoot.

Quite a different difficulty in the use of dual-labelled amino acids is the risk for extracellular cleavage of amino acids into ammonium and carbon skeletons in the soil (in the case of glycine, ammonium and glyoxylate would be the products), followed by simultaneous uptake of these compounds by plant roots. In this way, ^{13}C and ^{15}N label would move into the plant, but as different molecules. One way of assessing the importance of this process would be to measure labelling of individual amino acids to ensure that ^{13}C and ^{15}N are present in the same molecule after uptake. As for the problem with losses of ^{13}C, rapid metabolism of absorbed tracers would also lead to difficulties in detecting ^{13}C and ^{15}N tracers within the same molecule in the plant. However, preliminary results from our laboratory have confirmed, through gas chromatography-mass spectrometry (GC-MS) measurements of labelling patterns of individual amino acids, the presence of dual-labelled glycine in roots from field-grown plants supplied with dual-labelled glycine.

Conclusions and future prospects

Uptake of organic N is not a new area of research. Many studies have, over the years, added to an increasing body of knowledge about plant acquisition of N sources other than ammonium and nitrate. Still, models for N turnover in ecosystems like boreal forests failed to account for this process. A strong focus on the importance of ecto- and ericoid mycorrhizas for uptake of organic N in plants has now been supplemented with studies showing that both arbuscular mycorrhizal and non-mycorrhizal plants can take up organic N.

Apparently, the capacity to take up organic N is widespread in the plant kingdom. The central question is now to what extent it supplies plants with N needed for growth and development. Studies of competition between plants and microbes indicate that the latter outcompetes the former, which suggests a limited role of organic N for plant N acquisition. Such studies, however, have not separated N absorption by mycorrhizal fungi from N absorption by microorganisms that actually compete with plants. More-

over, because the turnover of microbial populations is faster than that of plants, even a small fraction of N absorbed by plants could result in a significant N uptake during a growing season. Finally, studies of competition between plants and microorganisms for inorganic N sources also shows a strong dominance of microorganisms, indicating that they are in fact superior competitors irrespective of N source – in the short term. Still, plants do survive and plant N is usually much greater than microbial N in an ecosystem like boreal forests. So where does this N come from?

Obviously, types of studies other than those measuring short-term partitioning of labelled N between plants and microorganisms are needed to address the question of the relative importance of different N sources for boreal forest plants. Looking back, many studies have shown that plants have a potential for uptake of a range of organic N sources, but very few studies have demonstrated that this potential is realized in the field. Theoretical models of the absorption of organic N in the arctic tundra, as well as in agricultural settings, have indicated that organic N may be important for plants. But these models suffer from a lack of information of production rates of different compounds in the soil and of the physical and chemical behaviour of these substances in the soil. As more data on root and hyphal uptake capacities, soil production and fluxes of organic N sources in the soil are gathered, the calculations of theoretical models will improve. We will still need to assess plant N acquisition when all players, i.e. plants, symbiotic and non-symbiotic microorganisms, as well as other soil organisms, are present. To this end, dual-labelled isotopes are useful because they may make it possible to differentiate between direct uptake of organic N and uptake of mineralization products. If plants acquire N through repeated competition events, however, other methods will be needed to determine which forms of N that boreal forest plants acquire in their natural environment.

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