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Low Nitrogen Losses with a New Source of Nitrogen for Cultivation of Conifer Seedlings

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Losses of nitrogen (N) when cultivating plants may cause a number of adverse environmental effects. N losses from conifer nurseries, for instance, may have a considerable impact on the local environment, and studies indicate that the bulk of added N is not recovered in the cultivated plants. This study was conducted to obtain insight into the causes of the low recovery and to test an alternative N fertilizer. Hence, growth of the economically important Scots pine (*Pinus sylvestris (L).*) seedlings and the recovery of different forms of added nitrogen (N) were investigated in a greenhouse experiment. Containerized seedlings were grown in peat for one summer, with two different N fertilizers, one organic (arginine) and one inorganic (a commercial fertilizer (CF) containing a mixture of ammonium and nitrate) each at an N concentration of 3 mM. At the end of the growth period, some seedlings were labeled with solutions containing either U-[$^{13}C_6$], [$^{15}N_4$]-arginine, $(^{15}NH_4)_2SO_4$, or $K^{15}NO_3$ supplied to the growth substrate. Labeled seedlings were harvested 1 h, 5 days, and 19 days after tracer addition, and the recovery of each added nitrogen source in both the seedlings and the growth substrate was measured. The retention of the three N forms during discharge of solutions from the growth substrate, peat, was tested in a separate experiment. Arginine-fed seedlings grew better and had higher needle N concentrations than the CF-fed seedlings. Isotopic data showed that the arginine treatment gave significantly higher N recoveries (80%) compared to the CF treatment (50%). The low recovery of N in the CF treatment was largely due to very low recovery (30%) of NO_3^- -N. The retention of the different N forms during discharge of solutions from the growth substrate was highest for arginine, somewhat lower for NH_4^+, and very low for NO_3^-. The high rate of seedling growth and the small nitrogen losses observed when using arginine suggest that this amino acid may be an efficient and environmentally favorable N source for cultivating conifer seedlings.

Introduction

Awareness of problems associated with nitrogen (N) leaching during various forms of plant cultivation has increased in recent years. Facilities responsible for such leaching include conifer nurseries, some of which show very low recoveries of added N (*1*). Traditionally, the principal N sources used for cultivating plants have been the inorganic N compounds ammonium (NH_4^+) and nitrate (NO_3^-) (*2*−*4*). A number of studies have shown that conifers have a preference for NH_4^+ and that NH_4^+ under certain conditions may inhibit the uptake of NO_3^- (*5*−*12*). Moreover, NO_3^- can easily be lost from the growth substrate due to its high mobility, further increasing conifer use of NH_4^+. Hence, it would seem logical that the main N form used for growth of conifers should be NH_4^+. However, a high fraction of NH_4^+ in the supplied fertilizer has been shown to cause serious problems such as nutrient imbalance, NH_4^+ toxicity, and acidification of the growth substrate (*13*−*16*). These problems have limited the use of NH_4^+ fertilizers for cultivating seedlings. To avoid the problems associated with NH_4^+ nutrition, a high fraction of NO_3^- is present in most commercial fertilizers, despite the obvious risk of low recovery of this N form. Thus, fertilizing conifer seedlings in large-scale facilities currently involves a compromise between the risk of ammonium toxicity for seedlings and the risk of N leaching to the environment. The need for alternative N sources is, therefore, compelling.

It has become increasingly apparent that organic N contributes to the N economy of a range of different plant species (*16*−*32*). The range of potential N sources that could be used to promote plant growth may, therefore, be substantially broader than previously thought and alternative N sources could be sought among a wide range of organic N forms.

In a previous work, we have shown that high growth and balanced nutrition can be obtained for both Scots pine and Norway spruce seedlings when amino acids are used as N sources (*16*).

In the current study, we focus on the retention of N when growing conifers on either arginine or a commercial fertilizer (CF) (containing a mixture of nitrate and ammonium). Thus, we test the hypothesis that the use of arginine as an N source could lead to lower N losses compared to the use of CF−N. Retention and growth on arginine N and CF−N were compared during cultivation of containerized Scots pine seedlings.

Materials and Methods

Plant Material and Growth Conditions. Seeds of Scots pine (*Pinus sylvestris* (L).) were obtained from seed orchard 410 (63°15′ N) Robertsfors, Sweden. Seedlings were grown for one summer in 20 cassettes (each containing 60 seedlings) in a greenhouse in the Gideå conifer nursery, Gideå, Sweden. Unfertilized peat (Sphagnum pH 5.5, humic degree H2−H4) was used as growth substrate. The seedlings were supplied with natural light, and the temperature was maintained at a constant 20 °C. The growth conditions were nonsterile and hence mycorrhizal infection was not regulated.

Two different nutrient solutions were supplied to the seedlings, one in which the N source was arginine and the other a commercial fertilizer (CF) containing 38.5% NH_4^+-N and 61.5% NO_3^- -N commonly used in conifer nurseries. The N concentration in the two nutrient solutions was 3 mM, and the concentration ratios between N and the other nutrients were identical for the two treatments. The ratios between the nutrients were based on the ratios in the commercial fertilizer, Superba S (Hygro Agil) which contains (% w/w): N 6.5 (NO_3^- -N 4.0, NH_4^+-N 2.5); P 1.0; K 4.7; Mg 0.6; S 0.5; B 0.01; Cu, 0.003; Fe 0.07; Mn 0.04; Mo 0.001; Zn 0.01. For each nutrient solution there were 10 cassettes, each containing 60 seedlings. Thus, each seedling received 16.7 mL or 0.7 mg N from the respective nutrient solutions twice a week with the help of a watering can. To prevent breakdown of arginine, the nutrient solutions containing this amino acid

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were stored in a refrigerator at a temperature of 5 °C. Moreover, the quality of the nutrient solution containing arginine was also repeatedly examined through HPLC-measurements (*33*) of the arginine and ammonium concentrations. The pH of the nutrient solutions was set to 5.0 with HCl, and the seedlings also received water twice a week between the nutrient solutions supliments.

Growth and Mineral Nutrient Analysis. At the end of the growth period, 30 randomly chosen actively growing seedlings from each treatment were harvested and dried at 60 °C for 48 h. Dry weights were measured for each seedling, and the mean dry weight per seedling was calculated.

N, C, P, K, Mg, S, Ca, and Fe concentrations were analyzed in seedlings from both treatments. For these analyses, six seedlings (i.e. $n = 6$) from each treatment were harvested at the end of the growth period and dried at 60 °C for 48 h. Seedlings were milled to a fine powder in a ball mill and dried at 40 °C for 24 h. Samples were analyzed using ICP-AES (Perkin-Elmer plasma 2000) (*34*) to determine their P, K, Mg, S, Ca, and Fe contents and an elemental analyzer (Perkin-Elmer 2400 CHN) to determine their C and N contents. All analyses were done at the Laboratory of Environmental research at the Department of Forest Ecology (SLU), Umeå, Sweden.

Retention Experiment. At the end of the growth period, 18 arginine-fed and 36 CF-fed actively growing seedlings were used in a labeling experiment. Each arginine-fed seedling was labeled with a solution containing 1.39 mg of U-[$^{13}C_6$], [$^{15}N_4$]-arginine (^{15}N 96−99%, ^{13}C 98%) and 1.10 mg of nonlabeled arginine. Half of the CF seedlings were labeled with a solution containing 3.14 mg of KNO_3 (^{15}N 98%) per seedling and the other half with a solution containing 1.29 mg of $(NH_4)_2SO_4$ (^{15}N 98%) per seedling. The concentrations and volumes of the tracers supplied were equal to the concentrations and volumes that were normally supplied to each seedling (i.e. 3 mM of the respective N compound in 17 mL of aqueous solution). Seedlings and their respective growth substrates were harvested 1 h, 5 days, and 19 days after tracer addition, and the roots were immediately separated from the growth substrate. For this, the seedling roots were first thoroughly washed with tap water and then immersed twice in 0.5 mM $CaCl_2$ solution to remove tracer adsorbed on the root surface. Both seedlings and their respective clumps of peat substrate were instantly frozen at −19 °C. The seedlings and peat clumps were then thawed and dried at 60 °C for 48 h. The dried seedlings were milled in a ball mill, while the dried peat clumps were milled in a Labassco type A 10 mill. To ensure total dryness of the samples the powders obtained from both the seedlings and peat clumps were dried a second time at 35 °C for 48 h. Finally, all samples were analyzed using a carbon−nitrogen analysis system (an ANCA-NT Solids/Liquids preparation Module coupled to an Europe 20−20 isotope ratio mass spectrometer (IRMS) from Europa Scientific) according to Ohlson and Wallmark (*35*).

Atom % excess values of ^{15}N and ^{13}C were calculated by subtracting the mean ^{15}N and ^{13}C abundances respectively of nonlabeled plants or peat clumps from the atom % of each labeled sample. The mole excess of the isotopes was then calculated by multiplying excess atom % ^{15}N and ^{13}C with the molar contents of N and C respectively of the seedlings or peat clumps. The recovery of added N and C was calculated as the ratio of moles recovered tracer to moles supplied tracer.

$$\text{recovery of added N (\%)} = ((\text{atom \% ls} - \text{atom \% nls}) * \text{molar content of N})/\text{moles supplied tracer}$$

$$\text{atom \% ls} = \text{atom \\% of labeled sample}$$

$$\text{atom \% nls} = \text{atom \\% of non labeled sample}$$

TABLE 1. Mineral Nutrient Concentrations in Needles of Scots Pine Seedlings from the Two Treatments (% of dw)[a]

	treatment	
element	arginine	CF
C	49.03 (±0.10)	49.98 (±0.26)
N	**2.13** (±0.10)	**1.70** (±0.07)
K	0.62 (±0.06)	0.68 (±0.02)
Ca	0.13 (±0.01)	0.13 (±0.01)
Mg	0.14 (±0.01)	0.15 (±0.01)
P	0.20 (±0.01)	0.22 (±0.01)
S	0.14 (±0.01)	0.12 (±0.01)
Fe	0.01 (±0.00)	0.01 (±0.00)

[a] Values are average of analyses of six seedlings and figures in parentheses indicate SE. Significant differences between treatments are indicated by boldface figures.

Recovery of added C was calculated identically as recovery of added N.

Laboratory Experiment. To examine the binding capacity of the substrate for the different N forms, a laboratory experiment was performed, in which 1 g of dry unfertilized peat (*Sphagnum* pH 5.5, humic degree H2−H4) was added to each of three 60 mL syringes (Plastipak) fitted with glass-fiber membranes (GF 30 Schleicher and Schuell) at the bottom. Five milliliters of ultrapure water was then added to the syringes in order to humidify the peat, and after 1 h excess water was removed using a vacuum manifold with a vacuum of −20 kPa. Another 2 mL of ultrapure water, set to pH 5.0 with HCl, was added to the syringes and after one minute the solution was collected, again using a −20 kPa vacuum as described above. Two milliliter portions of solutions containing arginine, NH_4Cl, or KNO_3, at an N concentration of 3 mM (pH 5.0), were then added to the prepared peat columns and after one minute the solutions were collected. This procedure was then repeated nine times. All samples were immediately frozen and stored at −19 °C until further processed. Ammonium and arginine in the samples were analyzed as their 9-fluorenylmethyl chloroformate derivatives by reversed-phase liquid chromatography following Näsholm et al. (*33*) using a Waters HPLC system and detected using a Waters 470 fluorescence detector. Individual components were separated on a LiChroCART 250-4 Superspher 100 RP-18 column using the following elution program: 0−10 min, 40% MeOH; 10−20 min, 50% MeOH, 20−30 min, 68% MeOH, 30−40 min; 90% MeOH; 40−45 min, 100% MeOH, the balance being provided by 1 mL of triethylamine and 6.5 mL HAc/1000 mL of ultrapure water, the pH set to 4.2 with NaOH.

Nitrate was analyzed using a FIA-Tecator 5012. These analyses were done at the Laboratory of Environmental research at the Department of Forest Ecology (SLU), Umeå, Sweden.

Statistical Analysis. The effect of N treatment on recovered tracers was tested using Analysis of Variance (ANOVA) followed by Tukeys post hoc test and unpaired *t*-tests were used to evaluate potential differences in dry weight between the arginine- and CF-fed seedlings.

Results

The arginine-fed seedlings had significantly higher mean dry weights (0.33 g) than the CF-fed seedlings (0.27 g) ($P = 0.0002$). Further, analyses of mineral nutrient content in the seedlings (Table 1) revealed a significantly higher N concentration in the arginine-fed seedlings compared to the CF-fed seedlings ($P = 0.0057$). Other differences in nutrient concentrations were generally small between the two treatments (Table 1).

An hour after tracer addition 66% and 78% of the added N was recovered in either the peat substrate or in the seedlings

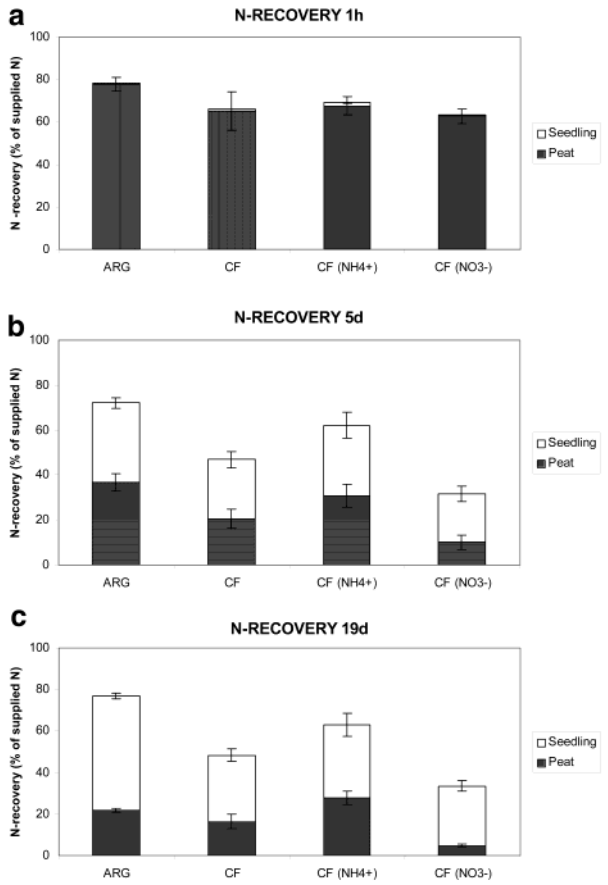

FIGURE 1. a−c. Total nitrogen recovery in seedlings and peat for the three labeled nitrogen sources: arginine, ammonium, and nitrate. The different figures show mean total nitrogen recovery in seedlings (white bars) and peat (black bars) 1 h (a), 5 days (b), and 19 days (c) after tracer addition. Error bars indicate SE ($n = 6$).

for the CF and arginine treatments, respectively. Hence, 22−34% of the added N had been lost already 1 h after N addition, possibly due to runoff of N-solutions after tracer addition. Most of the recovered N was recorded in the peat substrate for all N forms, and the amount that had been taken up in seedlings was minor (Figure 1a). However, significantly higher levels of recovered N were found in the peat substrate in the arginine treatment compared to the CF treatment ($P = 0.0118$). Moreover, in the peat, differences in recovered N between NH_4^+-N and NO_3^- -N in the CF treatment were small, while in the seedlings a significantly higher level of NH_4^+-N compared to NO_3^- -N was found ($P = 0.018$).

Five days after tracer addition, the amount of N recovered in the seedlings had increased for all N forms, while the N recovered in the peat substrate had decreased (Figure 1b). The total N recovery (i.e. the sum of N recovered in both the peat substrate and the seedlings) was significantly higher for the arginine treatment compared to the CF treatment ($P = 0.0077$). Significant differences in N recovery between the treatments were recorded in the peat substrate ($P = 0.0042$) but not in the seedlings at this harvest. Within the CF treatment, the NO_3^- -N recovery in peat was significantly lower than the NH_4^+-N recovery ($P = 0.0033$). Moreover, the recovery of NO_3^- -N in seedlings was also significantly lower than the NH_4^+-N recovery ($P = 0.0003$).

Nineteen days after tracer addition, the N recovery from seedlings given the arginine treatment had increased compared with the harvest 5 days after additions, while the corresponding increase in seedlings given the CF treatment was minor (Figure 1c). This resulted in a significantly higher total N recovery for the arginine treatment compared to the CF treatment ($P < 0.0001$). Moreover, the N recoveries in seedlings and peat substrate were both significantly higher for the arginine treatment than for the CF treatment ($P < 0.0001$ and $P = 0.0095$, respectively). Within the CF treatment both total and peat substrate N recovery were found to be significantly higher for NH_4^+-N compared to NO_3^- -N ($P < 0.0001$ and $P < 0.0001$, respectively). Thus, when comparing the three harvests it becomes clear that the largest loss of NO_3^- -N occurred during the first 5 days after tracer addition, while the loss of NH_4^+-N and especially arginine-N was small during the entire experiment.

In all harvests, total N concentration was significantly higher in arginine-fed compared to CF-fed seedlings (Figure 2). For arginine-fed seedlings, total N concentrations did not differ between the harvests. For CF-fed seedlings, however, a gradual decrease in total N concentration with time was recorded, resulting in increasing differences in total N concentration between arginine- and CF-fed seedlings (with P values ranging from 0.02 to 0.04).

The amount of the different N sources leached from peat samples differed markedly (Figure 3), being low for arginine, somewhat higher for NH_4^+, and high for NO_3^-. The initial proportions of retained N were 72% for NO_3^-, 100% for NH_4^+, and 99% for arginine. The amount of NO_3^- retained declined rapidly in successive fractions and even by the third portion, retention was close to zero. For both arginine and NH_4^+, a gradual decrease in retention was found. Further calculation based on these figures indicate a binding capacity of 9 μmol, 37 μmol, and 47 μmol/g dry weight for NO_3^-, NH_4^+, and arginine, respectively, at the end of the experiment.

As mentioned above, most ^{15}N was found in the peat substrate an hour after addition of U-[$^{13}C_6$], [$^{15}N_4$]-arginine (Figure 1a). The highest levels of ^{13}C were also found in peat (Figure 4a), while only minor fractions of both ^{13}C and ^{15}N were found in the seedlings at this stage (Figure 4b). Moreover, the ratio between excess ^{15}N and excess ^{13}C in peat did not change as much as the corresponding ratio in the seedlings during the course of the experiment (Figure 5a,b).

Discussion

Seedlings supplied with arginine grew more and had higher needle N concentrations (Table 1) compared to CF-fed seedlings. As no differences in concentrations of other macronutrients were recorded, and the total amounts of nutrients supplied to the seedlings were equal, the most probable cause to the recorded growth differences was the difference in N form supplied. We therefore suggest that the higher growth in the arginine treatment was caused by a combination of strong retention of N in the peat growth substrate combined with a high rate of arginine uptake by the seedling roots. This hypothesis is corroborated by the levels of ^{15}N remaining in the plant-peat system at the end of the retention study, showing that more than 67% of the supplied NO_3^- -N was lost while corresponding losses for NH_4^+-N and arginine-N were 37% and 23%, respectively (Figure 1c). Thus, the data from this study supports our original hypothesis that the retention of arginine in peat is high and that the use of this N source may lead to smaller N losses when cultivating conifer seedlings. Furthermore, it seems highly probable that the low retention of NO_3^- in the growth substrate combined with the slow rates of root uptake recorded in the previous study (*16*) are the causes of both the low growth rates and the high losses of N in the CF-treatment.

The advantage of using arginine should be greatest when seedlings are grown in small volumes of growth substrate, with frequent supplies of water and with fertilizer supplied in relatively high concentrations. Such conditions inevitably lead to large losses of NO_3^- -N, but are nevertheless commonly maintained in conifer seedling nurseries. Thus, the

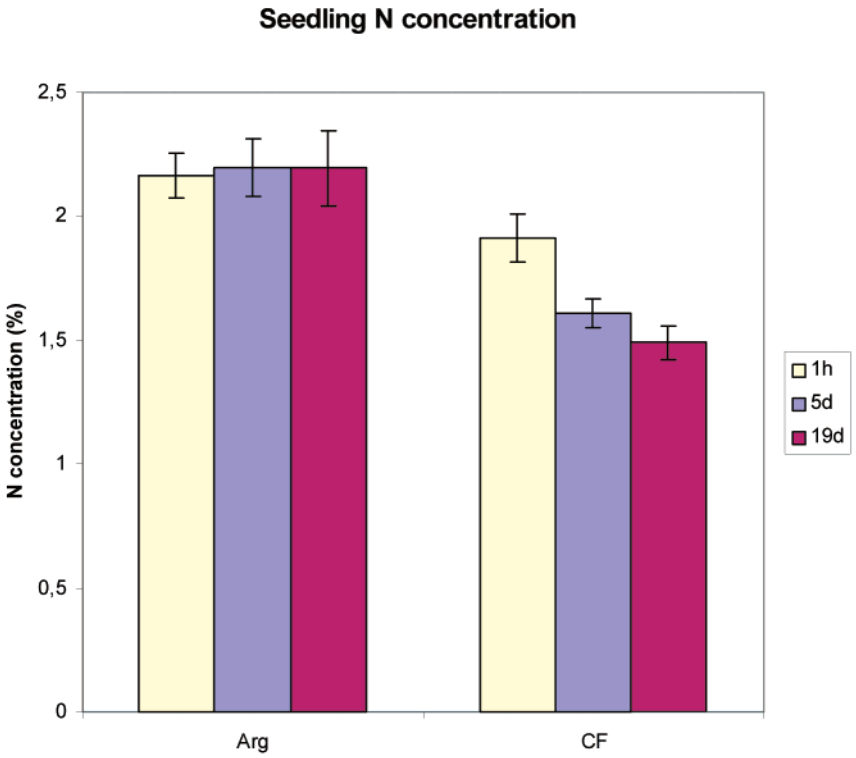

FIGURE 2. Nitrogen concentration in seedlings grown on arginine or a mixture of ammonium and nitrate 1 h, 5 days, and 19 days after the last fertilizing occasion. Error bars indicate SE ($n = 6$).

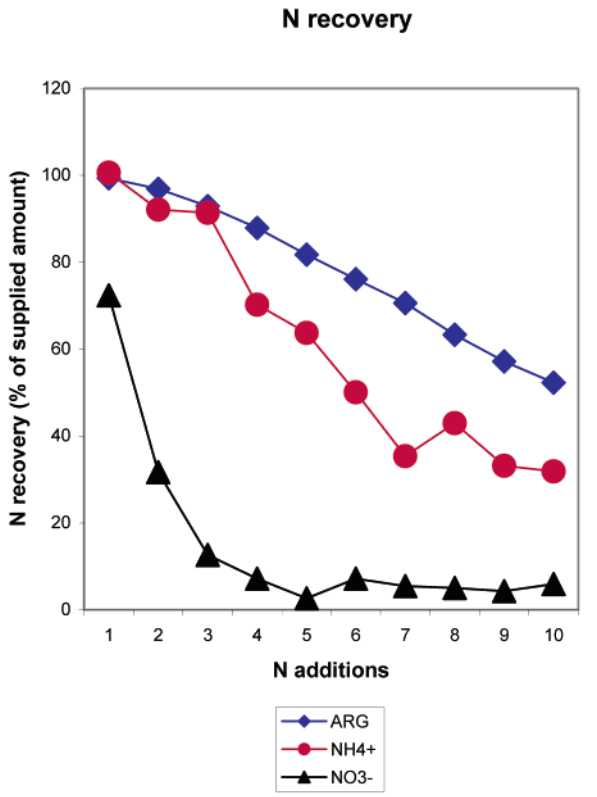

FIGURE 3. Recovery of arginine (◆), ammonium (●), and nitrate (▲) in peat. The *x*-axis shows the number of additions of the respective N forms.

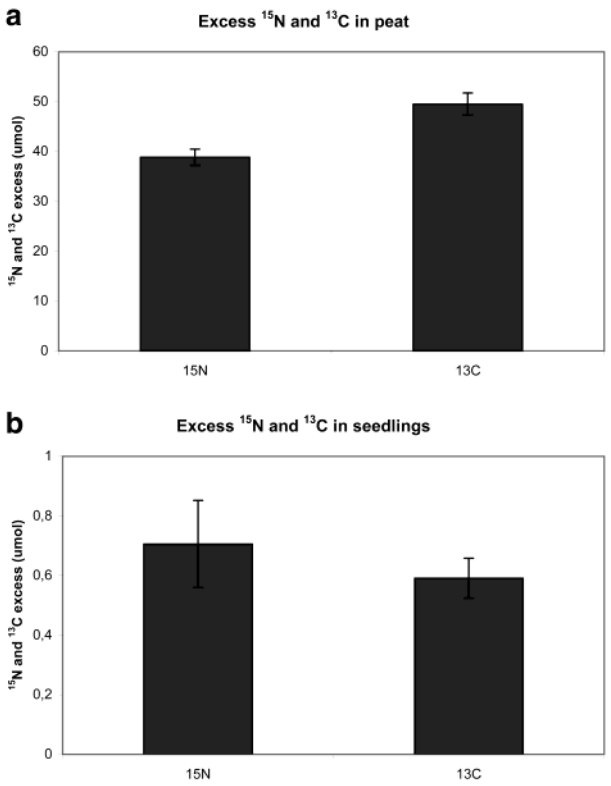

FIGURE 4. a,b. Total recovery of ^{15}N and ^{13}C in (a) peat and (b) seedlings 1 h after addition of U-[$^{13}C_6$], [$^{15}N_4$]-arginine tracer. Error bars indicate SE ($n = 6$).

difference between the commercial fertilizer and arginine could have been even greater if comparisons had been made at a commercial growth unit.

As mentioned above, the N losses from the arginine treatment at the end of the experiment were significantly smaller than corresponding losses of NO_3^- and NH_4^+ in the CF treatment (Figure 1c). These results indicate that peat has an equal or higher binding capacity for arginine than for NH_4^+, which itself tends to be bound at high capacities in diverse growth substrates. This conclusion is also supported

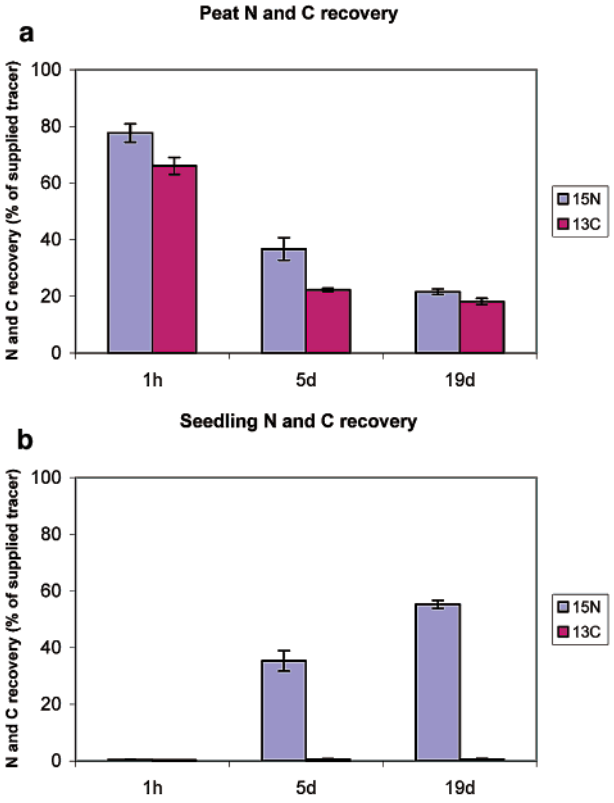

FIGURE 5. a,b. ^{15}N and ^{13}C recovery in (a) U-[$^{13}C_6$], [$^{15}N_4$]-arginine labeled seedlings and (b) peat harvested 1 h, 5 days, and 19 days after tracer addition. Error bars indicate SE ($n = 6$).

by results from the retention study and the measurements of total N content in the seedlings supplied with arginine, both of which showed that peat has a high binding capacity for arginine (Figures 3 and 2). The N concentration of seedlings grown on arginine remained constant during the whole labeling experiment (19 days), while the N concentration of the CF-grown seedlings declined markedly with time (Figure 2). These results suggest that the arginine-fed seedlings had access to N a long time after the supply of N had been terminated. Hence, it could be speculated that arginine accumulated in the peat substrate while it was being regularly added, which may explain how the seedlings were able to maintain high N contents during the whole labeling period. The findings also suggest that it may be possible to supply seedlings with a store of N in the growth substrate before planting. Such use of arginine may promote the establishment and early growth of seedlings following planting out in the field.

The arginine used in this experiment was of analytical grade and thus rather expensive. However, at conifer nurseries the cost for fertilizers are rather small compared to other costs i.e., heating of greenhouses, management, etc. As a consequence the extra cost for growing seedlings on equal amounts of analytical grade arginine instead of CF−N becomes rather small, below 1% of the total cost of producing a seedling. It is also likely that this extra cost for arginine-N could be reduced if an arginine source with lower quality is found.

A critical issue when comparing seedlings grown with the two types of N is the actual form of N utilized by the seedlings. Earlier attempts to separate uptake of amino acids from uptake of mineralized N have indicated that most, if not all, N is taken up as intact amino acids (*16*, *19*, *25*). However, this conclusion was drawn from short-term uptake experiments. Analysis of the arginine-fed seedlings 1 h after labeling showed that only a minor proportion, 0.45%, of the supplied tracer had been absorbed at this point (Figure 1). Hence, the actual form of N absorbed during the following 19 days is not known. However, 1 h after tracer additions, elevated levels of both ^{13}C and ^{15}N (Figure 4a) were detected, suggesting that 56% of the absorbed arginine-N was taken up as intact amino acid. At this harvest, almost 78% of the supplied arginine was recorded in the peat substrate and the relation of ^{13}C to ^{15}N indicated that 65% of the arginine remained intact at this point (Figure 4b).

In the seedlings, the time-dependent increase in ^{15}N was not followed by a corresponding increase in ^{13}C (Figure 5a), while in the peat substrate, the relationship between ^{15}N and ^{13}C remained approximately constant during the experiment (Figure 5b). This may have been due to the seedling taking up N solely in the mineralized N, or to high respiration of absorbed amino acid C within seedlings, or to dilution of ^{13}C by produced ^{12}C from photosynthesis, or a combination of these possibilities. Additional experiments are needed to characterize more fully the uptake of intact and mineralized amino acid by plants.

In conclusion, this study shows that Scots pine seedlings grown under conditions similar to those used in conifer nurseries accumulated higher dry weights and needle N contents when supplied with the organic N source arginine compared to seedlings supplied with a commercial fertilizer containing the inorganic nitrogen sources NH_4^+ and NO_3^-. Further, at the end of the retention experiment the losses of arginine-N were significantly lower than the losses of NO_3^- -N and NH_4^+-N. The high levels of recovery of arginine were probably due to the strong binding of the compound to the growth substrate, together with a high rate of root uptake. The use of arginine as an N source thus gives a desirable combination of high seedling growth rates together with low N losses. Arginine may therefore offer an attractive alternative to the commercial fertilizers used today to promote growth in conifer seedlings.

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