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Boreal forest plants take up organic nitrogen

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Plant growth in the boreal forest, the largest terrestrial biome, is generally limited by the availability of nitrogen. The presumed cause of this limitation is slow mineralization of soil organic nitrogen[1,2]. Here we demonstrate, to our knowledge for the first time, the uptake of organic nitrogen in the field by the trees *Pinus sylvestris* and *Picea abies*, the dwarf shrub *Vaccinium myrtillus* and the grass *Deschampsia flexuosa*. These results show that these plants, irrespective of their different types of root–fungal associations (mycorrhiza), bypass nitrogen mineralization. A trace of the amino acid glycine, labelled with the stable isotopes ^{13}C and ^{15}N, was injected into the organic (mor) layer of an old successional boreal coniferous forest. Ratios of ^{13}C:^{15}N in the roots showed that at least 91, 64 and 42% of the nitrogen from the absorbed glycine was taken up in intact glycine by the dwarf shrub, the grass and the trees, respectively. Rates of glycine uptake were similar to those of ^{15}N-ammonium. Our data indicate that organic nitrogen is important for these different plants, even when they are competing with each other and with non-symbiotic microorganisms. This has major implications for our understanding of the effects of nitrogen deposition, global warming and intensified forestry.

Current conceptual models of nitrogen cycling in boreal forests are based on the assumption that mineralization of organic nitrogen is a prerequisite for plant nitrogen acquisition. Laboratory studies have shown, however, that some plants can use amino acids[3,4] and proteins[5], thereby bypassing the common mineralization pathway. This has not been demonstrated in the field, where plants compete with soil microorganisms held to be superior in using organic substrates. It has been proposed that plants forming ericoid mycorrhiza and ectomycorrhiza are especially efficient at using organic nitrogen[6]. This would give them a competitive advantage over plants forming arbuscular mycorrhiza and over non-mycorrhizal plants, and help explain their dominance in boreal forests[6], in which levels of organic nitrogen are typically high in the soil (compare with Table 1). If so, anthropogenic inorganic nitrogen deposition, climate warming and intensified silviculture could alter the composition of plant communities by promoting a shift from organic- towards inorganic-nitrogen nutrition. Again, field evidence demonstrating that organic-nitrogen uptake occurs and is exclusive to some groups of plants is lacking. The interaction between the abiotic soil system, mycorrhizal fungi, other microorganisms and plants is complex[7], and studies showing higher gross turnover rates of inorganic nitrogen pools than previously assumed[8] call for intensified studies of the nitrogen cycle, taking into account higher levels of complexity[9].

The lack of data showing organic nitrogen uptake by plants in the field relates to methodological problems. Classic ^{15}N-tracer technique cannot separate uptake of intact amino acids from uptake of mineralized nitrogen. ^{14}C-labelling has been used in studies of hydroponic (soil-free, nutrient-water) systems[4], but not in the field. Double-labelled (^{13}C, ^{15}N) glycine was used in a macrocosm study of arctic tundra, showing that plant uptake of glycine-derived nitrogen was as fast as uptake of NH_4^+, but this gave no evidence that the ^{13}C-labelled tracer was taken up[10]. Variations in the natural abundance of ^{15}N among plants in the field may be related to use of different nitrogen sources, but analysis of these variations is unlikely to yield conclusive quantitative evidence of the relative importance of organic and inorganic nitrogen uptake[11].

We aimed to test whether uptake of organic nitrogen occurs in the field, and to test whether plants with different types of mycorrhiza differ in this respect. We performed a low-tracer-level study with minimal disturbance of the soil–plant system in a late successional coniferous forest in northern Sweden (Table 1). Double, universally labelled (U) amino acid (U-$^{13}C_2$, ^{15}N-glycine) injected at 1 kg nitrogen per hectare into the mor layer was used to study the importance of direct uptake of intact amino acid compared with uptake of nitrogen from mineralized amino acid, that is ^{15}N-NH_4^+. Typical European boreal forest species[12] (the ectomycorrhizal trees *Pinus sylvestris* L. and *Picea abies* (L) Karst., the ericoid-mycorrhizal dwarf shrub *Vaccinium myrtillus* L. and the arbuscular-mycorrhizal grass *Deschampsia flexuosa* (L) Trin.) were included in the study. Sequential sampling of above- and below-ground plant parts and mor layer soil was conducted after 6 h (2 h for above-ground parts), 1 day and 7 days. Respiration of ^{13}C-CO_2 was measured after 2 h, 1 day and 7 days.

Table 1 Characteristics of the mor layer at the site studied, Renberget

pH*	3.1 ± 0.0
C:N ratio	36.6 ± 0.9
Total soil nitrogen (kg ha^{-1})	302 ± 33
Microbial nitrogen† (kg ha^{-1})	27 ± 3
Extractable amino-acid nitrogen‡ (kg ha^{-1})	1.0 ± 0.1
Extractable inorganic nitrogen‡ (kg ha^{-1})	0.3 ± 0.05

* 0.04 M $CaCl_2$, soil:solution ratio 1:10.
† Method of ref. 20, using 0.45 as an estimate of the fraction of nitrogen released by fumigation.
‡ In 2 M KCl, soil:solution ratio 1:10.
Data (averages ±1 s.e.m.) are from control plots (n = 22–24).

The results clearly show that a significant proportion of the supplied tracer was absorbed as intact amino acid. This is illustrated by plots of excess ^{13}C against excess ^{15}N in the soluble nitrogen fraction in roots (Fig. 1), and the highly significant regressions produced ($P < 0.001$). By comparing the slopes of these lines with the slope of 2, derived from the ^{13}C:^{15}N ratio of the tracer, a conservative estimate of the fraction of nitrogen taken up as amino acid can be obtained. After 6 h, this fraction was at least 42%, 64% and 91% for the trees, the grass and the dwarf shrub, respectively. Corresponding values after 1 day were 45%, 50% and 61%. Seven days after tracer application, there was no clear linkage between ^{13}C and ^{15}N levels in the trees and the grass, although the association was still seen in the dwarf shrub.

The slopes of the regression lines from the first samples show that the dwarf shrub had higher ^{13}C:^{15}N values than the trees (analysis of covariance followed by Tukey's test; $P < 0.01$) (ref. 13). Tracer ^{15}N was also detected in shoots of the grass after only 2 h and in dwarf shrub shoots after 1 day (Table 2). The ^{15}N-tracer level of both roots (Fig. 1) and shoots (Table 2) was similar for glycine-treated and NH_4^+-treated plants, giving further support to the hypothesis that glycine-derived nitrogen was, to a large extent, taken up as the intact amino acid[10].

Metabolism of glycine in plants or microorganisms occurs mainly by the route of serine synthesis, whereby 2 mol glycine are converted into 1 mol serine, 1 mol NH_3 and 1 mol CO_2 (ref. 14). Ammonium is re-assimilated, whereas CO_2 is presumably not re-assimilated in non-photosynthetic tissue. We used glycine labelled at both carbon positions, and the likely loss of the carboxy group during incorporation could explain the very rapid shift in the ^{13}C:^{15}N ratio from 2:1 to, or towards, 1:1 (Figs 1 and 2). Hence, our estimates of organic nitrogen uptake are probably underestimates, and differences between species could be partly related to differences in rates of decarboxylation. In the previous study of tundra plants[10], only the carboxy carbon of the glycine used was labelled, which may explain the difficulties in detecting the ^{13}C-labelled tracer. Here, some of the variability in natural abundance of ^{13}C relates to differences between plants in shaded and unshaded environments[15]. Analysis of the chitin content[16] of the roots indicated that all plants were mycorrhizal, with values of 0.5, 1.1, and 2.2 mg chitin per g root dry weight in the dwarf shrub, the grass and the trees, respectively. These data reflect the large proportion of rhizomes found in the samples of dwarf shrub roots. We cannot deduce how much of the ^{13}C and ^{15}N label was located in either plant or fungal cells. However, as the fungus is an integral part of the mycorrhizal root, this distinction is not critical in the context of biogeochemical nitrogen cycling. The most important consideration is whether or not organic nitrogen is taken up from the soil by the mycorrhizal roots.

Measurements in sifted soil from the labelled plots (Fig. 2) show that 64 ± 2%, that is, most of the nitrogen applied as either glycine or NH_4^+, was retained in this compartment. Unlike in previous studies, we did not prevent leaching and lateral flow or transport of glycine or NH_4^+ by roots out of the labelled plots, to minimize disturbance to the studied system. The average ^{13}C:^{15}N ratio for the

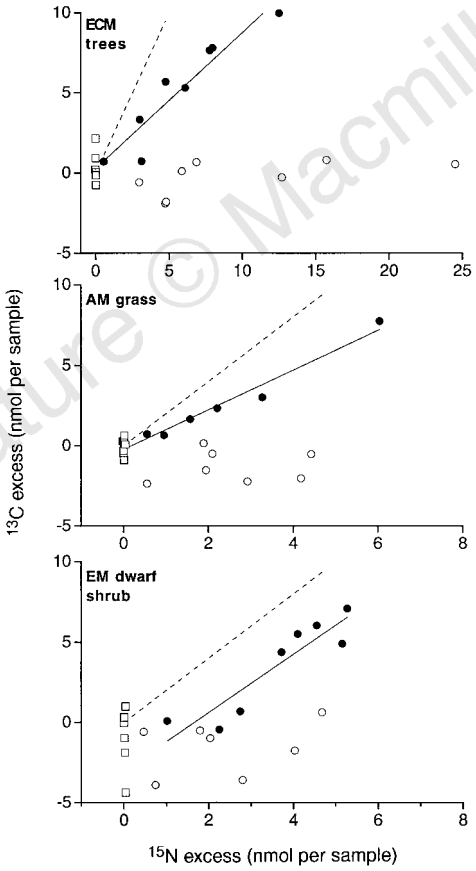

Figure 1 The relationship between excess ^{13}C and excess ^{15}N in the soluble nitrogen fraction in plant roots from the plots 6 h after injecting the mor layer with water (control plots, squares) or solutions containing (U-$^{13}C_2$, ^{15}N) glycine (filled circles) or $^{15}NH_4^+$ (open circles). The ^{13}C:^{15}N ratio of the glycine (2:1) injected (broken lines) and the regressions relating to glycine-treated plots (unbroken lines) are shown. ECM, ectomycorrhizal (slope = 0.85, $r^2 = 0.89$); AM, arbuscular mycorrhizal (slope = 1.24, $r^2 = 0.97$); EM, ericoid mycorrhizal (slope = 1.81, $r^2 = 0.84$); n = 6–8.

Table 2 $\partial^{15}N$ of shoots from the dwarf shrub *V. myrtillus* and the grass *D. flexuosa*

Species	Treatment	$\partial^{15}N$ of shoots*		
		2 h	1 day	7 days
Dwarf shrub	Control	− 1.5 ± 0.6	− 1.5 ± 0.6	− 1.1 ± 0.7
	$^{15}NH_4^+$	1.5 ± 1.5	2.5 ± 3.4	11.5 ± 7.0
	(U-$^{13}C_2$, ^{15}N) Glycine	− 0.5 ± 0.7	3.3 ± 1.7	3.8 ± 1.4
Grass	Control	− 0.1 ± 0.9	− 1.0 ± 0.7	− 0.3 ± 0.7
	$^{15}NH_4^+$	2.1 ± 0.8	12.5 ± 10	64.9 ± 36.5
	(U-$^{13}C_2$, ^{15}N) Glycine	2.6 ± 1.4	3.3 ± 1.5	20.5 ± 8.2

* Denotes part per thousand (‰) deviations form the ratio ^{15}N:^{14}N in atmospheric N_2. These results were obtained from plots treated with either water (control), $^{15}NH_4^+$ or (U-$^{13}C_2$, ^{15}N) glycine. (Averages ± s.e.m., n = 8.)

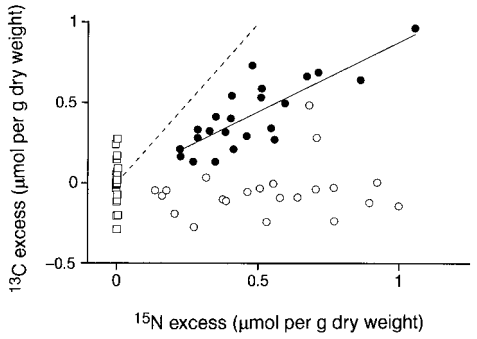

Figure 2 The relationship between excess ^{13}C and excess ^{15}N in the mor layer into which water, or solutions containing (U-$^{13}C_2$, ^{15}N) glycine or $^{15}NH_4^+$, was injected. The ^{13}C:^{15}N ratio of the glycine (2:1) injected (broken line) and the regression relating to glycine-injected plots are shown (unbroken line) (slope = 0.87, $r^2 = 0.67$). Data from all samplings are pooled as there were no major differences between samplings. Symbols as in Fig. 1; n = 24.

three samplings of the mor layer was 0.86, corresponding to 27% recovery of added glycine carbon (Fig. 2). The rapid decline in the ^{13}C:^{15}N ratio in the soil between 0 h and 1 day is corroborated by data showing that the ^{13}C-CO_2 respiration rates were 14-fold higher at 2 h than at 7 days. These results further strengthen the conclusion that the turnover of glycine is very dynamic, and support the idea that uptake and initial decarboxylation of the glycine is rapid. The magnitude of that process (Fig. 2) indicates that biotic immobilization of glycine-derived nitrogen is at least as important as any possible mechanism of abiotic immobilization. For example, if all of the 64% of glycine recovered in the mor layer was decarboxylated, there should be 32% recovery of the added ^{13}C. This is similar to the value found (27%, see above), which lends support to the idea that essentially all of the immobilization in the mor layer is biotic. In further support of this, release of ^{15}N after fumigation and extraction, and retention of ^{15}N-labelled tracer in the mor layer, were strongly correlated ($r^2 = 0.78$, $P < 0.001$). The biotic material of the sifted soil will include a fraction of ericaceous hair-roots and extramatrical mycelium, and therefore part of the nitrogen immobilized in this compartment is in the process of being transported to plants.

Evidence accumulated over a long period indicates that nitrogen is a key element regulating the production, structure and function of many terrestrial ecosystems[1]. Our data indicate, however, that current conceptual models may be overstating the importance of nitrogen mineralization. We have shown that the dominant plant species in these forests, irrespective of their type of mycorrhiza, all compete well for, and use, glycine as a N source. This demonstrates that nitrogen mineralization is not necessary before plants use organic nitrogen, and suggests that a separation between these groups of plants into those that are capable and incapable of using organic nitrogen should be made with care. However, there may be quantitative differences applying to different organic nitrogen sources, in which the apparently exclusive capacity of ectomycorrhizal and ericoid-mycorrhizal plants to excrete proteases may play a key role[6]. Differences in the capacity of these species to respond to variations in nitrogen supply rate are probably also important[17]. □

Methods

Field site. The experiment was conducted at Renberget (64° 14′ N, 19° 46′ E) in northern Sweden, 60 km northwest of Umeå. The area is located in the middle boreal zone in a late-successional *P. sylvestris/P. abies* forest of mesic dwarf shrub type, with a field layer dominated by the ericaceous dwarf shrub *V. myrtillus*. Other species present include the dwarf shrub *V. vitis-idaea* and the grass *D. flexuosa*. The total area used for the study was ~150 m × 50 m. The daytime temperature of the mor layer was ~7–8 °C during the study.

Experimental design. Circular plots (0.0464 m^2) were chosen so that shoots of both *V. myrtillus* and *D. flexuosa* plants were present within each plot. The site was divided into eight blocks, and within each block each treatment was replicated three times, allowing three samplings with eight repetitions each ($n = 8$).

Treatments. At 10:00 on 16 June 1996, the experiment was started by applying the labelled substances to the test plots, and water to the control plots. 250 ml 1.2 mM (U-$^{13}C_2$, 98 atom percentage; ^{15}N, 96–99 atom percentage) glycine or 1.2 mM ^{15}N-NH_4Cl (98 atom percentage) or water was injected into the mor layer (using a 50-ml syringe fitted with a 4-sideport needle) a few cm above the mineral soil. We made no attempts to stop solutions flowing either down into the mineral soil or laterally in the mor layer.

Sampling. The first step in sampling was cutting and recovering the above-ground vegetation on the plots. Thereafter, below-ground ^{13}C-CO_2 respiration was measured using a cuvette technique[18]. These measurements were taken within 10 min, on each of six plots simultaneously. The below-ground compartment was sampled by cutting out the treated mor layer soil core. Samples were put into plastic bags and transported on ice to the laboratory. Above-ground structures of *V. myrtillus* and *D. flexuosa* were sorted so that only fresh young leaves were taken from both species.

Roots of *V. myrtillus*, the two tree species (roots of which were not separated by species) and *D. flexuosa* were carefully picked from the soil core, thoroughly rinsed under tap water to remove soil material, and immersed thrice in 0.5 mM $CaCl_2$ to remove tracer adsorbed on root surfaces. Then, to obtain soil samples, the remaining cores were sifted through separate sieves (5-mm mesh) for each treatment. Samples were harvested (and stored at −22 °C) ~6 h (2 h for above-ground vegetation), 1 day and 7 days after application of the treatment solutions.

Analyses. Two sets of plant samples were prepared for analysis of ^{13}C and ^{15}N. The first set of plant samples and root-free soil samples were dried (70 °C, 24 h) and milled in a ball mill to a fine powder. The second set of plant samples was prepared by extraction of fresh and frozen roots in 10 mM phosphate buffer (pH 8.0), using 0.5 ml to 200 mg (fresh mass) plant material to obtain the soluble nitrogen fraction. The first set was used directly for isotopic analysis. The solutions of the second set were evaporated under reduced pressure to a final volume of ~50 μl, then transferred to tin capsules with Chromosorb absorbent. The extracts contained ~2–5 mg carbon and 0.02–0.05 mg nitrogen. The solutions were then dried (70 °C) before closing the capsules. Mass-spectrometric analysis was performed on a CF-IRMS[19] (continuous flow isotope ratio mass spectrometer). Microbial nitrogen levels were determined in moist, root-free soil samples after one day of chloroform fumigation and extraction[20,21]. The atom percentage of nitrogen released from microorganisms was measured after 48 h diffusion[22] of distilled nitrogen from Kjeldahl extracts onto acidified Al_2O_3 in tin capsules. The diffusion was done in 12-ml gas-tight vials. Corrections for background nitrogen were made using a calculated blank[23], by comparing diffused and non-diffused isotope standards treated as the original samples. Concentrations of amino acids and inorganic nitrogen species in soil were determined in soil extracts (Table 1) by HPLC.

Calculations. Data on nitrogen and carbon content, and atom percentage ^{15}N and atom percentage ^{13}C in excess of the natural abundances of ^{15}N and ^{13}C in control plots were used to calculate mol ^{15}N excess and mol ^{13}C excess for each sample from plots treated with glycine or NH_4^+.

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