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Growth of conifer seedlings on organic and inorganic nitrogen sources

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Summary Effects of organic and inorganic nitrogen sources on growth and mineral nutrient concentrations of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) seedlings were compared in a 100-day experiment in a greenhouse. Seedlings were grown in pots containing peat. Nutrient solutions differing in ammonium, nitrate, arginine and glycine composition were supplied to the seedlings at three nitrogen (N) concentrations: 1, 3 and 10 mM. We used dual (^{13}C, ^{15}N) and single (^{15}N) isotopic labeling to determine the uptake of organic and inorganic N at the end of the experiment. Seedling dry weights and mineral nutrient concentrations of the needles showed that both conifer species were able to grow well and maintain nutrient balance on all investigated N forms except for the ammonium-dominated nutrient mixtures at the 10 mM N concentration. In Scots pine, no significant differences in dry weights were found between seedlings grown on the amino acids and seedlings grown on a commercial fertilizer containing 61.5% NO_3^--N and 38.5% NH_4^+-N. Isotopic labeling of seedlings indicated that uptake rates of arginine-N, glycine-N and NH_4^+-N were similar, and 7–8 times greater than uptake rates for NO_3^--N in both species. In Scots pine seedlings, 100% of arginine-N, and at least 67% of glycine-N was derived from the uptake of intact amino acids through seedling roots or mycorrhizae. Corresponding figures for Norway spruce were 83% for arginine and 96% for glycine. The gas chromatography–mass spectrometry analyses confirmed the presence of intact labeled molecules of both arginine and glycine in seedlings. We conclude that arginine and glycine are comparable to inorganic N as N sources for growth of conifer seedlings.

Keywords: amino acids, arginine, gas chromatography–mass spectrometry, glycine nitrogen uptake.

Introduction

In all forms of plant cultivation, an abundant supply of nitrogen (N) is a prerequisite for good growth. Traditionally, inorganic N compounds (i.e., ammonium and nitrate) have been regarded as the principal N sources for plants (e.g., Runge 1983, Haynes 1986, Mengel and Kirkby 1987), but the relative importance of these substances differs among plant species (Stewart et al. 1988, Falkengren-Grerup and Lakkenborg-Kristensen 1993, Schmidt and Stewart 1997). It is well established that conifers have a strong preference for NH_4^+, the rate of uptake of this N form typically being 2–20 times greater than that of NO_3^- (Buchmann et al. 1995, Kronzucker et al. 1997). Moreover, NH_4^+ may inhibit the uptake of NO_3^-, further emphasizing the preference for NH_4^+ (Lee and Drew 1988, Marschner et al. 1991, Donaldsson Knoepp et al. 1993, Kamminga-van Wijk and Prins 1993). Nitrate, a mobile anion, can also be leached easily from the growth substrate if rates of plant or soil microbial uptake are low. It would therefore seem logical to supply NH_4^+ as the main N form when growing conifer seedlings or other plants with a strong preference for NH_4^+. However, NH_4^+ nutrition can be accompanied by several problems, such as nutrient imbalance, NH_4^+ toxicity and acidification of the growth substrate (Boxman and Roelofs 1988, Rollwagen and Zasoski 1988, Griffin et al. 1995). Typically, the uptake of base cations such as K^+, Mg^{2+} and Ca^{2+} is hampered in the presence of NH_4^+ (Boxman and Roelofs 1988, Marschner 1995). Thus, supplying N for growth of conifers, and for a range of other plants, may be problematic. If a large proportion of NO_3^- is present in the fertilizer, uptake by the plants may be low, and significant amounts of N may be lost to the surrounding environment. On the other hand, NH_4^+-dominated fertilizers may give rise to low growth rates, high mortality and low amounts of base cations in the plants. Alternative N sources for large scale production of plants are therefore needed.

Several studies have shown that a range of plant species, including conifers, can take up organic N, notably a range of amino acids (Virtanen and Linkola 1946, Melin and Nilsson 1953, Abuzinadah and Read 1986, Schobert and Komor 1987, Chapin et al. 1993, Kielland 1994, 1995, Turnbull et al. 1995, 1996, Raab et al. 1996, Näsholm et al. 1998, 2000, 2001, Lipson et al. 1999, 2000, Schmidt and Stewart 1999). Thus, the range of potential N sources for plants may be substantially broader than previously thought. If so, there is the potential to use alternative N sources for plant growth. Plants such as *Eriophorum vaginatum* (L.) (Chapin et al. 1993), *Eucalyptus grandis* Hill *ex* Maiden and *E. maculata* Hook (Turnbull et al. 1995) can grow on various organic N sources. However, these studies were either performed in solution culture (Chapin et al. 1993) or under sterile conditions on agar (Turnbull et al. 1995) and therefore may not provide conclusive evidence that plants can take up N from organic N sources when growing under natural conditions. Attempts to use complex N sources such as

keratin to promote the growth of conifer seedlings yielded negative results (Seith et al. 1996, George et al. 1999). Thus, the extent to which organic N sources can improve the growth of plants remains uncertain.

Our objective was to determine how different organic and inorganic N sources affect the growth and development of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) seedlings under non-sterile conditions. Two organic N forms, arginine and glycine, and the inorganic forms NH_4^+ and NO_3^- were tested, alone and in various combinations. Uptake rates of the different N sources, as well as the fraction of N taken up as intact amino acids, were also studied by means of stable isotope techniques.

Materials and methods

Plant material and growth conditions

Seeds of Scots pine were obtained from Seed Orchard 410 (63°15′ N), Robertsfors, Sweden and seeds of Norway spruce were obtained from native trees (63°15′ N, 250 m a.s.l.). The seedlings were grown for 100 days in 0.5-l pots (five seedlings per pot) in a greenhouse at the Swedish University of Agricultural Sciences in Umeå. Unfertilized peat (Sphagnum, pH 5.5, humic degree H2–H4) was the growth medium. The seedlings were supplied with constant light of 200 µmol m^{-2} s^{-1} (Philips Powertone SON-T Plus 400 W) and were kept at a temperature of 20 °C. The growth conditions were non-sterile and hence mycorrhizal infection was not regulated. Nevertheless, inspection of roots revealed that all plants were mycorrhizal at harvest, although the extent of infection decreased with increasing N supply.

A basal nutrient solution based on a commercial fertilizer (CF) (Superba S or Hygro Agil) commonly used in conifer nurseries was prepared without the N components and adjusted to pH 5.0 with HCl. This mixture contained (% w/w): 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; and Zn, 0.01. Twenty-four test solutions were then prepared by adding eight variations of N supply (in which one had the same N composition as the CF) each at three different total N concentrations: 1, 3 and 10 mM (Table 1). For each test solution there were six replicates (pots) for both Scots pine and Norway spruce. The concentrations of non-N nutrients in the test solutions were as listed for the basal nutrient solution, except for one of the NH_4^+ treatments and one of the 95/5 (95% NH_4^+, 5% NO_3^-) treatments, which were given Mg^{2+} and K^+ at double rates, and were denoted NH_4^+KMg and 95/5KMg, respectively. Nutrient solutions were supplied to the seedlings twice a week with a Vogel pipette (0.1 l pot^{-1}). Once a week the seedlings received water (pH 5.0).

Table 1. Composition of nutrient solutions.

Treatment	N Form
CF	38.5% NH_4^+, 61.5% NO_3^-
Mixture	33% Arginine, 33% NH_4^+, 33% NO_3^-
Arginine	100% Arginine
Glycine	100% Glycine
NH_4^+	100% NH_4^+
95/5	95% NH_4^+, 5% NO_3^-
NH_4^+KMg	100% NH_4^+
95/5KMg	95% NH_4^+, 5% NO_3^-

Growth parameters and pH

At the end of the experiment, seedlings were harvested and dried at 60 °C for 48 h. Shoot and root dry weights were measured for each seedling and the total mean dry weight per pot (i.e., the total dry weight of five seedlings) was calculated. Shoot dry weight per pot, root dry weight per pot and shoot/root ratios for each pot were also calculated. After harvesting the Scots pine seedlings, the pH of the soil solution was measured.

Mineral nutrient analysis

Nitrogen, C, P, K, Mg, S, Ca and Fe concentrations were analyzed in Scots pine needles from all treatments. Seedlings were harvested and dried for 48 h at 60 °C. All green needles from each of three pots per treatment (n = 3) were removed and milled to a fine powder in a ball mill. The powder was then dried at 40 °C for 24 h. Samples were analyzed by inductively coupled plasma atomic emission spectroscropy (Plasma 2000, PerkinElmer, Shelton, CT) to determine their P, K, Mg, S, Ca and Fe contents. The C and N contents of the samples were determined with an elemental analyzer (PerkinElmer 2400 CHN). Analyses were performed at the Laboratory of Environmental Research at the Department of Forest Ecology (SLU), Umeå, Sweden.

Labeling experiment

At the end of the growth experiment one Arginine, one Glycine, two CF and three Mixture pots (all in the 3 mM N regime) with Scots pine seedlings, and a corresponding set with Norway spruce seedlings, were used in a labeling experiment. The Arginine pots were labeled with a solution containing 22.3 mg U-[$^{13}C_6$], [$^{15}N_4$]-arginine (^{15}N 96–99%, ^{13}C 98%). The Glycine pots were labeled with a solution containing 22.4 mg U-[$^{13}C_2$], [^{15}N]-glycine (^{15}N 99%, ^{13}C 98%). One CF pot with Scots pine seedlings and one with Norway spruce seedlings were labeled with a solution containing 6.5 mg $^{15}NH_4Cl$ (^{15}N 98%) and the remaining CF pots were labeled with a solution containing 18.4 mg KNO_3 (^{15}N 98%). Finally, one Mixture pot for each species was labeled with 22.3 mg U-[$^{13}C_6$], [$^{15}N_4$]-arginine (^{15}N 96–99%, ^{13}C 98%) one with 5.4 mg NH_4Cl (^{15}N 98%) and the third with 10.2 mg KNO_3 (^{15}N 98%).

Seedlings were harvested 2 h after tracer additions. Roots were thoroughly washed with tap water to remove soil particles and immersed twice in 0.5 mM $CaCl_2$ solution to remove tracer adsorbed to the root surface. The seedlings were frozen at –19 °C. Roots were then removed at the first lateral branching and freeze dried. The dried roots were milled in a ball mill and the powder was dried at 40 °C for 24 h. Finally, samples were analyzed in a carbon-nitrogen analyzer (ANCA-NT sys-

tem, Solids/Liquids preparation Module, PDZ Europa, Cheshire, U.K.) coupled to a Europa 20-20 isotope ratio mass spectrometer as described by Ohlsson and Wallmark (1999).

Atom% excess of ^{13}C and ^{15}N was calculated by subtracting the mean ^{13}C abundance and the mean ^{15}N abundance, respectively, of unlabeled plants from the atom% of each labeled sample. The mole excess of isotopes (per gram of root) was then calculated by multiplying excess atom% ^{13}C and ^{15}N, respectively, with the molar content of C and N per gram of root. The ^{13}C and ^{15}N values were plotted against each other, and the slopes of the regression lines were calculated. By comparing these slopes with the ratio of ^{13}C to ^{15}N in the tracer (i.e., 1.5 for arginine and 2.0 for glycine) an estimate of the fraction of arginine N absorbed as intact amino acid by the roots was obtained (cf. Näsholm et al. 1998, Näsholm and Persson 2001).

Gas chromatography–mass spectrometry (GC–MS) analysis

To verify the presence of universally labeled arginine and glycine in seedling roots, amino acids extracted from roots were analyzed by GC–MS. Milled and dried roots were extracted in 10 mM HCl for 1 h at 5 °C. Each extract was centrifuged at 20,000 *g* for 20 min, and the supernatant was removed and evaporated under reduced pressure in an evacuated centrifuge. Samples from plants supplied with labeled glycine were directly derivatized for GC–MS analysis. Samples from plants supplied with labeled arginine were further processed as follows. The amino acids in the root extracts were purified by solid phase extraction with Alltech (Vejle, Denmark) strong cation exchange cartridges, containing 200 mg of ion-exchange resin. This involved drawing 0.2–0.5 ml of each root extract through a column that had been conditioned with 10 ml of ultrapure water. The columns were washed with 2 ml of a methanol:water mixture (8:1 v/v) and amino acids were eluted with 2 × 1 ml of 6 M NH_4OH. Extracts were evaporated to dryness under reduced pressure, redissolved in 40 µl of ultrapure water and again evaporated.

Amino acids from extracts (glycine samples) and column effluents (arginine samples) were dissolved in 40 µl of *N*,*N*-dimethylformamide and converted to tert-butyldimethylsilyl (tBDMS) derivatives by adding 20 µl of *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) (Mawhinney et al. 1986, Woo and Lee 1995). Samples were analyzed on a Varian Saturn 2000 GC ion-trap MS system (Varian, Palo Alto, CA) operating in the chemical ionization mode. Samples were injected with a split ratio of 10 onto a 30 m × 0.25 mm fused silica capillary column with a chemically bound 0.25-µm CP-CIL 5 CB low bleed/MS phase. The injector temperature was set at 270 °C and the temperature in the ion trap was set at 250 °C. At the start of each run, the temperature in the column was held at 110° C for 2 min, and then linearly increased by 40 °C min^{-1} to 290 °C. Helium was used as carrier gas, at a head pressure of 76.5 kPa. In this system, the retention times for tBDMS-glycine, tBDMS-serine and tBDMS-arginine were 5.3, 6.5 and 7.9 min, respectively. The mass spectrometer detected the molecular ion of tBDMS-glycine at m/z 304 and that of tBDMS-U-[$^{13}C_2$], [^{15}N]-glycine at m/z 307. Derivatization of arginine with MTBSTFA resulted in two products: 3S-tBDMS-arginine and 4s-tBDMS-arginine, with m/z ratios of 501 and 631, respectively (cf. Mahwinney et al. 1986). The former, which dominated under the derivatization conditions used, is produced through the loss of one silylated NH_2 moiety of arginine. Therefore, tBDMS-arginine and tBDMS-U-[$^{13}C_6$], [$^{15}N_4$]-arginine were detected at m/z 501 and m/z 510, respectively, i.e., with a difference of 9 mass units instead of the 10-unit difference seen with unlabeled arginine. Ratios of m/z 307:304 and 510:501 were used to indicate the occurrence (and relative abundance) of labeled glycine and arginine, respectively, in seedling roots. Moreover, in samples from glycine-treated plants, labeling of serine was detected by analysis of the ions with m/z values of 449 and 453, corresponding to tBDMS-serine and tBDMS-U-[$^{13}C_3$], [^{15}N]-serine, respectively.

Reference samples were taken from Scots pine and Norway spruce seedlings grown with unlabeled arginine and glycine that had been processed and analyzed as described for the labeled samples.

Statistical analysis

The effects of N treatment on biomass production and allocation (total dry weight, and shoot/root ratio), mineral nutrient concentrations and uptake rates of tracers were tested by analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Unpaired *t*-tests were used to evaluate potential differences between reference compounds and glycine and arginine samples, respectively, in the GC–MS analysis.

Results

As shown in Figure 1, growth of seedlings of both species increased with increasing N availability, for all types of N supplied, except for the NH_4^+-dominated treatments (i.e., NH_4^+, 95/5, NH_4^+KMg and 95/5KMg). In the 10 mM N regime, significantly lower growth (Scots pine) or even death of seedlings (Norway spruce) was recorded in the NH_4^+-dominated treatments compared with the other N forms. Apart from this, differences in seedling growth between the N forms were minor.

For all treatments, and for both species, the shoot/root ratio increased with increasing N availability and differences between N forms were small, except in the NH_4^+-dominated treatments. For Scots pine seedlings, the shoot/root ratio increased from 4.4 ± 0.21 (mean ± SE) in the 3 mM N regime to 9.9 ± 0.74 in the 10 mM N regime when NH_4^+ was the major form of N supplied, whereas the corresponding shoot/root ratios for the other treatments increased from 3.8 ± 0.21 to 5.9 ± 0.15. Similarly, among Norway spruce seedlings grown with a 3 mM N supply, the shoot/root ratio was highest in seedlings grown in the NH_4^+-dominated treatment. We observed a distinct change in root morphology, with smaller and thicker roots at high N concentrations.

For all N forms and all N treatments, the pH of the soil solutions in which Scots pine seedlings had grown was low, and differences among N forms were generally minor (Table 2). However, the pH was significantly lower in soil solutions sup-

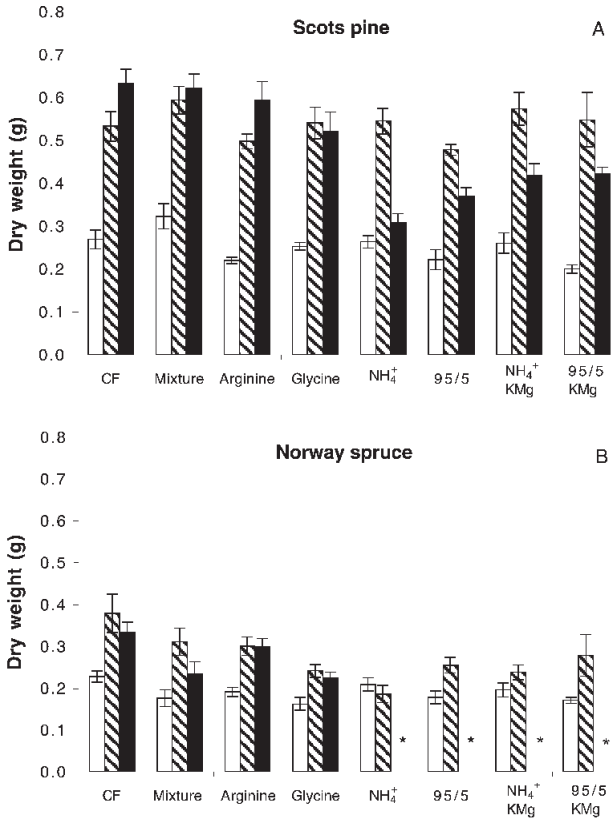

Figure 1. Treatment effects on dry weights of Scots pine (A) and Norway spruce seedlings (B). White, hatched and filled bars indicate 1, 3 and 10 mM N supply regimes, respectively. Values shown are means ± SE (n = 6). Asterisks indicate that no data is available because all plants in the treatment died.

plied with 3 and 10 mM N dominated by NH_4^+ than in the other treatments.

Nitrogen concentrations in needles of Scots pine seedlings were low in the 1 and 3 mM N regimes but high in seedlings grown in the 10 mM N regime (Table 3). In the 1 mM N regimes, no significant difference in N concentrations between N forms was recorded. In the 3 mM N regimes, a significantly higher N concentration was recorded in seedlings from the Mixture treatment compared with the other treatments. Calcium concentrations were significantly lower in seedlings grown in NH_4^+-dominated 3 and 10 mM N treatments than in seedlings grown in other treatments.

Table 2. Mean (± SE) pH of the soil solutions from pots with Scots pine seedlings (n = 4–6).

Treatment	pH		
	1 mM N	3 mM N	10 mM N
CF	3.6 ± 0.05	3.5 ± 0.03	3.4 ± 0.03
Mixture	3.5 ± 0.07	3.3 ± 0.05	3.2 ± 0.06
Arginine	3.4 ± 0.07	3.4 ± 0.06	3.3 ± 0.05
Glycine	3.3 ± 0.21	3.4 ± 0.04	3.6 ± 0.05
NH_4^+	3.3 ± 0.07	3.0 ± 0.05	2.8 ± 0.06
95/5	3.3 ± 0.06	3.0 ± 0.06	2.7 ± 0.07
NH_4^+KMg	3.3 ± 0.04	2.9 ± 0.03	2.7 ± 0.05
95/5KMg	3.4 ± 0.04	3.0 ± 0.06	2.6 ± 0.07

Tracer additions to the pots showed that uptake rates of seedlings in the Arginine, Glycine and CF treatments were similar, for both species (Table 4). Thus, the rate of uptake of amino acid N from both arginine and glycine was similar to the sum of NH_4^+ and NO_3^- uptake rates by seedlings in the CF treatment. Seedlings grown on CF took up little NO_3^--N compared with NH_4^+-N. The low uptake rate of NO_3^--N was even more pronounced in seedlings in the Mixture treatment. We found that Scots pine took up arginine-N at a significantly higher rate than NH_4^+-N from the CF mixture (Table 5).

Plotting values of excess ^{13}C versus excess ^{15}N in roots of seedlings supplied with dual-labeled arginine resulted in significant regressions. An estimate of the fraction of arginine N absorbed as intact amino acid by the roots was obtained by comparing the slopes of these regression lines with the slope representing 100% uptake as intact amino acid (which is equal to the ratio of ^{13}C to ^{15}N in the tracer, i.e., 1.5). Thus, in seedlings in the Arginine treatment, 100 and 83% of absorbed arginine N was derived from the uptake of intact arginine molecules in Scots pine and Norway spruce, respectively (Figure 2A). For seedlings grown on the Mixture and supplied with dual-labeled arginine, the corresponding values were 100 and 93% (Figure 2B). The fractions of glycine-N derived from the uptake of intact glycine were calculated in the same manner, but the ratio of isotopes ($^{13}C/^{15}N$) in the glycine tracer was 2.0. These calculations indicated that 67 and 96% of glycine N was absorbed as intact amino acid by Scots pine and Norway spruce, respectively (Figure 2C).

The GC–MS analysis of seedlings labeled with arginine or glycine tracers verified the occurrence of intact amino acids in both species (Figures 3A and 3B). For plants supplied with arginine tracer, the magnitude of the 510:501 m/z ratio was considered to indicate the presence of the labeled compound. Compared with reference samples, this ratio was significantly higher in plants of both species ($P < 0.0001$ for Scots pine and $P < 0.001$ for Norway spruce), being zero in reference samples and 24 and 3% in arginine-treated Scots pine and Norway spruce seedlings, respectively. Similarly, in glycine-treated plants, compared with reference samples, significant increases were found in the m/z 307:304 ion ratios ($P < 0.0001$ for Scots pine and $P < 0.01$ for Norway spruce). In reference samples of Scots pine and Norway spruce, ratios of m/z 307:304 were 0 and 2%, respectively, whereas corresponding values for seedlings labeled with U-[$^{13}C_2$], [^{15}N]-glycine were 445 and 400%. The relatively high ratios of m/z 307:304 in glycine-labeled seedlings compared with the m/z 510:501 ratios in arginine-labeled seedlings may be partially explained by the endogenous pool of arginine being much larger than that of glycine (data not shown).

In samples of glycine-treated plants, isotopic label was also detected in serine. The presence of universally labeled serine (U-[$^{13}C_3$], [^{15}N]-serine) was indicated by a significant increase in the m/z 453:449 ratio relative to reference samples (for which this ratio was zero) (Figure 3C). This ratio was 69 and

Table 3. Mean macronutrient concentrations (% dry weight) in needles of Scots pine seedlings from the different treatments. Values are means of three replicates. Significant differences between treatments are indicated by an asterisk.

Element	CF	Mixture	Arginine	Glycine	NH_4^+	95/5	NH_4^+KMg	95/5KMg
1 mM N								
C	47.73	48.53	47.47	47.33	47.97	47.80	47.47	47.40
N	1.03	1.22	1.07	1.04	0.92	0.94	1.04	0.85
K	0.70	0.67	0.67	0.70	0.71	0.73	0.78	0.71
Ca	0.18	0.16	0.18	0.16	0.15	0.15	0.16	0.16
Mg	0.13	0.15	0.14	0.14	0.13	0.14	0.14	0.13
P	0.12	0.09	0.11	0.11	0.10	0.11	0.11	0.07
S	0.13	0.12	0.12	0.12	0.12	0.14	0.14	0.12
3 mM N								
C	48.63	48.90	48.10	47.77	48.10	48.10	47.87	47.70
N	1.19	1.76*	1.22	1.04	1.10	1.11	1.02	1.05
K	0.79	0.83	0.78	0.73	0.77	0.80	0.76	0.87
Ca	0.18	0.21	0.17	0.16	0.13*	0.14*	0.14*	0.15*
Mg	0.14	0.15	0.16	0.15	0.15	0.16	0.13	0.15
P	0.15	0.12	0.14	0.12	0.14	0.14	0.13	0.13
S	0.13	0.14	0.12	0.10	0.13	0.15	0.13	0.16
10 mM N								
C	48.90	49.13	48.63	48.60	49.17	48.87	48.70	48.93
N	2.31	2.75	2.89	2.87	2.53	2.37	2.39	2.22
K	0.98	1.00	1.15	1.20	1.02	1.08	1.06	1.09
Ca	0.20	0.20	0.22	0.21	0.13*	0.12*	0.11*	0.11*
Mg	0.18	0.21	0.20	0.20	0.16	0.20	0.14	0.17
P	0.26	0.28	0.27	0.28	0.25	0.25	0.23	0.24
S	0.18	0.19	0.17	0.18	0.23	0.28	0.20	0.26

58% for glycine-treated Scots pine ($P < 0.001$) and Norway spruce ($P < 0.005$) seedlings, respectively.

Discussion

Both Scots pine and Norway spruce seedlings were able to grow well (Figure 1) and maintain nutrient balance (Table 2) on all nutrient solutions tested except those where N was supplied at high concentrations with NH_4^+ as the major form. Thus, plants supplied with arginine or glycine as sole N sources, and those supplied with a mixture of N forms (i.e., arginine, NH_4^+ and NO_3^-) performed as well as those supplied with a standard mixture of NH_4^+ and NO_3^-, and better than plants supplied with NH_4^+ as the predominant N source.

Isotopic analysis of seedling roots by isotope ratio mass spectrometry indicated that roots had elevated amounts of both ^{13}C and ^{15}N, suggesting that uptake of intact amino acids had occurred. To calculate the fraction of N derived from the uptake of intact amino acids we plotted excess ^{13}C versus excess ^{15}N on a molar basis and compared the slopes of the resulting regressions with the slope corresponding to the ratio of isotopes in the tracer (see Näsholm and Persson 2001 for a discussion of the method). Based on this technique we determined that all, or a large fraction, of the absorbed N from the two amino acids originated from the uptake of intact arginine and glycine molecules (Figures 2A–C). The GC–MS analysis of root extracts verified the presence of universally labeled arginine and glycine in seedling roots or mycorrhiza (Figures 3A and 3B), confirming that both Scots pine and Norway spruce absorbed the two amino acids as intact molecules when growing under non-sterile conditions in pots. Further, in seed-

Table 4. Mean (± SE) *in situ* uptake rates (μmol g_{dw}^{-1} h^{-1}) of NH_4^+-N, NO_3^--N, glycine-N and arginine-N for Scots pine and Norway spruce seedlings from the CF, Glycine and Arginine treatments ($n = 5$).

N Source	Scots pine	Norway spruce
CF	2.77 ± 0.20	6.89 ± 0.51
	(NH_4^+-N = 2.15 ± 0.20)	(NH_4^+-N = 5.92 ± 0.48)
	(NO_3^--N = 0.62 ± 0.03)	(NO_3^--N = 0.98 ± 0.14)
Arginine	3.04 ± 0.20	5.03 ± 0.64
Glycine	3.54 ± 0.18	4.82 ± 0.97

Table 5. Mean (± SE) *in situ* uptake rates (μmol g_{dw}^{-1} h^{-1}) of NH_4^+-N, NO_3^--N and arginine-N for Scots pine and Norway spruce seedlings from the Mixture treatment ($n = 5$).

Nitrogen form	Scots pine	Norway spruce
NH_4^+-N	1.19 ± 0.39	1.94 ± 0.21
NO_3^--N	0.14 ± 0.02	0.22 ± 0.04
Arg-N	3.97 ± 0.20	2.79 ± 0.41

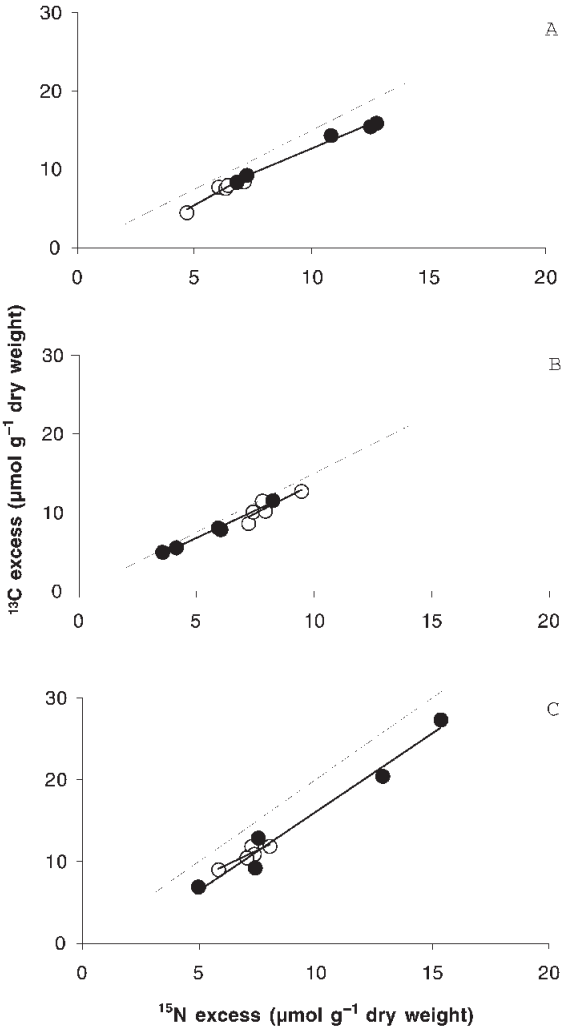

Figure 2. Relationship between excess ^{13}C and excess ^{15}N in Scots pine (○) and Norway spruce (●) roots from the Arginine (A), Mixture (B) and Glycine (C) treatments. Potted seedlings were supplied with U-[$^{13}C_6$], [$^{15}N_4$]-arginine (A and B) or U-[$^{13}C_2$], [^{15}N]-glycine (C) 2 h before harvest. Each symbol represents one analysis of roots from one seedling. Dashed lines indicate the slopes corresponding to 100% of amino-N absorbed as intact amino acid (arginine slope = 1.5, glycine slope = 2.0). Solid lines indicate the regressions for roots of seedlings treated with U-[$^{13}C_6$], [$^{15}N_4$]-arginine and U-[$^{13}C_2$], [^{15}N]-glycine. Regressions for Scots pine (slope = 1.66; r^2 = 0.91) and Norway spruce (slope = 1.25; r^2 = 0.99) from the Arginine treatment indicate that 100 and 83% of the arginine-N was taken up as intact amino acid, respectively. Regressions for Scots pine (slope = 1.53; r^2 = 0.76) and Norway spruce (slope = 1.40; r^2 = 0.99) from the Mixture treatment indicate that 100 and 93% of the arginine-N was taken up as intact amino acid, respectively. Regressions for Scots pine (slope = 1.35; r^2 = 0.83) and Norway spruce (slope = 1.93; r^2 = 0.97) from the Glycine treatment indicate that 67 and 96% of glycine-N was taken up as intact amino acid, respectively.

lings supplied with glycine tracer, isotopic label was also detected in serine (Figure 3C) implying that absorbed, labeled glycine had been metabolized to serine in the seedling roots or mycorrhizas. Because the plants were grown under non-sterile conditions, uptake by plant roots and mycorrhizas could not be distinguished. However, inspection of the roots showed that all the plants were mycorrhizal. Thus, it seems likely that plant amino acid uptake occurred mainly through their respective mycorrhizas.

Tracer studies can provide information only about the short-term uptake of a specific N source. Thus, the fraction of seedling N derived from the uptake of intact amino acids is unknown. If seedlings acquired N over a long period between two fertilization events, part of the supplied amino acid N could have been mineralized before uptake. Hence, a significant part of the supplied arginine-N and glycine-N could have been taken up as NH_4^+-N. However, several observations indicate that arginine-N and glycine-N were absorbed predominately as intact amino acids. First, in both the 3 and 10 mM N treatments, the pH of the soil solutions of Scots pine seedlings were significantly higher when they were supplied with arginine or glycine than when the major form of N supplied was NH_4^+ (Table 2). This suggests that when N was supplied as either arginine or glycine it was largely taken up as the intact amino acid rather than as mineralized N because NH_4^+ uptake is an acidifying process, whereas uptake of amino acids leads to alkalinization of the soil solution (Rygiewicz et al. 1984, Rollwagen and Zasoski 1988, Bush 1993, Fischer et al. 1998). Second, no decrease in Ca concentration was recorded in needles of the arginine- or glycine-grown Scots pine seedlings, whereas a significant decrease was found in all seedlings grown on NH_4^+-dominated nutrient solutions in both the 3 and 10 mM N treatments. Third, the shoot/root ratios of Scots pine seedlings grown with 10 mM N were twice as high when N was supplied as NH_4^+ than when N was supplied as arginine.

The labeling experiments showed that, for both species, total N uptake rates were similar in seedlings in the Arginine, Glycine and CF treatments (Table 4). However, in the CF treatment, uptake rates of NO_3^--N by seedlings were much lower than uptake rates of NH_4^+-N. These results are consistent with data from other N uptake studies performed on different conifer species (Boxman and Roelofs 1988, Marschner et al. 1991, Buchmann et al. 1995, Kronzucker et al. 1997, Bedell et al. 1999). Further, when comparing seedlings in the CF and Mixture treatments, the relative NO_3^--N uptake rate was much lower from the Mixture than from the CF treatment, suggesting that the presence of arginine in the Mixture treatment repressed, either directly or indirectly, the uptake of NO_3^-.

Large amounts of N, when supplied predominantly in the form of NH_4^+, resulted in decreased growth rates and chlorosis in Scots pine seedlings, and the death of Norway spruce seedlings. The chlorosis and low concentrations of Ca found in needles of Scots pine seedlings grown on NH_4^+ as the main N source (Table 3) are typical Ca-deficiency symptoms (Kirkby and Pilbeam 1984). Because low Ca concentrations were recorded only in seedlings in the NH_4^+-dominated treatments, we speculate that the high concentrations of NH_4^+ in these treatments hampered Ca uptake. In a study on *Zea mays* L. cv. Bastion, Engels and Marschner (1993) found that NH_4^+ nutrition resulted in low shoot concentrations of K and Ca. However, in contrast to results observed in previous studies (e.g., Rygiewicz et al. 1984), we found no significant decrease in K con-

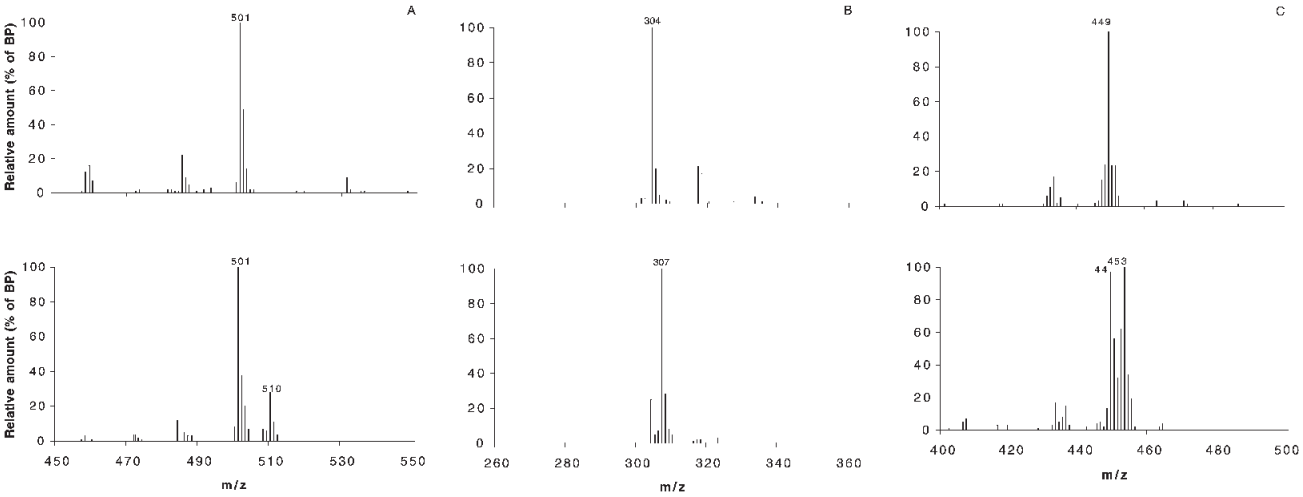

Figure 3. Gas chromatography–mass spectrometry spectra of tBDMS-amino acids extracted from Scots pine roots. (A) Chemical ionization spectra of tBDMS-arginine obtained from root extracts of reference (top) and U-[$^{13}C_6$], [$^{15}N_4$]-arginine labeled (bottom) seedlings. (B) Chemical ionization spectra of tBDMS-glycine obtained from root extracts of reference (top) and U-[$^{13}C_2$], [^{15}N]-glycine labeled (bottom) seedlings. (C) Chemical ionization spectra of tBDMS-serine obtained from root extracts of reference (top) and U-[$^{13}C_2$], [^{15}N]-glycine labeled (bottom) seedlings.

centration in seedlings grown on NH_4^+ as the main N source (Table 3). Possibly, inhibition of K uptake by NH_4^+ occurs mainly when high concentrations of Ca are available, whereas at lower concentrations, the uptake of Ca is prone to inhibition.

Several studies have shown that an increased supply of N increases shoot growth relative to root growth (e.g., Seith et al. 1996). This effect was also evident in our study. For seedlings in the CF, Arginine, Glycine and Mixture treatments, similar increases in shoot/root ratios were recorded with increasing N supply. However, the difference in shoot/root ratios between seedlings in the 3 and 10 mM N treatments was significantly greater when NH_4^+ was the principal N source than in all other cases. This difference may have been an effect of the low Ca concentrations (Ericsson 1995). Alternatively, it could have been associated with differences in the N sources, or to a combination of the two factors. Ammonium-N nutrition leads to excretion of excess H^+ and enrichment of anions at the root surface. Low pH at the root surface could decrease root growth or damage the roots, and thus contribute to a high shoot/root ratio (Runge 1983). For both species, high concentrations of NH_4^+ gave rise to nutrient imbalance, low pH values in the soil solution, decreased growth and, in some cases, seedling death. These results appear to conflict with numerous laboratory studies showing that conifers, and various other plants, grow well on NH_4^+ (e.g., Ingestad 1979). However, the concentrations of NH_4^+ supplied in these studies were generally much lower than in our study, which may explain the discrepancy. This conclusion is further supported by our finding that seedling growth at the lowest N supply was similar in all of the treatments (Figures 1A and 1B), indicating that the concentration of supplied NH_4^+ is critical when using NH_4^+ as the sole or dominant N source.

We conclude that seedlings of Scots pine and Norway spruce take up arginine and glycine intact when these compounds are supplied in a non-sterile peat substrate. These N sources supported growth to a similar extent as a commercial fertilizer based on NO_3^- and NH_4^+. Uptake of NH_4^+ by seedlings was much higher than uptake of NO_3^-, but a high proportion of the NH_4^+ in the fertilizer resulted in base cation deficiency, poor growth and high mortality of seedlings. These problems were not encountered in the amino acid treatments, even at high rates of supply. The good seedling growth on arginine is of interest because this amino acid is a strong cation; therefore, it is strongly adsorbed to negatively charged soil particles and hence the mobility of this N form in the growth substrate is low. Thus, the use of arginine as an N source for growth of conifer seedlings may combine high growth rates of seedlings with low N losses. Further studies of amino acid N nutrition are, therefore, warranted.

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